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(54)发明名称

用于TTR基因编辑及治疗ATTR淀粉样变性的组合物及方法

(57)摘要

提供一种用于在TTR基因内进行编辑(例如引入双链断裂)的组合物及方法。提供一种用于治疗患有与甲状腺素运载蛋白相关的淀粉样变性(ATTR)的个体的组合物及方法。

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1.一种诱导TTR基因内的双链断裂(DSB)的方法,其包含向细胞递送组合物,其中所述组合物包含

a.包含选自SEQ ID NO:5-82的引导序列的引导RNA;

b.包含选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸的引导RNA;或

c.包含与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列的引导RNA。

2.一种修饰TTR基因的方法,其包含向细胞递送组合物,其中所述组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列。

3.一种治疗与TTR相关的淀粉样变性(ATTR)的方法,其包含向有需要的个体施用组合物,其中所述组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列,

从而治疗ATTR。

4.一种降低TTR血清浓度的方法,其包含向有需要的个体施用组合物,其中所述组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列,

从而降低TTR血清浓度。

5.一种减少或预防个体中包含TTR的淀粉样蛋白或淀粉样原纤维的累积的方法,其包含向有需要的个体施用组合物,其中所述组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列,

从而减少淀粉样蛋白或淀粉样原纤维的累积。

6.一种组合物,其包含含有以下的引导RNA:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列。

7.一种包含编码引导RNA的载体的组合物,其中所述引导RNA包含:

a.选自SEQ ID NO:5-82的引导序列;

b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸;或

c.与选自SEQ ID NO:5-82的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列。

8.如权利要求6或7所述的组合物,其用于诱导细胞或个体中所述TTR基因内的双链断裂(DSB)。

9.如权利要求6或7所述的组合物,其用于修饰细胞或个体中的所述TTR基因。

10.如权利要求6或7所述的组合物,其用于治疗个体中与TTR相关的淀粉样变性(ATTR)。

11.如权利要求6或7所述的组合物,其用于降低个体的TTR血清浓度。

12.如权利要求6或7所述的组合物,其用于减少或预防个体中淀粉样蛋白或淀粉样原纤维的累积。

13.如权利要求1至5中任一项所述的方法或如权利要求8至12中任一项所使用的组合物,其中所述组合物降低血清TTR含量。

14.如权利要求13的方法或使用的组合物,其中与施用所述组合物之前的血清TTR含量相比,所述血清TTR含量降低至少50%。

15.如权利要求13的方法或使用的组合物,其中与施用所述组合物之前的血清TTR含量相比,所述血清TTR含量降低50-60%、60-70%、70-80%、80-90%、90-95%、95-98%、98-99%或99-100%。

16.如权利要求1至5或8至15中任一项所述的方法或使用的组合物,其中所述组合物导致所述TTR基因的编辑。

17.如权利要求16的方法或使用的组合物,其中所述编辑是计算经编辑群体的百分比(编辑百分比)。

18.如权利要求17的方法或使用的组合物,其中所述编辑百分比介于所述群体的30%与99%之间。

19.如权利要求17所述的方法或使用的组合物,其中所述编辑百分比介于所述群体的30%与35%之间、35%与40%之间、40%与45%之间、45%与50%之间、50%与55%之间、55%与60%之间、60%与65%之间、65%与70%之间、70%与75%之间、75%与80%之间、80%与85%之间、85%与90%之间、90%与95%之间或95%与99%之间。

20.如权利要求1至5中任一项所述的方法或如权利要求8至19中任一项所使用的组合物,其中所述组合物减少至少一种组织中的淀粉样沉积。

21.如权利要求20所述的方法或使用的组合物,其中所述至少一种组织包含胃、结肠、坐骨神经或背根神经节中的一项或多项。

22.如权利要求20或21所述的方法或使用的组合物,其中在施用所述组合物后8周测量淀粉样沉积。

23.如权利要求20至22中任一项所述的方法或使用的组合物,其中淀粉样沉积是与阴

性对照或施用所述组合物之前测量的含量进行比较。

24.如权利要求20至23中任一项所述的方法或使用的组合物，其中淀粉样沉积是在生检样本中和/或藉由免疫染色测量。

25.如权利要求20至24中任一项所述的方法或使用的组合物，其中淀粉样沉积的减少是介于阴性对照中所观察到的所述淀粉样沉积的30％与35％之间、35％与40％之间、40％与45％之间、45％与50％之间、50％与55％之间、55％与60％之间、60％与65％之间、65％与70％之间、70％与75％之间、75％与80％之间、80％与85％之间、85％与90％之间、90％与95％之间或95％与99％之间。

26.如权利要求20至25中任一项所述的方法或使用的组合物，其中淀粉样沉积的减少是介于施用所述组合物之前观察到的所述淀粉样沉积的30％与35％之间、35％与40％之间、40％与45％之间、45％与50％之间、50％与55％之间、55％与60％之间、60％与65％之间、65％与70％之间、70％与75％之间、75％与80％之间、80％与85％之间、85％与90％之间、90％与95％之间或95％与99％之间。

27.如权利要求1至5或8至26中任一项所述的方法或使用的组合物，其中施用或递送所述组合物至少两次。

28.如权利要求27所述的方法或使用的组合物，其中施用或递送所述组合物至少三次。

29.如权利要求27所述的方法或使用的组合物，其中施用或递送所述组合物至少四次。

30.如权利要求27所述的方法或使用的组合物，其中施用或递送所述组合物高达五次、六次、七次、八次、九次或十次。

31.如权利要求27至30中任一项所述的方法或使用的组合物，其中是依1、2、3、4、5、6、7、8、9、10、11、12、13、14或15天的间隔进行所述施用或递送。

32.如权利要求27至30中任一项所述的方法或使用的组合物，其中是依1、2、3、4、5、6、7、8、9、10、11、12、13、14或15周的间隔进行所述施用或递送。

33.如权利要求27至30中任一项所述的方法或使用的组合物，其中是依1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个月的间隔进行所述施用或递送。

34.如前述权利要求中任一项所述的方法或组合物，其中所述引导序列是选自SEQ ID NO:5-82。

35.如前述权利要求中任一项所述的方法或组合物，其中所述引导RNA与所述人类TTR基因中存在的靶序列至少部分互补。

36.如权利要求35所述的方法或组合物，其中所述靶序列是在所述人类TTR基因的外显子1、2、3或4中。

37.如权利要求35所述的方法或组合物，其中所述靶序列是在所述人类TTR基因的外显子1中。

38.如权利要求35所述的方法或组合物，其中所述靶序列是在所述人类TTR基因的外显子2中。

39.如权利要求35所述的方法或组合物，其中所述靶序列是在所述人类TTR基因的外显子3中。

40.如权利要求35所述的方法或组合物，其中所述靶序列是在所述人类TTR基因的外显子4中。

41.如权利要求1至40中任一项所述的方法或组合物,其中所述引导序列与TTR的正链中的靶序列互补。

42.如权利要求1至40中任一项所述的方法或组合物,其中所述引导序列与TTR的负链中的靶序列互补。

43.如权利要求1至40中任一项所述的方法或组合物,其中所述第一引导序列与所述TTR基因的正链中的第一靶序列互补,且其中所述组合物进一步包含与所述TTR基因的负链中的第二靶序列互补的第二引导序列。

44.如前述权利要求中任一项所述的方法或组合物,其中所述引导RNA包含含有所述引导序列的crRNA且进一步包含SEQ ID NO:126的核苷酸序列,其中SEQ ID NO:126的所述核苷酸在其3'端的所述引导序列之后。

45.如前述权利要求中任一项所述的方法或组合物,其中所述引导RNA是双重引导(dgRNA)。

46.如权利要求45所述的方法或组合物,其中所述双重引导RNA包含含有SEQ ID NO:126的核苷酸序列的crRNA(其中SEQ ID NO:126的所述核苷酸在其3'端的所述引导序列之后)及trRNA。

47.如权利要求1至43中任一项所述的方法或组合物,其中所述引导RNA是单引导(sgRNA)。

48.如权利要求47所述的方法或组合物,其中所述sgRNA包含具有SEQ ID NO:3的型式的引导序列。

49.如权利要求47所述的方法或组合物,其中所述sgRNA包含所述SEQ ID NO:3的序列。

50.如权利要求48或49所述的方法或组合物,其中SEQ ID NO:3中的每一N是任何天然或非天然核苷酸,其中所述N形成所述引导序列,且所述引导序列使Cas9靶向所述TTR基因。

51.如权利要求47至50中任一项所述的方法或组合物,其中所述sgRNA包含SEQ ID NO:5-82的所述引导序列及SEQ ID NO:126的所述核苷酸中的任一种。

52.如权利要求47至51中任一项所述的方法或组合物,其中所述sgRNA包含与选自SEQ ID No:87-124的序列具有至少99%、98%、97%、96%、95%、94%、93%、92%、91%或90%一致性的引导序列。

53.如权利要求47所述的方法或组合物,其中所述sgRNA包含选自SEQ ID No:87-124的序列。

54.如前述权利要求中任一项所述的方法或组合物,其中所述引导RNA包含至少一个修饰。

55.如权利要求54所述的方法或组合物,其中所述至少一个修饰包括2'-O-甲基(2'-O-Me)修饰的核苷酸。

56.如权利要求54或55所述的方法或组合物,其中所述至少一个修饰包括核苷酸之间的硫代磷酸酯(PS)键。

57.如权利要求54至56中任一项所述的方法或组合物,其中所述至少一个修饰包括2'-氟(2'-F)修饰的核苷酸。

58.如权利要求54至57中任一项所述的方法或组合物,其中所述至少一个修饰包括5'端之前五个所述核苷酸中的一项或多项的修饰。

59.如权利要求54至58中任一项所述的方法或组合物,其中所述至少一个修饰包括3'端的最后五个所述核苷酸中的一项或多项的修饰。

60.如权利要求54至59中任一项所述的方法或组合物,其中所述至少一个修饰包括前四个所述核苷酸之间的PS键。

61.如权利要求54至60中任一项所述的方法或组合物,其中所述至少一个修饰包括最后四个所述核苷酸之间的PS键。

62.如权利要求54至61中任一项所述的方法或组合物,其中所述至少一个修饰包括在5'端之前三个所述核苷酸的2'-O-Me修饰的核苷酸。

63.如权利要求54至62中任一项所述的方法或组合物,其中所述至少一个修饰包括在3'端的最后三个所述核苷酸的2'-O-Me修饰的核苷酸。

64.如权利要求54至63中任一项所述的方法或组合物,其中所述引导RNA包含SEQ ID NO:3的所述修饰的核苷酸。

65.如权利要求1至64中任一项所述的方法或组合物,其中所述组合物进一步包含医药上可接受的赋形剂。

66.如权利要求1至65中任一项所述的方法或组合物,其中所述引导RNA与脂质纳米颗粒(LNP)相联。

67.如权利要求66所述的方法或组合物,其中所述LNP包含CCD脂质。

68.如权利要求67所述的方法或组合物,其中所述CCD脂质是脂质A或脂质B。

69.如权利要求66至68所述的方法或组合物,其中所述LNP包含中性脂质。

70.如权利要求69所述的方法或组合物,其中所述中性脂质是DSPC。

71.如权利要求66至70中任一项所述的方法或组合物,其中所述LNP包含辅助脂质。

72.如权利要求71所述的方法或组合物,其中所述辅助脂质是胆固醇。

73.如权利要求66至72中任一项所述的方法或组合物,其中所述LNP包含隐密脂质。

74.如权利要求73所述的方法或组合物,其中所述隐密脂质是PEG2k-DMG。

75.如前述权利要求中任一项所述的方法或组合物,其中所述组合物进一步包含RNA引导的DNA结合剂。

76.如前述权利要求中任一项所述的方法或组合物,其中所述组合物进一步包含编码RNA引导的DNA结合剂的mRNA。

77.如权利要求75或76所述的方法或组合物,其中所述RNA引导的DNA结合剂是Cas裂解酶。

78.如权利要求77所述的方法或组合物,其中所述RNA引导的DNA结合剂是Cas9。

79.如权利要求75至78中任一项所述的方法或组合物,其中所述RNA引导的DNA结合剂经修饰。

80.如权利要求75至79中任一项所述的方法或组合物,其中所述RNA引导的DNA结合剂是切口酶(nickase)。

81.如权利要求79或80所述的方法或组合物,其中所述经修饰RNA引导的DNA结合剂包含核定位信号(NLS)。

82.如权利要求75至81中任一项所述的方法或组合物,其中所述RNA引导的DNA结合剂是来自II型CRISPR/Cas系统的Cas。

83.如前述权利要求中任一项所述的方法或组合物,其中所述组合物是医药调配物且进一步包含医药上可接受的载体。

84.如权利要求1至5或8至83中任一项所述的方法或使用的组合物,其中所述组合物减少或防止包含TTR的淀粉样蛋白或淀粉样原纤维。

85.如权利要求84所述的方法或使用的组合物,其中所述淀粉样蛋白或淀粉样原纤维是在神经、心脏或胃肠道中。

86.如权利要求1至5或8至83中任一项所述的方法或使用的组合物,其中非同源末端接合(NHEJ)在所述TTR基因中的DSB修复期间导致突变。

87.如权利要求86所述的方法或使用的组合物,其中NHEJ在所述TTR基因中的DSB修复期间导致核苷酸(群)的缺失或插入。

88.如权利要求87所述的方法或使用的组合物,其中所述核苷酸(群)的缺失或插入诱导所述TTR基因中的框移或无义突变。

89.如权利要求87所述的方法或使用的组合物,其中在至少50%的肝细胞的所述TTR基因中诱导框移或无义突变。

90.如权利要求89所述的方法或使用的组合物,其中在50%-60%、60%-70%、70%或80%、80%-90%、90-95%、95%-99%或99%-100%的肝细胞的所述TTR基因中诱导框移或无义突变。

91.如权利要求87至90中任一项所述的方法或使用的组合物,其中在所述TTR基因中出现的核苷酸的缺失或插入是脱靶位点中的至少50倍或更多。

92.如权利要求91所述的方法或使用的组合物,其中在所述TTR基因中出现的核苷酸的所述缺失或所述插入是脱靶位点中的50倍至150倍、150倍至500倍、500倍至1500倍、1500倍至5000倍、5000倍至15000倍、15000倍至30000倍或30000倍至60000倍。

93.如权利要求87至92中任一项所述的方法或使用的组合物,其中核苷酸的所述缺失或所述插入出现在原代人类肝细胞中少于或等于3、2、1或0个脱靶位点处,视情况其中所述脱靶位点不出现在所述原代人类肝细胞的基因组中的蛋白质编码区中。

94.如权利要求93所述的方法或使用的组合物,其中核苷酸的所述缺失或所述插入出现在原代人类肝细胞中的一定数目脱靶位点处,所述数目少于在Cas9过度表达细胞中出现核苷酸的缺失或插入的脱靶位点的数目,视情况其中所述脱靶位点不出现在所述原代人类肝细胞的基因组中的蛋白质编码区中。

95.如权利要求94所述的方法或使用的组合物,其中所述Cas9过度表达细胞是稳定表达Cas9的HEK293细胞。

96.如权利要求93至95中任一项所述的方法或使用的组合物,其中所述藉由分析来自经Cas9 mRNA及所述引导RNA活体外转染的原代人类肝细胞的基因组DNA来测定原代人类肝细胞中脱靶位点的数目,视情况其中所述脱靶位点不出现在所述原代人类肝细胞的所述基因组中的蛋白质编码区中。

97.如权利要求93至95中任一项所述的方法或使用的组合物,其中所述藉由寡核苷酸插入分析法测定原代人类肝细胞中脱靶位点的数目,所述分析法包括:分析来自经Cas9 mRNA、所述引导RNA及供体寡核苷酸活体外转染的原代人类肝细胞的基因组DNA,视情况其中所述脱靶位点不出现在所述原代人类肝细胞的所述基因组中的蛋白质编码区中。

98.如权利要求1至43或47至97中任一项所述的方法或组合物,其中所述引导RNA的序列是:

a)SEQ ID NO:92或104;

b)SEQ ID NO:87、89、96或113;

c)SEQ ID NO:100、102、106、111或112;或

d)SEQ ID NO:88、90、91、93、94、95、97、101、103、108或109,

视情况其中所述引导RNA不在出现在原代人类肝细胞的所述基因组中的蛋白质编码区中的脱靶位点产生插入或缺失(indel)。

99.如权利要求1至5或8至98中任一项所述的方法或使用的组合物,其中施用所述组合物以降低所述个体中的TTR含量。

100.如权利要求99所述的方法或使用的组合物,其中所述TTR的含量降低至少50%。

101.如权利要求100所述的方法或使用的组合物,其中所述TTR的含量降低50%-60%、60%-70%、70%或80%、80%-90%、90-95%、95%-99%或99%-100%。

102.如权利要求100或101所述的方法或使用的组合物,其中在血清、血浆、血液、脑脊液或痰中测量所述TTR的含量。

103.如权利要求100或101所述的方法或使用的组合物,其中在肝、脉络丛和/或视网膜中测量所述TTR的含量。

104.如权利要求99至103中任一项所述的方法或使用的组合物,其中经由酶联免疫吸附分析(E LISA)测量所述TTR的含量。

105.如权利要求1至5或8至104中任一项所述的方法或使用的组合物,其中所述个体患有ATTR。

106.如权利要求1至5或8至105中任一项所述的方法或使用的组合物,其中所述个体是人类。

107.如权利要求105或106所述的方法或使用的组合物,其中所述个体患有ATTRwt。

108.如权利要求105或106所述的方法或使用的组合物,其中所述个体患有遗传性ATTR。

109.如权利要求1至5、8至106或108中任一项所述的方法或使用的组合物,其中所述个体具有ATTR家族史。

110.如权利要求1至5、8至106或108至109中任一项所述的方法或使用的组合物,其中所述个体患有家族性淀粉样多神经病变。

111.如权利要求1至5或8至110中任一项所述的方法或使用的组合物,其中所述个体仅具有或主要具有ATTR的神经症状。

112.如权利要求1至5或8至110中任一项所述的方法或使用的组合物,其中所述个体患有家族性淀粉样心肌病。

113.如权利要求1至5、8至109或112中任一项所述的方法或使用的组合物,其中所述个体仅具有或主要具有ATTR的心脏症状。

114.如权利要求1至5或8至113中任一项所述的方法或使用的组合物,其中所述个体表达具有V30突变的TTR。

115.如权利要求114所述的方法或使用的组合物,其中所述V30突变是V30A、V30G、V30L

或V30M。

116.如权利要求1至5或8至113中任一项所述的方法或使用的组合物，其中所述个体表达具有T60突变的TTR。

117.如权利要求116所述的方法或使用的组合物，其中所述T60突变是T60A。

118.如权利要求1至5或8至113中任一项所述的方法或使用的组合物，其中所述个体表达具有V122突变的TTR。

119.如权利要求118所述的方法或使用的组合物，其中所述V122突变是V122A、V122I或V122(-)。

120.如权利要求1至5或8至119中任一项所述的方法或使用的组合物，其中所述个体表达野生型TTR。

121.如权利要求1至5、8至107或120中任一项所述的方法或使用的组合物，其中所述个体不表达具有V30、T60或V122突变的TTR。

122.如权利要求1至5、8至107或120至121中任一项所述的方法或使用的组合物，其中所述个体不表达具有病理突变的TTR。

123.如权利要求121所述的方法或使用的组合物，其中所述个体的野生型TTR是同型接合。

124.如权利要求1至5或8至123中任一项所述的方法或使用的组合物，其中在施用后，所述个体具有改善、稳定或减缓变化的感觉运动神经病变的症状。

125.如权利要求124所述的方法或使用的组合物，其中使用肌电图、神经传导测试或患者报告的结果测量所述感觉神经病变的改善、稳定或减缓变化。

126.如权利要求1至5或8至125中任一项所述的方法或使用的组合物，其中所述个体具有改善、稳定或减缓变化的郁血性心脏衰竭的症状。

127.如权利要求126所述的方法或使用的组合物，其中使用心脏生物标记测试、肺功能测试、胸部x射线或心电图测量所述郁血性心脏衰竭的改善、稳定或减缓变化。

128.如权利要求1至5或8至127中任一项所述的方法或使用的组合物，其中所述组合物或所述医药调配物是经由病毒载体施用。

129.如权利要求1至5或8至127中任一项所述的方法或使用的组合物，其中所述组合物或所述医药调配物是经由脂质纳米颗粒施用。

130.如权利要求1至5或8至129中任一项所述的方法或使用的组合物，其中在施用所述组合物或所述调配物之前测试所述个体的所述TTR基因的特异性突变。

131.如权利要求1至130中任一项所述的方法或组合物，其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:5。

132.如权利要求1至130中任一项所述的方法或组合物，其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:6。

133.如权利要求1至130中任一项所述的方法或组合物，其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:7。

134.如权利要求1至130中任一项所述的方法或组合物，其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:8。

135.如权利要求1至130中任一项所述的方法或组合物，其中选自SEQ ID NO:5-82的所

述序列是SEQ ID NO:9。

136.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:10。

137.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:11。

138.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:12。

139.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:13。

140.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:14。

141.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:15。

142.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:16。

143.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:17。

144.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:18。

145.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:19。

146.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:20。

147.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:21。

148.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:22。

149.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:23。

150.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:24。

151.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:25。

152.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:26。

153.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:27。

154.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:28。

155.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:29。

156.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:30。

157.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:31。

158.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:32。

159.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:33。

160.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:34。

161.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:35。

162.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:36。

163.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:37。

164.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:38。

165.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:39。

166.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:40。

167.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:41。

168.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:42。

169.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:43。

170.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:44。

171.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:45。

172.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:46。

173.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:47。

174.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所

述序列是SEQ ID NO:48。

175.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:49。

176.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:50。

177.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:51。

178.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:52。

179.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:53。

180.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:54。

181.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:55。

182.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:56。

183.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:57。

184.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:58。

185.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:59。

186.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:60。

187.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:61。

188.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:62。

189.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:63。

190.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:64。

191.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:65。

192.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:66。

193.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:67。

194.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:68。

195.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:69。

196.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:70。

197.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:71。

198.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:72。

199.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:73。

200.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:74。

201.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:75。

202.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:76。

203.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:77。

204.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:78。

205.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:79。

206.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:80。

207.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:81。

208.如权利要求1至130中任一项所述的方法或组合物,其中选自SEQ ID NO:5-82的所述序列是SEQ ID NO:82。

209.一种如权利要求6至208中任一项所述的组合物或调配物的用途,其用于制备用于治疗患有ATTR的人类个体的药剂。

用于TTR基因编辑及治疗ATTR淀粉样变性的组合物及方法

[0001] 本申请要求于2017年9月29日提出申请的美国临时申请案第62/556,236号以及于2018年5月15日提出申请的美国临时申请案第62/671,902号的优先权益,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27日创建的该ASCII拷贝命名为2018-09-27_01155-0013-PCT_ST25.txt且大小为417,471个字节。

[0003] 甲状腺素运载蛋白(TTR)是由TTR基因产生的蛋白质,其通常用于在整个身体内转运视黄醇及甲状腺素。TTR主要在肝中合成,在脉络丛及视网膜中产生小部分。TTR通常在血液中作为可溶性四聚体蛋白循环。

[0004] 可破坏四聚体稳定性的TTR的病原变体可由TTR基因的突变等位基因编码。突变TTR可导致错误折叠的TTR,其可产生淀粉样蛋白(亦即,错误折叠的TTR蛋白的聚集物)。在一些情形下,TTR的病原变体可导致淀粉样变性或由淀粉样蛋白的累积引起的疾病。例如,错误折叠的TTR单体可在组织(例如周围神经、心脏及胃肠道)内聚合成淀粉样原纤维。淀粉样斑块亦可包含沉积于错误折叠的TTR上的野生型TTR。

[0005] 在60岁或以上的男性中亦观察到野生型TTR的错误折叠及沉积,且其与心律问题、心脏衰竭及腕管相关。

[0006] 特征在于TTR沉积的淀粉样变性可称作“ATTR”、“TTR相关的淀粉样变性”、“TTR淀粉样变性”或“ATTR淀粉样变性”、“ATTR家族性淀粉样变性”(当与家族中的基因突变相关时)、或“ATTRwt”或“野生型ATTR”(当由野生型TTR的错误折叠及沉积产生时)。

[0007] ATTR可呈现广谱症状,且具有不同类别的ATTR的患者可具有不同特征及预后。一些类别的ATTR包括家族性淀粉样多神经病变(FAP)、家族性淀粉样心肌病(FAC)及野生型TTR淀粉样变性(wt-TTR淀粉样变性)。FAP通常与感觉运动神经病变一起呈现,而FAC及wt-TTR淀粉样变性通常与郁血性心脏衰竭一起呈现。FAP及FAC通常与TTR基因的遗传突变相关,且TTR基因的超过100种不同突变与ATTR相关。相比之下,wt-TTR淀粉样变性与衰老相关,而与TTR的基因突变无关。据估计,世界范围内约50,000名患者可能受到FAP及FAC的影响。

[0008] 尽管TTR的超过100种突变与ATTR相关,但某些突变与神经病变和/或心肌病更紧密相关。例如,TTR的T60的突变与心肌病及神经病变二者相关;V30的突变与神经病变相关性更大;且V122的突变与心肌病相关性更大。

[0009] 已研究一系列用于治疗ATTR的治疗方法,但无批准的药物可停止疾病进展并改善生活质量。尽管肝移植已被研究用于治疗ATTR,但由于其涉及显著风险且在移植后疾病进展有时继续,其使用正在下降。小分子稳定剂(例如二氢尼柳(diflunisal)及他发米帝司(tafamidis))似乎减缓ATTR进展,但该药剂并未阻止疾病进展。

[0010] 使用小干扰RNA(siRNA)减弱、反义减弱或单株抗体靶向淀粉样原纤维进行破坏的方法目前亦正在研究中,但是尽管TTR表达的短期抑制结果显示令人鼓舞的初步数据,但需要可产生长效TTR抑制的治疗。

[0011] 因此，提供以下实施例。在一些实施例中，本发明提供使用引导RNA与RNA引导的DNA结合剂(例如CRISPR/Cas系统)来显著减少或剔除TTR基因的表达从而显著减少或消除与ATTR相关的TTR蛋白的产生的组合物及方法。经由改变TTR基因显著减少或消除与ATTR相关的TTR蛋白的产生可为长期减少或消除。

[0012] 发明概述

[0013] 实施例1是诱导TTR基因内的双链断裂(DSB)的方法，其包含向细胞递送组合物，其中该组合物包含

[0014] a.包含选自SEQ ID NO:5-82的引导序列的引导RNA；

[0015] b.包含选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸的引导RNA；或

[0016] c.包含与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列的引导RNA。

[0017] 实施例2是修饰TTR基因的方法，其包含向细胞递送组合物，其中该组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA：

[0018] a.选自SEQ ID NO:5-82的引导序列；

[0019] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0020] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0021] 实施例3是治疗与TTR相关的淀粉样变性(ATTR)的方法，其包含向有需要的个体施用组合物，其中该组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA：

[0022] a.选自SEQ ID NO:5-82的引导序列；

[0023] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0024] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列，

[0025] 从而治疗ATTR。

[0026] 实施例4是降低TTR血清浓度的方法，其包含向有需要的个体施用组合物，其中该组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA：

[0027] a.选自SEQ ID NO:5-82的引导序列；

[0028] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0029] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列，

[0030] 从而降低TTR血清浓度。

[0031] 实施例5是减少或预防个体中包含TTR的淀粉样蛋白或淀粉样原纤维的累积的方法，其包含向有需要的个体施用组合物，其中该组合物包含(i)RNA引导的DNA结合剂或编码RNA引导的DNA结合剂的核酸及(ii)包含以下的引导RNA：

[0032] a.选自SEQ ID NO:5-82的引导序列；

[0033] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0034] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列，

[0035] 从而减少淀粉样蛋白或淀粉样原纤维的累积。

[0036] 实施例6是包含引导RNA的组合物，该引导RNA包含：

[0037] a.选自SEQ ID NO:5-82的引导序列；

[0038] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0039] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0040] 实施例7是包含编码引导RNA的载体的组合物，其中该引导RNA包含：

[0041] a.选自SEQ ID NO:5-82的引导序列；

[0042] b.选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸；或

[0043] c.与选自SEQ ID NO:5-82的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0044] 实施例8是实施例6或7的组合物，其用于诱导细胞或个体中的TTR基因内的双链断裂(DSB)。

[0045] 实施例9是实施例6或7的组合物，其用于修饰细胞或个体中的TTR基因。

[0046] 实施例10是实施例6或7的组合物，其用于治疗个体的与TTR相关的淀粉样变性(ATTR)。

[0047] 实施例11是实施例6或7的组合物，其用于降低个体的TTR血清浓度。

[0048] 实施例12是实施例6或7的组合物，其用于减少或预防个体中淀粉样蛋白或淀粉样原纤维的累积。

[0049] 实施例13是实施例1至5中任一实施例的方法或实施例8至12中任一实施例使用的组合物，其中该组合物降低血清TTR含量。

[0050] 实施例14是实施例13的方法或使用的组合物，其中与施用该组合物之前的血清TTR含量相比，该血清TTR含量降低至少50％。

[0051] 实施例15是实施例13的方法或使用的组合物，其中与施用该组合物之前的血清TTR含量相比，该血清TTR含量降低50-60％、60-70％、70-80％、80-90％、90-95％、95-98％、98-99％或99-100％。

[0052] 实施例16是实施例1至5或8至15中任一实施例的方法或使用的组合物，其中该组合物导致该TTR基因的编辑。

[0053] 实施例17是实施例16的方法或使用的组合物，其中该编辑计算为经编辑群体的百分比(编辑百分比)。

[0054] 实施例18是实施例17的方法或使用的组合物，其中该编辑百分比介于群体的30％与99％之间。

[0055] 实施例19是实施例17的方法或使用的组合物，其中该编辑百分比介于群体的30％与35％之间、35％与40％之间、40％与45％之间、45％与50％之间、50％与55％之间、55％与60％之间、60％与65％之间、65％与70％之间、70％与75％之间、75％与80％之间、80％与85％之间、85％与90％之间、90％与95％之间或95％与99％之间。

[0056] 实施例20是实施例1至5中任一实施例的方法或实施例8至19中任一实施例使用的

组合物，其中该组合物减少至少一种组织中的淀粉样沉积。

[0057] 实施例21是实施例20的方法或使用的组合物，其中该至少一种组织包含胃、结肠、坐骨神经或背根神经节中的一项或多项。

[0058] 实施例22是实施例20或21的方法或使用的组合物，其中在施用该组合物后8周测量淀粉样沉积。

[0059] 实施例23是实施例20至22中任一实施例的方法或使用的组合物，其中淀粉样沉积是与阴性对照或施用该组合物之前测量的含量进行比较。

[0060] 实施例24是实施例20至23中任一实施例的方法或使用的组合物，其中淀粉样沉积是在生检样本中和/或藉由免疫染色测量。

[0061] 实施例25是实施例20至24中任一实施例的方法或使用的组合物，其中淀粉样沉积的减少是介于阴性对照中所观察到的淀粉样沉积的30％与35％之间、35％与40％之间、40％与45％之间、45％与50％之间、50％与55％之间、55％与60％之间、60％与65％之间、65％与70％之间、70％与75％之间、75％与80％之间、80％与85％之间、85％与90％之间、90％与95％之间或95％与99％之间。

[0062] 实施例26是实施例20至25中任一实施例的方法或使用的组合物，其中淀粉样沉积的减少是介于施用该组合物之前所观察到的淀粉样沉积的30％与35％之间、35％与40％之间、40％与45％之间、45％与50％之间、50％与55％之间、55％与60％之间、60％与65％之间、65％与70％之间、70％与75％之间、75％与80％之间、80％与85％之间、85％与90％之间、90％与95％之间或95％与99％之间。

[0063] 实施例27是实施例1至5或8至26中任一实施例的方法或使用的组合物，其中施用或递送该组合物至少两次。

[0064] 实施例28是实施例27的方法或使用的组合物，其中施用或递送该组合物至少三次。

[0065] 实施例29是实施例27的方法或使用的组合物，其中施用或递送该组合物至少四次。

[0066] 实施例30是实施例27的方法或使用的组合物，其中施用或递送该组合物高达五次、六次、七次、八次、九次或十次。

[0067] 实施例31是实施例27至30中任一实施例的方法或使用的组合物，其中该施用或递送是以1、2、3、4、5、6、7、8、9、10、11、12、13、14或15天的间隔发生。

[0068] 实施例32是实施例27至30中任一实施例的方法或使用的组合物，其中该施用或递送是以1、2、3、4、5、6、7、8、9、10、11、12、13、14或15周的间隔发生。

[0069] 实施例33是实施例27至30中任一实施例的方法或使用的组合物，其中该施用或递送是以1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个月的间隔发生。

[0070] 实施例34是前述实施例中任一实施例的方法或组合物，其中该引导序列是选自SEQ ID NO:5-82。

[0071] 实施例35是前述实施例中任一实施例的方法或组合物，其中该引导RNA与人类TTR基因中存在的靶序列至少部分互补。

[0072] 实施例36是实施例35的方法或组合物，其中该靶序列在人类TTR基因的外显子1、2、3或4中。

[0073] 实施例37是实施例35的方法或组合物，其中该靶序列在人类TTR基因的外显子1中。

[0074] 实施例38是实施例35的方法或组合物，其中该靶序列在人类TTR基因的外显子2中。

[0075] 实施例39是实施例35的方法或组合物，其中该靶序列在人类TTR基因的外显子3中。

[0076] 实施例40是实施例35的方法或组合物，其中该靶序列在人类TTR基因的外显子4中。

[0077] 实施例41是实施例1至40中任一实施例的方法或组合物，其中该引导序列与TTR的正链中的靶序列互补。

[0078] 实施例42是实施例1至40中任一实施例的方法或组合物，其中该引导序列与TTR的负链中的靶序列互补。

[0079] 实施例43是实施例1至40中任一实施例的方法或组合物，其中第一引导序列与该TTR基因的该正链中的第一靶序列互补，且其中该组合物进一步包含与该TTR基因的该负链中的第二靶序列互补的第二引导序列。

[0080] 实施例44是前述实施例中任一实施例的方法或组合物，其中该引导RNA包含含有该引导序列的crRNA且进一步包含SEQ ID NO:126的核苷酸序列，其中SEQ ID NO:126的核苷酸在其3'端引导序列之后。

[0081] 实施例45是前述实施例中任一实施例的方法或组合物，其中该引导RNA是双重引导(dgRNA)。

[0082] 实施例46是实施例45的方法或组合物，其中该双重引导RNA包含含有SEQ ID NO:126的核苷酸序列的crRNA及trRNA，其中SEQ ID NO:126的核苷酸在其3'端引导序列之后。

[0083] 实施例47是实施例1至43中任一实施例的方法或组合物，其中该引导RNA是单引导(sgRNA)。

[0084] 实施例48是实施例47的方法或组合物，其中该sgRNA包含具有SEQ ID NO:3的型式的引导序列。

[0085] 实施例49是实施例47的方法或组合物，其中该sgRNA包含SEQ ID NO:3的序列。

[0086] 实施例50是实施例48或49的方法或组合物，其中SEQ ID NO:3中的每一N是任何天然或非天然核苷酸，其中该N形成该引导序列，且该引导序列使Cas9靶向该TTR基因。

[0087] 实施例51是实施例47至50中任一实施例的方法或组合物，其中该sgRNA包含SEQ ID NO:5-82的引导序列及SEQ ID NO:126的核苷酸中的任一种。

[0088] 实施例52是实施例47至51中任一实施例的方法或组合物，其中该sgRNA包含与选自SEQ ID No:87-124的序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0089] 实施例53是实施例47的方法或组合物，其中该sgRNA包含选自SEQ ID No:87-124的序列。

[0090] 实施例54是前述实施例中任一实施例的方法或组合物，其中该引导RNA包含至少一个修饰。

[0091] 实施例55是实施例54的方法或组合物，其中该至少一个修饰包括2'-O-甲基(2'-

O-Me)修饰的核苷酸。

[0092] 实施例56是实施例54或55的方法或组合物,其中该至少一个修饰包括核苷酸之间的硫代磷酸酯(PS)键。

[0093] 实施例57是实施例54至56中任一实施例的方法或组合物,其中该至少一个修饰包括2'-氟(2'-F)修饰的核苷酸。

[0094] 实施例58是实施例54至57中任一实施例的方法或组合物,其中该至少一个修饰包括5'端之前五个核苷酸中的一项或多项的修饰。

[0095] 实施例59是实施例54至58中任一实施例的方法或组合物,其中该至少一个修饰包括3'端的最后五个核苷酸中的一项或多项的修饰。

[0096] 实施例60是实施例54至59中任一实施例的方法或组合物,其中该至少一个修饰包括前四个核苷酸之间的PS键。

[0097] 实施例61是实施例54至60中任一实施例的方法或组合物,其中该至少一个修饰包括最后四个核苷酸之间的PS键。

[0098] 实施例62是实施例54至61中任一实施例的方法或组合物,其中该至少一个修饰包括在5'端之前三个核苷酸的2'-O-Me修饰的核苷酸。

[0099] 实施例63是实施例54至62中任一实施例的方法或组合物,其中该至少一个修饰包括在3'端的最后三个核苷酸的2'-O-Me修饰的核苷酸。

[0100] 实施例64是实施例54至63中任一实施例的方法或组合物,其中该引导RNA包含SEQ ID NO:3的修饰的核苷酸。

[0101] 实施例65是实施例1至64中任一实施例的方法或组合物,其中该组合物进一步包含医药上可接受的赋形剂。

[0102] 实施例66是实施例1至65中任一实施例的方法或组合物,其中该引导RNA与脂质纳米颗粒(LNP)相联。

[0103] 实施例67是实施例66的方法或组合物,其中该LNP包含CCD脂质。

[0104] 实施例68是实施例67的方法或组合物,其中该CCD脂质是脂质a或脂质B。

[0105] 实施例69是实施例66至68的方法或组合物,其中该LNP包含中性脂质。

[0106] 实施例70是实施例69的方法或组合物,其中该中性脂质是DSPC。

[0107] 实施例71是实施例66至70中任一实施例的方法或组合物,其中该LNP包含辅助脂质。

[0108] 实施例72是实施例71的方法或组合物,其中该辅助脂质是胆固醇。

[0109] 实施例73是实施例66至72中任一实施例的方法或组合物,其中该LNP包含隐密脂质(stealth lipid)。

[0110] 实施例74是实施例73的方法或组合物,其中该隐密脂质是PEG2k-DMG。

[0111] 实施例75是前述实施例中任一实施例的方法或组合物,其中该组合物进一步包含RNA引导的DNA结合剂。

[0112] 实施例76是前述实施例中任一实施例的方法或组合物,其中该组合物进一步包含编码RNA引导的DNA结合剂的mRNA。

[0113] 实施例77是实施例75或76的方法或组合物,其中该RNA引导的DNA结合剂是Cas裂解酶。

[0114] 实施例78是实施例77的方法或组合物，其中该RNA引导的DNA结合剂是Cas9。

[0115] 实施例79是实施例75至78中任一实施例的方法或组合物，其中该RNA引导的DNA结合剂经修饰。

[0116] 实施例80是实施例75至79中任一实施例的方法或组合物，其中该RNA引导的DNA结合剂是切口酶。

[0117] 实施例81是实施例79或80的方法或组合物，其中该经修饰的RNA引导的DNA结合剂包含核定位信号(NLS)。

[0118] 实施例82是实施例75至81中任一实施例的方法或组合物，其中该RNA引导的DNA结合剂是来自II型CRISPR/Cas系统的Cas。

[0119] 实施例83是前述实施例中任一实施例的方法或组合物，其中该组合物是医药调配物且进一步包含医药上可接受的载体。

[0120] 实施例84是实施例1至5或8至83中任一实施例的方法或使用的组合物，其中该组合物减少或防止包含TTR的淀粉样蛋白或淀粉样原纤维。

[0121] 实施例85是实施例84的方法或使用的组合物，其中该淀粉样蛋白或淀粉样原纤维是在神经、心脏或胃肠道中。

[0122] 实施例86是实施例1至5或8至83中任一实施例的方法或使用的组合物，其中非同源末端接合(NHEJ)在该TTR基因中的DSB修复期间导致突变。

[0123] 实施例87是实施例86的方法或使用的组合物，其中NHEJ在该TTR基因中的DSB修复期间导致核苷酸的缺失或插入。

[0124] 实施例88是实施例87的方法或使用的组合物，其中核苷酸的缺失或插入诱导该TTR基因中的框移或无义突变。

[0125] 实施例89是实施例87的方法或使用的组合物，其中在至少50％的肝细胞的该TTR基因中诱导框移或无义突变。

[0126] 实施例90是实施例89的方法或使用的组合物，其中在50％-60％、60％-70％、70％或80％、80％-90％、90-95％、95％-99％或99％-100％的肝细胞的该TTR基因中诱导框移或无义突变。

[0127] 实施例91是实施例87至90中任一实施例的方法或使用的组合物，其中在该TTR基因中出现的核苷酸的缺失或插入是脱靶位点中的至少50倍或更多。

[0128] 实施例92是实施例91的方法或使用的组合物，其中在该TTR基因中出现的核苷酸的缺失或插入是脱靶位点中的50倍至150倍、150倍至500倍、500倍至1500倍、1500倍至5000倍、5000倍至15000倍、15000倍至30000倍或30000倍至60000倍。

[0129] 实施例93是实施例87至92中任一实施例的方法或使用的组合物，其中核苷酸的缺失或插入是在原代人类肝细胞中的少于或等于3、2、1或0个脱靶位点处出现，视情况其中该(等)脱靶位点不出现在该原代人类肝细胞的基因组中的蛋白质编码区中。

[0130] 实施例94是实施例93的方法或使用的组合物，其中核苷酸的缺失或插入是在原代人类肝细胞中的一定数目脱靶位点处出现，该数目少于在Cas9过度表达细胞中出现核苷酸的缺失或插入的脱靶位点的数目，视情况其中该(等)脱靶位点不出现在该原代人类肝细胞的基因组中的蛋白质编码区中。

[0131] 实施例95是实施例94的方法或使用的组合物，其中该Cas9过度表达细胞是稳定表

达Cas9的HEK293细胞。

[0132] 实施例96是实施例93至95中任一实施例的方法或使用的组合物，其中藉由分析来自经Cas9 mRNA及该引导RNA活体外转染的原代人类肝细胞的基因组DNA测定原代人类肝细胞中脱靶位点的数目，视情况其中该(等)脱靶位点不出现在该原代人类肝细胞的基因组中的蛋白质编码区中。

[0133] 实施例97是实施例93至95中任一实施例的方法或使用的组合物，其中藉由包含以下的寡核苷酸插入分析测定原代人类肝细胞中脱靶位点的数目：分析来自经Cas9 mRNA、该引导RNA及供体寡核苷酸活体外转染的原代人类肝细胞的基因组DNA，视情况其中该(等)脱靶位点不出现在该原代人类肝细胞的基因组中的蛋白质编码区中。

[0134] 实施例98是实施例1至43或47至97中任一实施例的方法或组合物，其中该引导RNA的序列是：

[0135] a)SEQ ID NO:92或104；

[0136] b)SEQ ID NO:87、89、96或113；

[0137] c)SEQ ID NO:100、102、106、111或112；或

[0138] d)SEQ ID NO:88、90、91、93、94、95、97、101、103、108或109，

[0139] 视情况其中该引导RNA在脱靶位点不产生插入或缺失(indel)，该(等)脱靶位点出现在原代人类肝细胞的基因组中的蛋白质编码区中。

[0140] 实施例99是实施例1至5或8至98中任一实施例的方法或使用的组合物，其中施用该组合物降低该个体中的TTR含量。

[0141] 实施例100是实施例99的方法或使用的组合物，其中TTR的含量降低至少50％。

[0142] 实施例101是实施例100的方法或使用的组合物，其中TTR的含量降低50％-60％、60％-70％、70％或80％、80％-90％、90-95％、95％-99％或99％-100％。

[0143] 实施例102是实施例100或101的方法或使用的组合物，其中在血清、血浆、血液、脑脊液或痰中测量TTR的含量。

[0144] 实施例103是实施例100或101的方法或使用的组合物，其中在肝、脉络丛和/或视网膜中测量TTR的含量。

[0145] 实施例104是实施例99至103中任一实施例的方法或使用的组合物，其中经由酶联免疫吸附分析(ELISA)测量TTR的含量。

[0146] 实施例105是实施例1至5或8至104中任一实施例的方法或使用的组合物，其中该个体患有ATTR。

[0147] 实施例106是实施例1至5或8至105中任一实施例的方法或使用的组合物，其中该个体是人类。

[0148] 实施例107是实施例105或106的方法或使用的组合物，其中该个体患有ATTRwt。

[0149] 实施例108是实施例105或106的方法或使用的组合物，其中该个体患有遗传性ATTR。

[0150] 实施例109是实施例1至5、8至106或108中任一实施例的方法或使用的组合物，其中该个体具有ATTR家族史。

[0151] 实施例110是实施例1至5、8至106或108至109中任一实施例的方法或使用的组合物，其中该个体患有家族性淀粉样多神经病变。

[0152] 实施例111是实施例1至5或8至110中任一实施例的方法或使用的组合物，其中该个体仅具有或主要具有ATTR的神经症状。

[0153] 实施例112是实施例1至5或8至110中任一实施例的方法或使用的组合物，其中该个体患有家族性淀粉样心肌病。

[0154] 实施例113是实施例1至5、8至109或112中任一实施例的方法或使用的组合物，其中该个体仅具有或主要具有ATTR的心脏症状。

[0155] 实施例114是实施例1至5或8至113中任一实施例的方法或使用的组合物，其中该个体表达具有V30突变的TTR。

[0156] 实施例115是实施例114的方法或使用的组合物，其中该V30突变是V30A、V30G、V30L或V30M。

[0157] 实施例116是实施例1至5或8至113中任一实施例的方法或使用的组合物，其中该个体表达具有T60突变的TTR。

[0158] 实施例117是实施例116的方法或使用的组合物，其中该T60突变是T60A。

[0159] 实施例118是实施例1至5或8至113中任一实施例的方法或使用的组合物，其中该个体表达具有V122突变的TTR。

[0160] 实施例119是实施例118的方法或使用的组合物，其中该V122突变是V122A、V122I或V122(-)。

[0161] 实施例120是实施例1至5或8至119中任一实施例的方法或使用的组合物，其中该个体表达野生型TTR。

[0162] 实施例121是实施例1至5、8至107或120中任一实施例的方法或使用的组合物，其中该个体不表达具有V30、T60或V122突变的TTR。

[0163] 实施例122是实施例1至5、8至107或120至121中任一实施例的方法或使用的组合物，其中该个体不表达具有病理突变的TTR。

[0164] 实施例123是实施例121的方法或使用的组合物，其中该个体对于野生型TTR是同型接合的。

[0165] 实施例124是实施例1至5或8至123中任一实施例的方法或使用的组合物，其中在施用后，该个体具有感觉运动神经病变的症状的改善、稳定或减缓其变化。

[0166] 实施例125是实施例124的方法或使用的组合物，其中使用肌电图、神经传导测试或患者报告的结果测量感觉神经病变的改善、稳定或减缓其变化。

[0167] 实施例126是实施例1至5或8至125中任一实施例的方法或使用的组合物，其中该个体具有郁血性心脏衰竭的症状的改善、稳定或减缓其变化。

[0168] 实施例127是实施例126的方法或使用的组合物，其中使用心脏生物标记测试、肺功能测试、胸部x射线或心电图测量郁血性心脏衰竭的改善、稳定或减缓其变化。

[0169] 实施例128是实施例1至5或8至127中任一实施例的方法或使用的组合物，其中该组合物或医药调配物是经由病毒载体施用。

[0170] 实施例129是实施例1至5或8至127中任一实施例的方法或使用的组合物，其中该组合物或医药调配物是经由脂质纳米颗粒施用。

[0171] 实施例130是实施例1至5或8至129中任一实施例的方法或使用的组合物，其中在施用该组合物或调配物之前测试该个体的该TTR基因的特异性突变。

[0172] 实施例131是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:5。

[0173] 实施例132是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:6。

[0174] 实施例133是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:7。

[0175] 实施例134是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:8。

[0176] 实施例135是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:9。

[0177] 实施例136是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:10。

[0178] 实施例137是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:11。

[0179] 实施例138是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:12。

[0180] 实施例139是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:13。

[0181] 实施例140是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:14。

[0182] 实施例141是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:15。

[0183] 实施例142是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:16。

[0184] 实施例143是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:17。

[0185] 实施例144是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:18。

[0186] 实施例145是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:19。

[0187] 实施例146是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:20。

[0188] 实施例147是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:21。

[0189] 实施例148是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:22。

[0190] 实施例149是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:23。

[0191] 实施例150是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:

5-82的序列是SEQ ID NO:24。

[0192] 实施例151是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:25。

[0193] 实施例152是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:26。

[0194] 实施例153是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:27。

[0195] 实施例154是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:28。

[0196] 实施例155是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:29。

[0197] 实施例156是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:30。

[0198] 实施例157是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:31。

[0199] 实施例158是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:32。

[0200] 实施例159是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:33。

[0201] 实施例160是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:34。

[0202] 实施例161是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:35。

[0203] 实施例162是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:36。

[0204] 实施例163是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:37。

[0205] 实施例164是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:38。

[0206] 实施例165是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:39。

[0207] 实施例166是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:40。

[0208] 实施例167是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:41。

[0209] 实施例168是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:42。

[0210] 实施例169是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:43。

[0211] 实施例170是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:44。

[0212] 实施例171是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:45。

[0213] 实施例172是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:46。

[0214] 实施例173是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:47。

[0215] 实施例174是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:48。

[0216] 实施例175是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:49。

[0217] 实施例176是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:50。

[0218] 实施例177是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:51。

[0219] 实施例178是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:52。

[0220] 实施例179是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:53。

[0221] 实施例180是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:54。

[0222] 实施例181是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:55。

[0223] 实施例182是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:56。

[0224] 实施例183是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:57。

[0225] 实施例184是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:58。

[0226] 实施例185是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:59。

[0227] 实施例186是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:60。

[0228] 实施例187是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:61。

[0229] 实施例188是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:62。

[0230] 实施例189是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:

5-82的序列是SEQ ID NO:63。

[0231] 实施例190是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:64。

[0232] 实施例191是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:65。

[0233] 实施例192是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:66。

[0234] 实施例193是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:67。

[0235] 实施例194是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:68。

[0236] 实施例195是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:69。

[0237] 实施例196是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:70。

[0238] 实施例197是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:71。

[0239] 实施例198是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:72。

[0240] 实施例199是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:73。

[0241] 实施例200是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:74。

[0242] 实施例201是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:75。

[0243] 实施例202是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:76。

[0244] 实施例203是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:77。

[0245] 实施例204是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:78。

[0246] 实施例205是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:79。

[0247] 实施例206是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:80。

[0248] 实施例207是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:81。

[0249] 实施例208是实施例1至130中任一实施例的方法或组合物,其中选自SEQ ID NO:5-82的序列是SEQ ID NO:82。

[0250] 实施例209是实施例6至208中任一实施例的组合物或调配物的用途，其用于制备用于治疗患有ATTR的人类个体的药剂。

[0251] 亦揭示上述实施例中任一实施例的组合物或调配物的用途，其用于制造用于治疗患有ATTR的人类个体的药剂。亦揭示上述组合物或调配物中的任一种，其用于治疗ATTR或用于修饰TTR基因(例如，在其中形成插入或缺失或形成框移或无义突变)。

[0252] 附图简述

[0253] 图1显示具有由表1中提供的引导序列靶向的TTR基因的区的染色体18的示意图。

[0254] 图2显示靶向TTR的某些双重引导RNA的HEK293_Cas9细胞中的脱靶分析。对于测试的每一双重引导RNA，靶上位点用实心方块表示，而封闭圆圈代表潜在脱靶位点。

[0255] 图3显示靶向TTR的某些单引导RNA的HEK_Cas9细胞中的脱靶分析。对于测试的每一单引导RNA，靶上位点用实心方块表示，而开口圆圈代表潜在脱靶位点。

[0256] 图4显示原代人类肝细胞上脂质纳米颗粒调配的人类TTR特异性sgRNA的剂量反应曲线。

[0257] 图5显示原代食蟹猴肝细胞上脂质纳米颗粒调配的人类TTR特异性sgRNA的剂量反应曲线。

[0258] 图6显示原代食蟹猴肝细胞上脂质纳米颗粒调配的食蟹猴TTR特异性sgRNA的剂量反应曲线。

[0259] 图7显示于HepG2细胞中在施用x轴上提供的引导序列后TTR的编辑百分比(编辑%)及分泌的TTR的减少。该值正规化至α-1-抗胰蛋白酶(AAT)蛋白的量。

[0260] 图8显示在HUH7细胞中在施用靶向引导(表1中所列示)后细胞内TTR的西方墨点分析。

[0261] 图9显示在向具有人类化(G481-G499)或鼠类(G282)TTR的小鼠施用LNP调配物后观察到的TTR的肝编辑百分比。注意：图中省略了每一引导ID中之前三个“0”，例如“G481”是表2及3中的“G000481”。

[0262] 图10A-B显示在水平轴上指示的投药方案后观察到的作为μg/ml(图10A)或TSS对照的百分比(图10B)的血清TTR含量。贯穿本文，MPK＝mg/kg。

[0263] 图11A-B显示在水平轴上指示的1mg/kg(图11A)或0.5mg/kg剂量(图11B)的投药方案后观察到的血清TTR含量。单一2mg/kg剂量的数据以两个组包括作为右栏。

[0264] 图12A-B显示在水平轴上指示的0.5mg/kg(图12A)或1mg/kg剂量(图12B)的投药方案后观察到的肝编辑百分比。图12C显示在0.5、1或2mg/kg的单剂量后观察到的肝编辑百分比。

[0265] 图13显示在向关于TTR基因人类化的小鼠施用LNP调配物后观察到的肝编辑百分比。注意：图中省略了每一引导ID中之前三个“0”，例如“G481”是表2及3中的“G000481”。

[0266] 图14A-B显示在向关于TTR基因人类化的小鼠施用LNP调配物后肝编辑(图14A)与血清人类TTR含量(图14B)之间存在相关性。注意：图中省略了每一引导ID中之前三个“0”，例如“G481”是表2及3中的“G000481”。

[0267] 图15A-B显示在施用包含引导G502的LNP调配物后，野生型小鼠中存在相对于编辑百分比(图15A)及血清TTR含量(图15B)的剂量反应，该引导G502在小鼠与食蟹猴之间交叉同源。

[0268] 图16显示原代食蟹猴肝细胞上脂质纳米颗粒调配的人类TTR特异性sgRNA的剂量反应曲线。

[0269] 图17显示原代人类肝细胞上脂质纳米颗粒调配的食蟹猴TTR特异性sgRNA的剂量反应曲线。

[0270] 图18显示原代食蟹猴肝细胞上脂质纳米颗粒调配的食蟹猴TTR特异性sgRNA的剂量反应曲线。

[0271] 图19A-D显示在以指示比率及量投用LNP调配物后的血清TTR(%TSS;图19A及19C)及编辑结果(图19B及19D)。

[0272] 图20显示靶向TTR的原代人类肝细胞(PHH)中某些单引导RNA的脱靶分析。在图中,实心方块代表靶上切割位点的鉴别,而开口圆圈代表潜在脱靶位点的鉴别。

[0273] 图21A-B显示在施用脂质纳米颗粒调配的G000480后原代人类肝细胞中的靶上(ONT,图21A)及两个脱靶位点(OT2及OT4)的编辑百分比。图21B是图21A中的OT2、OT4及阴性对照(Neg Cont)数据的重新缩放版本。

[0274] 图22A-B显示在施用脂质纳米颗粒调配的G000486后原代人类肝细胞中的靶上(ONT,图22A)及脱靶位点(OT4)的编辑百分比。图22B是图22A中的OT4及阴性对照(Neg Cont)数据的重新缩放版本。

[0275] 图23A-B显示编辑百分比(图23A)及TTR基因座处的插入及缺失事件的数目(图23B)。图23A显示对照及治疗(投用脂质纳米颗粒调配的TTR特异性sgRNA)组中的TTR基因座的编辑百分比。图23B显示当在图23A的治疗组中观察到编辑时在TTR基因座的插入及缺失事件的数目。

[0276] 图24A-B分别显示对照及治疗(投用脂质纳米颗粒调配的TTR特异性sgRNA)组的循环血清(图24A)及脑脊髓液(CSF)(图24B)中的TTR含量,以μg/mL表示。治疗导致血清中TTR含量减少>99%。

[0277] 图25A-D显示来自对照及治疗(投用脂质纳米颗粒调配的TTR特异性sgRNA)小鼠的胃(图25A)、结肠(图25B)、坐骨神经(图25C)及背根神经节(DRG)(图25D)中针对TTR染色的免疫组织化学影像。在右边,条形图显示在经治疗小鼠中治疗后8周TTR染色的减少,如藉由每一组织类型的占据面积百分比所测量。

[0278] 图26A-C分别显示来自投用跨越一系列剂量的LNP调配物的人类化TTR小鼠的肝TTR编辑(图26A)及血清TTR结果(以μg/mL表示(图26B)及作为TSS治疗的对照的百分比(图26C)),该LNP调配物具有引导G000480、G000488、G000489及G000502且含有以重量计与引导呈1:1比率的Cas9 mRNA(SEQ ID NO:1)。

[0279] 图27A-C分别显示来自投用跨越一系列剂量的LNP调配物的人类化TTR小鼠的肝TTR编辑(图27A)及血清TTR结果(以μg/mL表示(图27B)及作为TSS治疗的对照的百分比(图27C)),该LNP调配物具有引导G000481、G000482、G000486及G000499且含有以重量计与引导呈1:1比率的Cas9 mRNA(SEQ ID NO:1)。

[0280] 图28A-C分别显示来自投用跨越一系列剂量的LNP调配物的人类化TTR小鼠的肝TTR编辑(图28A)及血清TTR结果(以μg/mL表示(图28B)及作为TSS治疗的对照的百分比(图28C)),该LNP调配物具有引导G000480、G000481、G000486、G000499及G000502且含有以重量计与引导呈1:2比率的Cas9 mRNA(SEQ ID NO:1)。

[0281] 图29显示与阴性(未治疗)对照相比,用包含Cas9 mRNA及gRNA(如指示)的LNP治疗后原始人类肝细胞(PHH)中TTR mRNA的相对表达。

[0282] 图30显示与阴性(未治疗)对照相比,用包含Cas9 mRNA及gRNA(如指示)的LNP治疗后原代人类肝细胞(PHH)中TTR mRNA的相对表达。

[0283] 发明详述

[0284] 现在将详细参考本发明的某些实施例,在附图中图解说明该实施例的实例。尽管将结合所阐释实施例来阐述本发明,但应理解,该实施例并不意欲将本发明限于这些实施例。相反,本发明意欲涵盖可包括于随附申请专利范围所界定的本发明内的所有替代、修饰及等效形式。

[0285] 在详细阐述本教示之前,应理解,本揭示内容并不限于特定组合物或过程步骤,因此可变。应注意,除非上下文另外明确指定,否则本说明书及随附申请专利范围中所用的单数形式"一(a、an)"及"该"皆包括复数个指示物。因此,例如,在提及"结合物"时包括复数个结合物且在提及"细胞"时包括复数个细胞及诸如此类。

[0286] 数字范围包括界定该范围的数字。测量值及可测量值应理解为近似值,考虑有效数字及与测量相关的误差。"包含(comprise、comprises、comprising)"、"含有(contain、contains、containing)"、"包括(include、includes及including)的使用亦不意欲具有限制性。应了解,前述一般说明及详细说明二者仅为示例性及解释性且并不限制教示内容。

[0287] 除非在以上说明书中特别指出,否则说明书中列举"包含"各种组分的实施例亦涵盖"由所列举组分组成"或"基本上由所列举组分组成";说明书中列举"由各种组分组成"的实施例亦涵盖"包含"所列举组分或"基本上由所列举组分组成";且说明书中列举"基本上由各种组分组成"的实施例亦涵盖"由所列举组分组成"或"包含"所列举组分(此可互换性不适用于在申请专利范围中使用该术语)。除非上下文另有明确说明,否则术语"或"以包括性含义使用,亦即等同于"和/或"。

[0288] 本文所用的各部分标题仅出于组织目的,而不能解释为以任何方式限制所述标的物。倘若以引用方式并入的任何材料与本说明书中定义的任何术语或本说明书的任何其它明确表达内容相矛盾,则以本说明书为准。尽管连同各种实施例阐述本发明教示,但并不意欲将本发明教示限制于该实施例。相反,本发明教示囊括各种替代、修改及等效形式,如所属领域的技术人员将了解。

[0289] I.定义

[0290] 除非另外陈述,否则本文所用的下列术语及片语意欲具有下列含义:

[0291] "多核苷酸"及"核酸"在本文中用于指包含核苷或核苷类似物的多聚体化合物,其具有沿主链连接在一起的含氮杂环碱基或碱基类似物,包括习用RNA、DNA、混合RNA-DNA及是其类似物的聚合物。核酸"主链"可由多个链接构成,包括一或多个糖-磷酸二酯链接、肽-核酸键("肽核酸"或PNA;PCT第WO 95/32305号)、硫代磷酸酯链接、甲基膦酸酯链接或其组合。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'卤化物取代)的类似化合物。含氮碱基可为习用碱基(A、G、C、T、U)、其类似物(例如,经修饰尿苷,例如5-甲氧基尿苷、假尿苷或N1-甲基假尿苷或者其它);肌苷;嘌呤或嘧啶的衍生物(例如,N^4-甲基脱氧鸟苷、去氮杂-或氮杂-嘌呤、去氮杂-或氮杂-嘧啶、在5或6位具有取代基的嘧啶碱基(例如,5-甲基胞嘧啶)、在2、6、或8位具有取代基的嘌呤碱基、2-胺基-6-甲基胺基嘌呤、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)。对于一般讨论,参见The Biochemistry of the Nucleic Acids 5-36,Adams等人编辑,第11版,1992)。核酸可包括一或多个"无碱基"残基,其中主链对于聚合物的位置不包括含氮碱基(美国专利第5,585,481号)。核酸可仅包含习用RNA或DNA糖、碱基及链接,或可包括习惯组分及取代(例如,具有2'甲氧基链接的习用碱基、或含有习用碱基及一或多种碱基类似物的聚合物)。核酸包括"锁核酸"(LNA),一种含有一或多个LNA核苷酸单体的类似物,其中二环呋喃糖单元锁定在仿真糖构象的RNA中,其增强对互补RNA及DNA序列的杂交亲和性(Vester及Wengel,2004,Biochemistry 43(42):13233-41)。RNA及DNA具有不同的糖部分,且可因RNA中存在尿嘧啶或其类似物及DNA中存在胸腺嘧啶或其类似物而不同。

[0292] "引导RNA"、"gRNA"及"引导"在本文中可互换使用,指crRNA(亦称为CRISPR RNA)或crRNA与trRNA的组合(亦称为tracrRNA)。crRNA及trRNA可作为单一RNA分子(单引导RNA,sgRNA)或以两个单独RNA分子(双重引导RNA,dgRNA)缔合。"引导RNA"或"gRNA"是指每一类型。trRNA可为天然序列、或与天然序列相比具有修饰或变化的trRNA序列。

[0293] 如本文所用,"引导序列"是指引导RNA内的序列,其与靶序列互补且用于将引导RNA引导至靶序列以藉由RNA引导的DNA结合剂结合或修饰(例如,裂解)。"引导序列"亦可称为"靶向序列"或"间隔序列"。例如,在酿脓链球菌(Streptococcus pyogenes)(亦即,Spy Cas9))及相关的Cas9同是物/直向同源物的情形下,引导序列的长度可为20个碱基对。亦可使用更短或更长的序列作为引导,例如,长度为15、16、17、18、19、21、22、23、24或25个核苷酸。例如,在一些实施例中,引导序列包含选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸。在一些实施例中,例如,靶序列在基因中或在染色体上,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或一致性程度可为约75%、80%、85%、88%、90%、95%、96%、97%、98%、99%或100%。例如,在一些实施例中,引导序列包含与选自SEQ ID NO:5-82的序列的至少17、18、19或20个邻接核苷酸具有约75%、80%、85%、88%、90%、95%、96%、97%、98%、99%或100%一致性的序列。在一些实施例中,引导序列及靶区可为100%互补或一致。在其它实施例中,引导序列及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,引导序列及靶区可含有1-4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。

[0294] Cas蛋白的靶序列包括基因组DNA的正链及负链(亦即,给出的序列及序列的反向补体),此乃因Cas蛋白的核酸底物是双链核酸。因此,在据称引导序列"与靶序列互补"的情况下,应理解,引导序列可引导引导RNA结合至靶序列的反向补体。因此,在引导序列结合靶序列的反向补体的情况下,引导序列与靶序列的某些核苷酸一致(例如,靶序列不包括PAM),只是在导向序列中用U取代T。

[0295] 如本文所用,"RNA引导的DNA结合剂"意指具有RNA及DNA结合活性的多肽或多肽的复合物、或该复合物的DNA结合亚单位,其中DNA结合活性具有序列特异性且取决于RNA的序列。实例性RNA引导的DNA结合剂包括Cas裂解酶/切口酶及其灭活形式("dCas DNA结合剂")。如本文所用,"Cas核酸酶",亦称为"Cas蛋白",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亚单位、I型CRISPR系统的级联复合物、其Cas3亚单位及2类Cas核酸酶。如本文所用，“2类Cas核酸酶”是具有RNA引导的DNA结合活性的单链多肽，例如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变体)(其进一步具有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/切口酶活性不活化)。2类Cas核酸酶包括(例如)Cas9、Cpf1、C2c1、C2c2、C2c3、HF Cas9(例如，N497A、R661A、Q695A、Q926A变体)、HypaCas9(例如，N692A、M694A、Q695A、H698A变体)、eSPCas9(1.0)(例如，K810A、K1003A、R1060A变体)及eSPCas9(1.1)(例如，K848A、K1003A、R1060A变体)蛋白及其修饰形式。Cpf1蛋白(Zetsche等人，Cell，163:1-13(2015))与Cas9同源，且含有RuvC样核酸酶结构域。Zetsche的Cpf1序列以引用方式整体并入。参见，例如，Zetsche，表S1及S3。“Cas9”涵盖Spy Cas9、本文列示的Cas9的变体及其等效物。参见(例如)Makarova等人，Nat Rev Microbiol，13(11):722-36(2015)；Shmakov等人，Molecular Cell，60:385-397(2015)。

[0296] “经修饰尿苷”在本文中用于指除胸苷以外的核苷，其具有与尿苷相同的氢键受体及与尿苷的一或多个结构差异的核苷。在一些实施例中，经修饰尿苷是经取代尿苷，亦即其中一或多个非质子取代基(例如，烷氧基，例如甲氧基)代替质子的尿苷。在一些实施例中，经修饰尿苷是假尿苷。在一些实施例中，经修饰尿苷是经取代假尿苷，亦即其中一或多个非质子取代基(例如，烷基，例如甲基)代替质子的假尿苷。在一些实施例中，经修饰尿苷是任何经取代尿苷、假尿苷或经取代假尿苷。

[0297] 如本文所用的“尿苷位置”是指由尿苷或经修饰尿苷占据的多核苷酸中的位置。因此，例如，其中“100％的尿苷位置是经修饰尿苷”的多核苷酸在每个位置含有经修饰尿苷，其将为相同序列的习用RNA(其中所有碱基皆是标准A、U、C或G碱基)中的尿苷。除非另有说明，否则本揭示内容中或随附的序列表格或序列表的多核苷酸序列中的U可为尿苷或经修饰尿苷。

[0298] 如本文所用，若第一序列与第二序列的比对显示第二序列的X％或更多的位置整体与第一序列匹配，则认为第一序列“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，此乃因比对将给出100％的一致性，其中与第二序列的所有三个位置皆匹配。RNA与DNA之间的差异(通常将尿苷更换为胸苷或反之亦然)及核苷类似物(例如经修饰尿苷)的存在不会导致多核苷酸间的一致性或互补性的差异，只要相关核苷酸(例如胸苷、尿苷或经修饰尿苷)具有相同的补体(例如，对于所有胸苷、尿苷或经修饰尿苷皆为腺苷；另一实例是胞嘧啶及5-甲基胞嘧啶，二者皆具有鸟苷或经修饰鸟苷作为补体)。因此，例如，认为序列5’-AXG(其中X是任何经修饰尿苷，例如假尿苷、N1-甲基假尿苷或5-甲氧基尿苷)与AUG 100％一致，其中二者皆与相同序列(5’-CAU)完美互补。实例性比对算法是Smith-Waterman及Needleman-Wunsch算法，其为业内所熟知。熟习此项技术者将了解哪种算法选择及参数设置适合于欲比对的给测序列对；对于通常类似长度及对于氨基酸预期一致性＞50％或对于核苷酸＞75％的序列，具有在www.ebi.ac.uk网站服务器上由EBI提供的Needleman-Wunsch算法接口的默认设置的Needleman-Wunsch算法通常是合适的。

[0299] “mRNA”在本文中用于指多核苷酸，其不是DNA且包含可转译成多肽(亦即，可用作

由核糖体及胺基-酰化tRNA转译的底物)的开放阅读框。mRNA可包含磷酸酯-糖主链,包括核糖残基或其类似物,例如2'-甲氧基核糖残基。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,mRNA不含有大量的胸苷残基(例如0个残基或少于30、20、10、5、4、3或2个胸苷残基;或少于10%、9%、8%、7%、6%、5%、4%、4%、3%、2%、1%、0.5%、0.2%或0.1%胸苷含量)。mRNA可在其一些或所有尿苷位置含有经修饰尿苷。

[0300] 如本文所用,给定的开放阅读框(ORF)的"最小尿苷含量"是如下ORF的尿苷含量:(a)在每个位置使用最小尿苷密码子且(b)编码与给定的ORF相同的氨基酸序列。给定氨基酸的最小尿苷密码子是具有最少尿苷(通常0或1,其中最小尿苷密码子具有2个尿苷酸的苯丙氨酸的密码子除外)的密码子。出于评估最小尿苷含量的目的,认为经修饰尿苷残基等效于尿苷。

[0301] 如本文所用,给定的开放阅读框(ORF)的"最小尿苷二核苷酸含量"是如下ORF的最低可能的尿苷二核苷酸(UU)含量:(a)在每个位置使用最小尿苷密码子(如上所论述)且(b)编码与给定的ORF相同的氨基酸序列。尿苷二核苷酸(UU)含量可在绝对意义上表示为ORF中UU二核苷酸的列举,或基于由尿苷二核苷酸的尿苷占据的位置的百分比表示(例如,AUUAU的尿苷二核苷酸含量为40%,此乃因5个位置中的2个由尿苷二核苷酸的尿苷占据)。出于评估最小尿苷二核苷酸含量的目的,认为经修饰尿苷残基等效于尿苷。

[0302] 如本文所用,"TTR"是指甲状腺素运载蛋白,其是TTR基因的基因产物。

[0303] 如本文所用,"淀粉样蛋白"是指通常可溶的蛋白质或肽的异常聚集物。淀粉样蛋白不溶,且淀粉样蛋白可在器官及组织中产生蛋白质性沉积。淀粉样蛋白中的蛋白质或肽可被错误折叠成容许蛋白质的许多拷贝黏在一起以形成原纤维的形式。尽管一些形式的淀粉样蛋白可能在人类体中具有正常功能,但本文所用的"淀粉样蛋白"是指蛋白质的异常或病理性聚集物。淀粉样蛋白可包含单一蛋白质或肽,例如TTR,或其可包含多个蛋白质或肽,例如TTR及额外蛋白质。

[0304] 如本文所用,"淀粉样原纤维"是指抗降解的淀粉样蛋白的不溶性纤维。淀粉样原纤维可基于特定的蛋白质或肽以及其所聚集的组织及细胞类型产生症状。

[0305] 如本文所用,"淀粉样变性"是指特征在于由淀粉样蛋白或淀粉样原纤维的沉积引起的症状的疾病。淀粉样变性可影响众多器官,包括心脏、肾、肝、脾、神经系统及消化道。

[0306] 如本文所用,"ATTR"、"TTR相关的淀粉样变性"、"TTR淀粉样变性"、"ATTR淀粉样变性"或"与TTR相关的淀粉样变性"是指与TTR的沉积相关的淀粉样变性。

[0307] 如本文所用,"家族性淀粉样心肌病"或"FAC"是指特征主要在于限制性心肌病的遗传性甲状腺素运载蛋白淀粉样变性(ATTR)。郁血性心脏衰竭在FAC中常见。平均发病年龄约为60-70岁,估计预期寿命为诊断后4-5年。

[0308] 如本文所用,"家族性淀粉样多神经病变"或"FAP"是指特征主要在于感觉运动神经病变的遗传性甲状腺素运载蛋白淀粉样变性(ATTR)。自主神经病变在FAP中常见。尽管神经病变是主要特征,但FAP的症状亦可包括恶病质、肾衰竭及心脏病。FAP的平均发病年龄约为30-50岁,估计预期寿命为诊断后5-15年。

[0309] 如本文所用,"野生型ATTR"及"ATTRwt"是指与病理TTR突变(例如T60A、V30M、V30A、V30G、V30L、V122I、V122A或V122(-))无关的ATTR。ATTRwt亦称为老年全身性淀粉样变

性。发作通常发生在60岁或以上的男性中，其中最常见的症状是郁血性心脏衰竭及异常的心律，例如心房震颤。额外症状包括心脏功能不良之后果，例如呼吸短促、疲劳、眩晕、肿胀(尤其是腿部)、恶心、心绞痛、睡眠中断及体重减轻。腕隧道症候群的病史指示ATTRwt的风险增加，且在一些情况下可指示早期疾病。ATTRwt通常导致心脏功能随着时间的推移而降低，但可具有比传遗性ATTR更佳的预后，此乃因野生型TTR沉积物累积得更慢。现有的治疗类似于其它形式的ATTR(肝移植除外)，且通常是关于支持或改善心脏功能(自利尿剂及有限的液体及盐摄入至抗凝剂的范围)且在严重情形下心脏移植。尽管如此，与FAC一样，ATTRwt可导致有时在诊断后的3-5年内因心脏衰竭死亡。

[0310] 可用于本文所述引导RNA组合物及方法中的引导序列示于表1及整个申请案中。

[0311] 如本文所用，“遗传性ATTR”是指与TTR基因的序列的突变相关的ATTR。与ATTR相关的TTR基因的已知突变包括导致TTR具有T60A、V30M、V30A、V30G、V30L、V122I、V122A或V122(-)取代的这些。

[0312] 如本文所用，“插入或缺失”是指由多个在靶核酸中的双链断裂(DSB)位点插入或缺失的核苷酸组成的插入/缺失突变。

[0313] 如本文所用，“减弱”是指特定基因产物(例如，蛋白质、mRNA或二者)的表达降低。蛋白质的减弱可藉由检测组织或细胞群体分泌的蛋白质(例如，在血清或细胞培养基中)或藉由检测来自所关注组织或细胞群的蛋白质的总细胞量来测量。用于测量mRNA的减弱的方法是已知的，且包括自所关注组织或的细胞群体分离的mRNA的测序。在一些实施例中，“减弱”可指特定基因产物的表达的一些损失，例如，转录的mRNA的量减少或细胞群体(包括活体内群体，例如组织中发现的这些)表达或分泌的蛋白质的量减少。

[0314] 如本文所用，“剔除”是指细胞中特定蛋白质的表达损失。剔除可藉由检测组织或细胞群体的蛋白质分泌量(例如，在血清或细胞培养基中)或藉由检测组织或细胞群体的蛋白质的总细胞量来测量。在一些实施例中，本揭示内容的方法“剔除”一或多个细胞中(例如，细胞群体，包括活体内群体，例如在组织中发现的这些中)的TTR。在一些实施例中，剔除不形成突变TTR蛋白，例如，由插入或缺失产生，而是在细胞中完全丧失TTR蛋白的表达。

[0315] 如本文所用，“突变TTR”是指TTR的基因产物(亦即，TTR蛋白)，其与TTR的野生型氨基酸序列相比具有TTR的氨基酸序列的变化。人类野生型TTR序列可在NCBI Gene ID:7276；Ensembl:Ensembl:ENSG00000118271获得。例如人类中与ATTR相关的TTR的突变形式包括T60A、V30M、V30A、V30G、V30L、V122I、V122A或V122(-)。

[0316] 如本文所用，“突变TTR”或“突变体TTR等位基因”是指与野生型序列相比具有TTR的核苷酸序列变化的TTR序列(NCBI Gene ID:7276;Ensembl:ENSG00000118271)。

[0317] 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，例如Cas核酸酶，例如Cas裂解酶、Cas切口酶或dCas DNA结合剂(例如，Cas9)。在一些实施例中，引导RNA引导RNA引导的DNA结合剂(例如Cas9)至靶序列，且引导RNA与靶序列杂交且试剂结合至靶序列；在试剂是裂解酶或切口酶的情形下，结合后可以切割或切口形成。

[0318] 如本文所用，“靶序列”是指靶基因中与gRNA的引导序列具有互补性的核酸序列。靶序列及引导序列的相互作用引导RNA引导的DNA结合剂结合，且可能在靶序列内形成切口或切割(取决于试剂的活性)。

[0319] 如本文所用，“治疗”是指对个体中疾病或病症的治疗剂的任何施用及施加，且包括抑制疾病、停止其发展、缓解疾病的一或多种症状、治愈疾病或防止疾病的一或多种症状再次出现。例如，ATTR的治疗可包含缓和ATTR的症状。

[0320] “经修饰尿苷”在本文中用于指除胸苷以外的核苷，其具有与尿苷相同的氢键受体及与尿苷的一或多个结构差异。在一些实施例中，经修饰尿苷是经取代尿苷，亦即其中一或多个非质子取代基(例如，烷氧基，例如甲氧基)代替质子的尿苷。在一些实施例中，经修饰尿苷是假尿苷。在一些实施例中，经修饰尿苷是经取代假尿苷，亦即其中一或多个非质子取代基(例如，烷基，例如甲基)代替质子的假尿苷，例如N1-甲基假尿苷。在一些实施例中，经修饰尿苷是任何经取代尿苷、假尿苷或经取代假尿苷。

[0321] 如本文所用，若第一序列与第二序列的比对显示第二序列的X％或更多的位置整体与第一序列匹配，则认为第一序列“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，此乃因比对将给出100％的一致性，其中与第二序列的所有三个位置皆匹配。RNA与DNA之间的差异(通常将尿苷更换为胸苷或反之亦然)及核苷类似物(例如经修饰尿苷)的存在不会导致多核苷酸间的一致性或互补性的差异，只要相关核苷酸(例如胸苷、尿苷或经修饰尿苷)具有相同的补体(例如，对于所有胸苷、尿苷或经修饰尿苷皆为腺苷；另一实例是胞嘧啶及5-甲基胞嘧啶，二者皆具有鸟苷作为补体)。因此，例如，认为序列5’-AXG(其中X是任何经修饰尿苷，例如假尿苷、N1-甲基假尿苷或5-甲氧基尿苷)与AUG 100％一致，其中二者皆与相同序列(5’-CAU)完美互补。实例性比对算法是Smith-Waterman及Needleman-Wunsch算法，其为业内所熟知。熟习此项技术者将了解哪种算法选择及参数设置适合于欲比对的给测序列对；对于通常类似长度及对于氨基酸预期一致性＞50％或对于核苷酸＞75％的序列，具有在www.ebi.ac.uk网站服务器上由EBI提供的Needleman-Wunsch算法接口的默认设置的Needleman-Wunsch算法通常是合适的。

[0322] 术语“约”或“近似地”意指熟习此项技术者所测定的特定值的可接受误差，其部分取决于该值的测量或测定方式。

[0323] II.组合物

[0324] A.包含引导RNA(gRNA)的组合物

[0325] 本文提供(例如)使用引导RNA与RNA引导的DNA结合剂(例如，CRISPR/Cas系统)用于编辑TTR基因的组合物。组合物可施用具有野生型或非野生型TTR基因序列的个体，例如患有ATTR(其可为ATTR wt或遗传性或家族性形式的ATTR)的个体。靶向TTR基因的引导序列示于表1中的SEQ ID No:5-82。

[0326] 表1:TTR靶向引导序列、命名法、染色体坐标及序列.

[0327]

SEQ ID No.	引导ID	说明	物种	染色体位置	引导序列*
5	CR003335	TTR (外显子1)	人类	chr18:31591917-31591937	CUGCUCCUCCUCUGCCUUGC
6	CR003336	TTR (外显子1)	人类	chr18:31591922-31591942	CCUCCUCUGCCUUGCUGGAC
7	CR003337	TTR (外显子1)	人类	chr18:31591925-31591945	CCAGUCCAGCAAGGCAGAGG
8	CR003338	TTR (外显子1)	人类	chr18:31591928-31591948	AUACCAGUCCAGCAAGGCAG
9	CR003339	TTR (外显子1)	人类	chr18:31591934-31591954	ACACAAAUACCAGUCCAGCA

[0328]

10	CR003340	TTR (外显子1)	人类	chr18:31591937-31591957	UGGACUGGUAUUUGUGUCUG
11	CR003341	TTR (外显子1)	人类	chr18:31591941-31591961	CUGGUAUUUGUGUCUGAGGC
12	CR003342	TTR (外显子2)	人类	chr18:31592880-31592900	CUUCUCUACACCCAGGGCAC
13	CR003343	TTR (外显子2)	人类	chr18:31592902-31592922	CAGAGGACACUUGGAUUCAC
14	CR003344	TTR (外显子2)	人类	chr18:31592911-31592931	UUUGACCAUCAGAGGACACU
15	CR003345	TTR (外显子2)	人类	chr18:31592919-31592939	UCUAGAACUUUGACCAUCAG
16	CR003346	TTR (外显子2)	人类	chr18:31592928-31592948	AAAGUUCUAGAUGCUGUCCG
17	CR003347	TTR (外显子2)	人类	chr18:31592948-31592968	CAUUGAUGGCAGGACUGCCU
18	CR003348	TTR (外显子2)	人类	chr18:31592948-31592968	AGGCAGUCCUGCCAUCAAUG
19	CR003349	TTR (外显子2)	人类	chr18:31592958-31592978	UGCACGGCCACAUUGAUGGC
20	CR003350	TTR (外显子2)	人类	chr18:31592962-31592982	CACAUGCACGGCCACAUUGA
21	CR003351	TTR (外显子2)	人类	chr18:31592974-31592994	AGCCUUUCUGAACACAUGCA

[0329]

22	CR003352	TTR (外显子2)	人类	chr18:31592986-31593006	GAAAGGCUGCUGAUGACACC
23	CR003353	TTR (外显子2)	人类	chr18:31592987-31593007	AAAGGCUGCUGAUGACACCU
24	CR003354	TTR (外显子2)	人类	chr18:31593003-31593023	ACCUGGGAGCCAUUUGCCUC
25	CR003355	TTR (外显子2)	人类	chr18:31593007-31593027	CCCAGAGGCAAAUGGCUCCC
26	CR003356	TTR (外显子2)	人类	chr18:31593015-31593035	GCAACUUACCCAGAGGCAAA
27	CR003357	TTR (外显子2)	人类	chr18:31593022-31593042	UUCUUUGGCAACUUACCCAG
28	CR003358	TTR (外显子3)	人类	chr18:31595127-31595147	AUGCAGCUCUCCAGACUCAC
29	CR003359	TTR (外显子3)	人类	chr18:31595126-31595146	AGUGAGUCUGGAGAGCUGCA
30	CR003360	TTR (外显子3)	人类	chr18:31595127-31595147	GUGAGUCUGGAGAGCUGCAU
31	CR003361	TTR (外显子3)	人类	chr18:31595140-31595160	GCUGCAUGGGCUCACAACUG
32	CR003362	TTR (外显子3)	人类	chr18:31595143-31595163	GCAUGGGCUCACAACUGAGG
33	CR003363	TTR (外显子3)	人类	chr18:31595156-31595176	ACUGAGGAGGAAUUUGUAGA

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34	CR003364	TTR (外显子3)	人类	chr18:31595157-31595177	CUGAGGAGGAAUUUGUAGAA
35	CR003365	TTR (外显子3)	人类	chr18:31595170-31595190	UGUAGAAGGGAUAUACAAAG
36	CR003366	TTR (外显子3)	人类	chr18:31595193-31595213	AAAUAGACACCAAAUCUUAC
37	CR003367	TTR (外显子3)	人类	chr18:31595197-31595217	AGACACCAAAUCUUACUGGA
38	CR003368	TTR (外显子3)	人类	chr18:31595205-31595225	AAGUGCCUUCCAGUAAGAUU
39	CR003369	TTR (外显子3)	人类	chr18:31595235-31595255	CUCUGCAUGCUCAUGGAAUG
40	CR003370	TTR (外显子3)	人类	chr18:31595236-31595256	CCUCUGCAUGCUCAUGGAAU
41	CR003371	TTR (外显子3)	人类	chr18:31595237-31595257	ACCUCUGCAUGCUCAUGGAA
42	CR003372	TTR (外显子3)	人类	chr18:31595242-31595262	UACUCACCUCUGCAUGCUCA
43	CR003373	TTR (外显子4)	人类	chr18:31598570-31598590	GUAUUCACAGCCAACGACUC
44	CR003374	TTR (外显子4)	人类	chr18:31598583-31598603	GCGGCGGGGGCCGGAGUCGU
45	CR003375	TTR (外显子4)	人类	chr18:31598592-31598612	AAUGGUGUAGCGGCGGGGGC

[0331]

46	CR003376	TTR (外显子4)	人类	chr18:31598596-31598616	CGGCAAUGGUGUAGCGGCGG
47	CR003377	TTR (外显子4)	人类	chr18:31598597-31598617	GCGGCAAUGGUGUAGCGGCG
48	CR003378	TTR (外显子4)	人类	chr18:31598598-31598618	GGCGGCAAUGGUGUAGCGGC
49	CR003379	TTR (外显子4)	人类	chr18:31598599-31598619	GGGCGGCAAUGGUGUAGCGG
50	CR003380	TTR (外显子4)	人类	chr18:31598602-31598622	GCAGGGCGGCAAUGGUGUAG
51	CR003381	TTR (外显子4)	人类	chr18:31598610-31598630	GGGGCUCAGCAGGGCGGCAA
52	CR003382	TTR (外显子4)	人类	chr18:31598616-31598636	GGAGUAGGGGCUCAGCAGGG
53	CR003383	TTR (外显子4)	人类	chr18:31598619-31598639	AUAGGAGUAGGGGCUCAGCA
54	CR003384	TTR (外显子4)	人类	chr18:31598620-31598640	AAUAGGAGUAGGGGCUCAGC
55	CR003385	TTR (外显子4)	人类	chr18:31598626-31598646	CCCCUACUCCUAUUCCACCA
56	CR003386	TTR (外显子4)	人类	chr18:31598629-31598649	CCGUGGUGGAAUAGGAGUAG
57	CR003387	TTR (外显子4)	人类	chr18:31598630-31598650	GCCGUGGUGGAAUAGGAGUA

[0332]

58	CR003388	TTR (外显子4)	人类	chr18:31598637-31598657	GACGACAGCCGUGGUGGAAU
59	CR003389	TTR (外显子4)	人类	chr18:31598643-31598663	AUUGGUGACGACAGCCGUGG
60	CR003390	TTR (外显子4)	人类	chr18:31598646-31598666	GGGAUUGGUGACGACAGCCG
61	CR003391	TTR (外显子4)	人类	chr18:31598647-31598667	GGCUGUCGUCACCAAUCCCA
62	CR003392	TTR (外显子4)	人类	chr18:31598661-31598681	AGUCCCUCAUUCCUUGGGAU
63	CR005298	TTR (外显子1)	人类	chr18:31591883-31591903	UCCACUCAUUCUUGGCAGGA
64	CR005299	TTR (外显子4)	人类	chr18:31598631-31598651	AGCCGUGGUGGAAUAGGAGU
65	CR005300	TTR (外显子1)	人类	chr18:31591967-31591987	UCACAGAAACACUCACCGUA
66	CR005301	TTR (外显子1)	人类	chr18:31591968-31591988	GUCACAGAAACACUCACCGU
67	CR005302	TTR (外显子2)	人类	chr18:31592874-31592894	ACGUGUCUUCUCUACACCCA
68	CR005303	TTR (外显子2)	人类	chr18:31592903-31592923	UGAAUCCAAGUGUCCUCUGA
69	CR005304	TTR (外显子2)	人类	chr18:31592969-31592989	GGCCGUGCAUGUGUUCAGAA

[0333]

70	CR005305	TTR (外显子3)	人类	chr18:31595114-31595134	UAUAGGAAAACCAGUGAGUC
71	CR005306	TTR (外显子3)	人类	chr18:31595204-31595224	AAAUCUUACUGGAAGGCACU
72	CR005307	TTR (外显子4)	人类	chr18:31598548-31598568	UGUCUGUCUUCUCUCAUAGG
73	CR000689	TTR	食蟹猴	chr18:50681533-50681553	ACACAAAUACCAGUCCAGCG
74	CR005364	TTR	食蟹猴	chr18:50680481-50680501	AAAGGCUGCUGAUGAGACCU
75	CR005365	TTR	食蟹猴	chr18:50680520-50680540	CAUUGACAGCAGGACUGCCU
76	CR005366	TTR	食蟹猴	chr18:50681539-50681559	AUACCAGUCCAGCGAGGCAG
77	CR005367	TTR	食蟹猴	chr18:50681542-50681562	CCAGUCCAGCGAGGCAGAGG
78	CR005368	TTR	食蟹猴	chr18:50681545-50681565	CCUCCUCUGCCUCGCUGGAC
79	CR005369	TTR	食蟹猴	chr18:50680540-50680560	AAAGUUCUAGAUGCCGUCCG
80	CR005370	TTR	食蟹猴	chr18:50680594-50680614	ACUUGUCUUCUCUAUACCCA
81	CR005371	TTR	食蟹猴	chr18:50678216-50678236	AAGUGACUUCCAGUAAGAUU
82	CR005372	TTR	食蟹猴	chr18:50680482-50680502	AAAAGGCUGCUGAUGAGACC

[0334] 上述引导序列中的每一种可进一步包含额外核苷酸以形成例如在其3′端的引导序列之后具有以下实例性核苷酸序列的crRNA:GUUUUAGAGCUAUGCUGUUUUG(SEQ ID NO:126)。在sgRNA的情形下,上述引导序列可以进一步包含额外核苷酸以形成例如在引导序列的3′端之后具有以下实例性核苷酸序列的sgRNA:在5’至3’方向上GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU(SEQ ID NO:125)。

[0335] 在一些实施例中,sgRNA经修饰。在一些实施例中,sgRNA包含以下SEQ ID NO:3中所示的修饰模式,其中N是任何天然或非天然核苷酸,且其中全体N包含如本文所述的引导

序列且经修饰sgRNA包含以下序列:mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU(SEQ ID NO:3),其中"N"可为任何天然或非天然核苷酸。例如,本文涵盖SEQ ID NO:3,其中N经本文揭示的引导序列中的任一种置换。尽管N取代了引导的核苷酸,但修饰仍然如SEQ ID NO:3中所示。亦即,尽管引导的核苷酸置换了"N",但前三个核苷酸经2'OMe修饰,且在第一核苷酸与第二核苷酸、第二核苷酸与第三核苷酸以及第三核苷酸与第四核苷酸之间存在硫代磷酸酯链接。

[0336] 在一些实施例中,涵盖表2中列举的序列中的任一种。

[0337] 表2:TTR靶向的sgRNA序列

[0338]

SEQ ID No.	引导ID	靶标及说明	物种	序列
87	G000480	TTR sgRNA 修饰的序列	人类	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0339]

88	G000481	TTR sgRNA 修饰的序列	人类	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
89	G000482	TTR sgRNA 修饰的序列	人类	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
90	G000483	TTR sgRNA 修饰的序列	人类	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
91	G000484	TTR sgRNA 修饰的序列	人类	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
92	G000485	TTR sgRNA 修饰的序列	人类	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
93	G000486	TTR sgRNA 修饰的序列	人类	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0340]

94	G000487	TTR sgRNA 修饰的序列	人类	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
95	G000488	TTR sgRNA 修饰的序列	人类	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
96	G000489	TTR sgRNA 修饰的序列	人类	mU*mU*mU*GACCAUCAGAGGACACUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
97	G000490	TTR sgRNA 修饰的序列	人类	mA*mA*mA*UAGACACCAAAUCUUACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
98	G000491	TTR sgRNA 修饰的序列	人类	mA*mU*mA*CCAGUCCAGCAAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
99	G000492	TTR sgRNA 修饰的序列	人类	mC*mU*mU*CUCUACACCCAGGGCACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0341]

100	G000493	TTR sgRNA 修饰的序列	人类	mA*mA*mG*UGCCUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
101	G000494	TTR sgRNA 修饰的序列	人类	mG*mU*mG*AGUCUGGAGAGCUGCAUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
102	G000495	TTR sgRNA 修饰的序列	人类	mC*mA*mG*AGGACACUUGGAUUCACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
103	G000496	TTR sgRNA 修饰的序列	人类	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
104	G000497	TTR sgRNA 修饰的序列	人类	mC*mU*mG*CUCCUCCUCUGCCUUGCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
105	G000498	TTR sgRNA 修饰的序列	人类	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0342]

106	G000499	TTR sgRNA 修饰的序列	人类	mU*mG*mA*AUCCAAGUGUCCUCUG AGUUUUAGAmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCAmAmCmUmUmGm AmAmAmAmAmGmUmGmGmCmAmCm CmGmAmGmUmCmGmGmUmGmCmU* mU*mU*mU
107	G000500	TTR sgRNA 修饰的序列	人类	mC*mC*mA*GUCCAGCAAGGCAGAGG GUUUUAGAmGmCmUmAmGmAmAmA mUmAmGmCAAGUUAAAAUAAGGCU AGUCCGUUAUCAmAmCmUmUmGmA mAmAmAmAmGmUmGmGmCmAmCmC mGmAmGmUmCmGmGmUmGmCmU*m U*mU*mU
108	G000501	TTR sgRNA 修饰的序列	人类	mU*mC*mA*CAGAAACACUCACCGUA GUUUUAGAmGmCmUmAmGmAmAmA mUmAmGmCAAGUUAAAAUAAGGCU AGUCCGUUAUCAmAmCmUmUmGmA mAmAmAmAmGmUmGmGmCmAmCmC mGmAmGmUmCmGmGmUmGmCmU*m U*mU*mU
109	G000567	TTR sgRNA 修饰的序列	人类	mG*mA*mA*AGGCUGCUGAUGACAC CGUUUUAGAmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCAmAmCmUmUmGm AmAmAmAmAmGmUmGmGmCmAmCm CmGmAmGmUmCmGmGmUmGmCmU* mU*mU*mU
110	G000568	TTR sgRNA 修饰的序列	人类	mG*mG*mC*UGUCGUCACCAAUCCCA GUUUUAGAmGmCmUmAmGmAmAmA mUmAmGmCAAGUUAAAAUAAGGCU AGUCCGUUAUCAmAmCmUmUmGmA mAmAmAmAmGmUmGmGmCmAmCmC mGmAmGmUmCmGmGmUmGmCmU*m U*mU*mU
111	G000570	TTR sgRNA 修饰的序列	人类	mC*mA*mU*UGAUGGCAGGACUGCC UGUUUUAGAmGmCmUmAmGmAmAm AmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCAmAmCmUmUmGm AmAmAmAmAmGmUmGmGmCmAmCm CmGmAmGmUmCmGmGmUmGmCmU* mU*mU*mU

[0343]

112	G000571	TTR sgRNA 修饰的序列	人类	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
113	G000572	TTR sgRNA 修饰的序列	人类	mC*mC*mC*CUACUCCUAUUCCACCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
114	G000502	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
115	G000503	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
116	G000504	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
117	G000505	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0344]

118	G000506	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
119	G000507	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
120	G000508	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
121	G000509	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
122	G000510	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
123	G000511	TTR 食蟹猴特异性 sgRNA 修饰的序列	食蟹猴	mA*mA*mG*UGACUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0345]

124	G000282	TTR	小鼠	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

[0346] *＝PS链接;“m”＝2′-O-Me核苷酸

[0347] 在表3中提供引导ID与相应sgRNA ID的比对定位以及与食蟹猴基因组及食蟹猴匹配的引导ID的同源性。

[0348] 表3:TTR靶向的引导序列ID定位及食蟹猴同源性

[0349]

说明	人类双重引导ID	人类单引导ID	与食蟹猴基因组的错配数目	食蟹猴匹配的dgRNA ID	食蟹猴匹配的 sgRNA ID
TTR	CR003335	G000497	1		
TTR	CR003336	G000485	1	CR005368	G000508
TTR	CR003337	G000500	1	CR005367	G000507
TTR	CR003338	G000491	1	CR005366	G000506
TTR	CR003339	G000486	1	CR000689	G000502
TTR	CR003340		0		
TTR	CR003341		0		
TTR	CR003342	G000492	食蟹猴中无PAM		
TTR	CR003343	G000495	食蟹猴中无PAM		
TTR	CR003344	G000489	0		
TTR	CR003345	G000481	0		
TTR	CR003346	G000488	1	CR005369	G000509
TTR	CR003347	G000570	2	CR005365	G000505
TTR	CR003348		2		
TTR	CR003349		>3		
TTR	CR003350		食蟹猴中无PAM		
TTR	CR003351		食蟹猴中无PAM		
TTR	CR003352	G000567	2	CR005372	G000503
TTR	CR003353	G000480	1	CR005364	G000504
TTR	CR003354		1		
TTR	CR003355		1		

[0350]

TTR	CR003356		3		
TTR	CR003357	G000487	>3		
TTR	CR003358		0		
TTR	CR003359	G000498	0		
TTR	CR003360	G000494	0		
TTR	CR003361		0		
TTR	CR003362		0		
TTR	CR003363		0		
TTR	CR003364		0		
TTR	CR003365	G000482	0		
TTR	CR003366	G000490	0		
TTR	CR003367	G000484	食蟹猴中无PAM		
TTR	CR003368	G000493	1	CR005371	G000511
TTR	CR003369		0		
TTR	CR003370		0		
TTR	CR003371		0		
TTR	CR003372		0		
TTR	CR003373		1		
TTR	CR003374		2		
TTR	CR003375		2		
TTR	CR003376		2		
TTR	CR003377		2		
TTR	CR003378		2		
TTR	CR003379		2		
TTR	CR003380		1		
TTR	CR003381		1		
TTR	CR003382		0		
TTR	CR003383		0		
TTR	CR003384		0		
TTR	CR003385	G000572	0		
TTR	CR003386		0		
TTR	CR003387		0		
TTR	CR003388		0		
TTR	CR003389	G000569	0		
TTR	CR003390		0		
TTR	CR003391	G000568	0		
TTR	CR003392		0		
TTR	CR005298	G000483	1		
TTR	CR005299		0		
TTR	CR005300	G000501	食蟹猴中无PAM		
TTR	CR005301	G000571	0		

[0351]

TTR	CR005302		2	CR005370	G000510
TTR	CR005303	G000499	0		
TTR	CR005304	G000496	>3		
TTR	CR005305		0		
TTR	CR005306		1		
TTR	CR005307		0		

[0352] 在一些实施例中，本发明提供包含一或多个引导RNA(gRNA)的组合物，该引导RNA包含将RNA引导的DNA结合剂(其可为核酸酶(例如，Cas核酸酶，例如Cas9))引导至TTR中的靶DNA序列的引导序列。gRNA可包含含有表1中所示的引导序列的crRNA。gRNA可包含含有表1中所示的引导序列的17、18、19或20个邻接核苷酸的crRNA。在一些实施例中，gRNA包含含有与表1中所示的引导序列的至少17、18、19或20个邻接核苷酸具有约75％、80％、85％、90％、95％、96％、97％、98％、99％或100％一致性的序列的crRNA。在一些实施例中，gRNA包含含有与表1中所示的引导序列具有约75％、80％、85％、90％、95％、96％、97％、98％、99％或100％一致性的序列的crRNA。gRNA可进一步包含trRNA。在本文所述的每一组合物及方法实施例中，crRNA及trRNA可作为单一RNA(sgRNA)缔合，或可在单独RNA(dgRNA)上。在sgRNA的上下文中，crRNA及trRNA组分可经由(例如)磷酸二酯键或其它共价键共价连接。

[0353] 在本文所述组合物、用途及方法实施例的每一种中，引导RNA可包含两个RNA分子作为“双重引导RNA”或“dgRNA”。dgRNA包含含有包含(例如)表1中所示的引导序列的crRNA的第一RNA分子及包含trRNA的第二RNA分子。第一及第二RNA分子可不共价连接，但可经由crRNA与trRNA的各部分之间的碱基配对形成RNA双链体。

[0354] 在本文所述组合物、用途及方法实施例的每一种中，引导RNA可包含单一RNA分子作为“单引导RNA”或“sgRNA”。sgRNA可包含含有与trRNA共价连接的表1中所示的引导序列的crRNA(或其部分)。sgRNA可包含表1中所示的引导序列的17、18、19或20个邻接核苷酸。在一些实施例中，crRNA及trRNA经由连接体共价连接。在一些实施例中，sgRNA经由crRNA与trRNA的各部分之间的碱基配对形成茎环结构。在一些实施例中，crRNA及trRNA经由一或多个不为磷酸二酯键的键共价连接。

[0355] 在一些实施例中，trRNA可包含衍生自天然CRISPR/Cas系统的trRNA序列的全部或一部分。在一些实施例中，trRNA包含经截短或修饰的野生型trRNA。trRNA的长度取决于所用的CRISPR/Cas系统。在一些实施例中，trRNA包含5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、25、30、40、50、60、70、80、90、100或超过100个核苷酸或由其组成。在一些实施例中，trRNA可包含某些二级结构，例如一或多个发夹或茎环结构或一或多个凸起结构。

[0356] 在一些实施例中，本发明提供包含一或多个包含SEQ ID NO:5-82中任一种的引导序列的引导RNA的组合物。

[0357] 在一方面中，本发明提供包含gRNA的组合物，该gRNA包含与SEQ ID NO:5-82的核酸中的任一种具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0358] 在其它实施例中，组合物包含至少一个、例如至少两个gRNA，该(等)gRNA包含选自SEQ ID NO:5-82的引导序列中的任两种或更多种的引导序列。在一些实施例中，组合物包含至少两个gRNA，其各自包含与SEQ ID NO:5-82的核酸中的任一种具有至少99％、98％、

97％、96％、95％、94％、93％、92％、91％或90％一致性的引导序列。

[0359] 在一些实施例中，gRNA是包含表2中所示的序列(SEQ ID No.87-124)中的任一种的sgRNA。在一些实施例中，gRNA是包含表2中所示的序列(SEQ ID No.87-124)中的任一种的sgRNA，但无如所示修饰(亦即，未经修饰的SEQ ID No.87-124)。在一些实施例中，sgRNA包含与SEQ ID No.87-124的核酸中的任一种具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的序列。在一些实施例中，sgRNA包含与SEQ ID No.87-124的核酸中的任一种具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性的序列，但无如所示修饰(亦即，未经修饰的SEQ ID No.87-124)。在一些实施例中，sgRNA包含表1中所示的引导序列中的任一种代替表2中SEQ ID No:87-124的sgRNA序列中所示的引导序列，具有或无修饰。

[0360] 本发明的引导RNA组合物经设计以识别(例如，杂交)TTR基因中的靶序列。例如，TTR靶序列可由提供的包含引导RNA的Cas裂解酶识别及切割。在一些实施例中，RNA引导的DNA结合剂(例如Cas裂解酶)可由引导RNA引导至TTR基因的靶序列，其中引导RNA的引导序列与靶序列杂交，且RNA引导的DNA结合剂(例如Cas裂解酶)切割靶序列。

[0361] 在一些实施例中，基于TTR基因内的靶序列确定一或多种引导RNA的选择。

[0362] 不受任何特定理论的限制，基因的某些区域的突变(例如，由于核酸酶介导的DSB而出现的插入或缺失所引起的框移突变)可能比基因的其它区域的突变更不可耐受，因此DSB的位置是可能导致的蛋白质减弱的量或类型的重要因素。在一些实施例中，与TTR内的靶序列互补或具有互补性的gRNA用于将RNA引导的DNA结合剂引导至TTR基因中的特定位置。在一些实施例中，gRNA经设计以具有与TTR的外显子1、外显子2、外显子3或外显子4中的靶序列互补或具有互补性的引导序列。

[0363] 在一些实施例中，引导序列与人类TTR基因中存在的靶序列具有至少99％、98％、97％、96％、95％、94％、93％、92％、91％或90％一致性。在一些实施例中，靶序列可与引导RNA的引导序列互补。在一些实施例中，引导RNA的引导序列与其相应靶序列之间的互补或一致性程度可为至少80％、85％、90％、95％、96％、97％、98％、99％或100％。在一些实施例中，gRNA的靶序列及导向序列可100％互补或一致。在其它实施例中，gRNA的靶序列及引导序列可含有至少一个错配。例如，gRNA的靶序列及引导序列可含有1、2、3或4个错配，其中引导序列的总长度为20。在一些实施例中，gRNA的靶序列及引导序列可含有1-4个错配，其中引导序列为20个核苷酸。

[0364] 在一些实施例中，本文揭示的组合物或调配物包含含有编码RNA引导的DNA结合剂(例如如本文所述的Cas核酸酶)的开放阅读框(ORF)的mRNA。在一些实施例中，提供、使用或施用包含编码RNA引导的DNA结合剂(例如Cas核酸酶)的ORF的mRNA。

[0365] 在一些实施例中，RNA引导的DNA结合剂是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，其亦可称为双链内核酸酶活性。在一些实施例中，RNA引导的DNA结合剂包含Cas核酸酶，例如2类Cas核酸酶(其可为例如II、V或VI型Cas核酸酶)。2类Cas核酸酶包括(例如)Cas9、Cpf1、C2c1、C2c2及C2c3蛋白及其修饰形式。Cas9核酸酶的实例包括酿脓链球菌(S.pyogenes)、金黄色葡萄球菌(S.aureus)及其它原核生物(例如，参见下一段中的清单)及其修饰(例如，工程化或突变体)型式的II型CRISPR系统的这些。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亚单位;及I型CRISPR系统的级联复合物或其Cas3亚单位。在一些实施例中,Cas核酸酶可来自IIA型、IIB型或IIC型系统。关于各种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)。

[0366] Cas核酸酶可源自的非限制实例性物种包括酿脓链球菌(Streptococcus pyogenes)、嗜热链球菌(Streptococcus thermophilus)、链球菌属(Streptococcus sp.)、金黄色葡萄球菌(Staphylococcus aureus)、无害李斯特菌(Listeria innocua)、加氏乳杆菌(Lactobacillus gasseri)、新凶手弗朗西斯氏菌(Francisella novicida)、琥珀酸弧菌(Wolinella succinogenes)、华德萨特菌(Sutterella wadsworthensis)、Y-变形杆菌(Gammaproteobacterium)、脑膜炎双球菌(Neisseria meningitidis)、空肠弯曲杆菌(Campylobacter jejuni)、多杀巴斯德菌(Pasteurella multocida)、产琥珀酸丝状杆菌(Fibrobacter succinogene)、红红螺菌(Rhodospirillum rubrum)、达松维尔类诺卡菌(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)、酸热脂环酸芽孢杆菌(Alicyclobacillus acidocaldarius)、假真菌样芽孢杆菌(Bacillus pseudomycoides)、还原硒酸盐芽孢杆菌(Bacillus selenitireducens)、西伯利亚微小杆菌(Exiguobacterium sibiricum)、戴白氏乳杆菌(Lactobacillus delbrueckii)、唾液乳酸杆菌(Lactobacillus salivarius)、布氏乳杆菌(Lactobacillus buchneri)、齿垢密螺旋体(Treponema denticola)、海洋微颤菌(Microscilla marina)、伯克霍尔德氏菌(Burkholderiales bacterium)、食萘极单胞菌(Polaromonas naphthalenivorans)、极单胞菌属(Polaromonas sp.)、海洋固氮菌(Crocosphaera watsonii)、蓝杆藻属(Cyanothece sp.)、铜绿微囊藻(Microcystis aeruginosa)、聚球藻属(Synechococcus sp.)、阿拉伯糖醋杆菌(Acetohalobium arabaticum)、制氨菌(Ammonifex degensii)、热角军纤维素菌(Caldicelulosiruptor becscii)、金矿菌(Candidatus Desulforudis)、肉毒梭菌(Clostridium botulinum)、难养芽胞梭菌(Clostridium diifficile)、大芬戈尔德菌(Finegoldia magna)、嗜热盐碱厌氧菌(Natranaerobius thermophilus)、丙酸互营细菌(Pelotomaculum thermopropionicum)、喜温嗜酸硫杆菌(Acidithiobacillus caldus)、嗜酸氧化亚铁硫杆菌(Acidithiobacillus ferrooxidans)、红色硫黄细菌(Allochromatium vinosum)、海洋杆菌属(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 evestigatum)、多变鱼腥藻(Anabaena variabilis)、泡沫节球藻(Nodularia spumigena)、念珠藻属(Nostoc sp.)、极大节旋藻(Arthrospira maxima)、纯顶节旋藻(Arthrospira platensis)、节旋藻属(Arthrospira sp.)、鞘丝藻属(Lyngbya sp.)、原形微鞘藻(Microcoleus chthonoplastes)、颤藻属(Oscillatoria sp.)、运动石袍菌(Petrotoga mobilis)、非洲栖热腔菌(Thermosipho africanus)、巴氏链球菌(Streptococcus pasteurianus)、灰色奈瑟菌(Neisseria cinerea)、红嘴鸥弯曲杆菌(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)及海洋无核绿藻(Acaryochloris marina)。

[0367] 在一些实施例中,Cas核酸酶是来自酿脓链球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自嗜热链球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自脑膜炎双球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自金黄色葡萄球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自新凶手弗朗西斯氏菌的Cpf1核酸酶。在一些实施例中,Cas核酸酶是来自氨基酸球菌属的Cpf1核酸酶。在一些实施例中,Cas核酸酶是来自毛螺科菌ND2006的Cpf1核酸酶。在其它实施例中,Cas核酸酶是来自以下的Cpf1核酸酶:土伦病弗朗西斯氏菌(Francisella tularensis)、毛螺科菌(Lachnospiraceae bacterium)、亨氏丁酸弧菌(Butyrivibrio proteoclasticus)、异域门菌(Peregrinibacteria bacterium)、Parcubacteria菌、密斯氏菌属(Smithella)、氨基酸球菌属(Acidaminococcus)、暂定白蚁产甲烷支原体(Candidatus Methanoplasma termitum)、挑剔真杆菌(Eubacterium eligens)、牛眼莫拉氏菌(Moraxella bovoculi)、稻田氏钩端螺旋体(Leptospira inadai)、狗口腔红棕色单胞菌(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核酸酶是来自氨基酸球菌属或毛螺菌科的Cpf1核酸酶。

[0368] 野生型Cas9具有两个核酸酶结构域:RuvC及HNH。RuvC结构域切割非靶DNA链,且HNH结构域切割DNA的靶链。在一些实施例中,Cas9核酸酶包含一个以上RuvC结构域和/或一个以上HNH结构域。在一些实施例中,Cas9核酸酶是野生型Cas9。在一些实施例中,Cas9能诱导靶DNA中的双链断裂。在某些实施例中,Cas核酸酶可切割dsDNA,其可切割dsDNA的一条链,或其可无DNA裂解酶或切口酶活性。实例性Cas9氨基酸序列提供为SEQ ID NO:203。包括起始及终止密码子的实例性Cas9mRNA ORF序列提供为SEQ ID NO:204。适于包括于融合蛋白中的实例性Cas9 mRNA编码序列提供为SEQ ID NO:210。

[0369] 在一些实施例中,使用嵌合Cas核酸酶,其中蛋白质的一个结构域或区域由不同蛋白质的一部分置换。在一些实施例中,Cas核酸酶结构域可经来自不同核酸酶(例如Fok1)的结构域置换。在一些实施例中,Cas核酸酶可为经修饰核酸酶。

[0370] 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。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。

[0371] 在一些实施例中,RNA引导的DNA结合剂具有单链切口酶活性,亦即可切割一条DNA链以产生单链断裂,亦称为“切口”。在一些实施例中,RNA引导的DNA结合剂包含Cas切口酶。切口酶是一种在dsDNA中产生切口、亦即切割DNA双螺旋的一条链但非另一链的酶。在一些实施例中,Cas切口酶是Cas核酸酶的一种形式(例如,如上所论述的Cas核酸酶),其中内核核溶解活性位点因(例如)催化结构域的一或多个变化(例如点突变)而不活化。关于Cas切口酶及实例性催化结构域变化的论述,参见(例如)美国专利第8,889,356号。在一些实施例中,Cas切口酶(例如Cas9切口酶)具有不活化的RuvC或HNH结构域。实例性Cas9切口酶氨基酸序列提供为SEQ ID NO:206。包括起始及终止密码子的实例性Cas9切口酶mRNA ORF序列

提供为SEQ ID NO:207。适于包括于融合蛋白中的实例性Cas9切口酶mRNA编码序列提供为SEQ ID NO:211。

[0372] 在一些实施例中,RNA引导的DNA结合剂经修饰以仅含有一个功能性核酸酶结构域。例如,试剂蛋白可经修饰,使得核酸酶结构域中的一个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结构域的切口酶。在一些实施例中,使用具有无活性RuvC结构域的切口酶。在一些实施例中,使用具有活性降低的HNH结构域的切口酶。在一些实施例中,使用具有无活性HNH结构域的切口酶。

[0373] 在一些实施例中,Cas蛋白核酸酶结构域内的保守胺基经取代以降低或改变核酸酶活性。在一些实施例中,Cas核酸酶可包含RuvC或RuvC样核酸酶结构域中的氨基酸取代。RuvC或RuvC样核酸酶结构域中的实例性氨基酸取代包括D10A(基于酿脓链球菌Cas9蛋白)。例如,参见Zetsche等人(2015)Cell Oct 22:163(3):759-771。在一些实施例中,Cas核酸酶可包含HNH或HNH样核酸酶结构域中的氨基酸取代。HNH或HNH样核酸酶结构域中的实例性氨基酸取代包括E762A、H840A、N863A、H983A及D986A(基于酿脓链球菌Cas9蛋白)。例如,参见Zetsche等人(2015)。其它实例性氨基酸取代包括D917A、E1006A及D1255A(基于新凶手弗朗西斯氏菌U112 Cpf1(FnCpf1)序列(UniProtKB-A0Q7Q2(CPF1_FRATN))。

[0374] 在一些实施例中,编码切口酶的mRNA与一对分别与靶序列的正义链及反义链互补的引导RNA组合提供。在此实施例中,引导RNA将切口酶引导至靶序列并藉由在靶序列的相对链上产生切口引入DSB(亦即,双切口形成)。在一些实施例中,使用双切口形成可提高特异性并减少脱靶效应。在一些实施例中,切口酶与两个靶向DNA的相反链的单独引导RNA一起使用以在靶DNA中产生双切口。在一些实施例中,切口酶与经选择以紧密靠近的两个单独的引导RNA一起使用以在靶DNA中产生双切口。

[0375] 在一些实施例中,RNA引导的DNA结合剂无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,而基本上无催化(裂解酶/切口酶)活性。在一些实施例中,dCas多肽是dCas9多肽。在一些实施例中,无裂解酶及切口酶活性或dCas DNA结合多肽的RNA引导的DNA结合剂是Cas核酸酶的一种形式(例如,上文论述的Cas核酸酶),其中其内核溶解活性位点因其催化结构域的一或多个变化(例如,点突变)不活化。例如,参见US 2014/0186958 A1;US 2015/0166980 A1。实例是dCas9氨基酸序列提供为SEQ ID NO:208。包括起始及终止密码子的实例性dCas9 mRNA ORF序列提供为SEQ ID NO:209。适于包括于融合蛋白中的实例性dCas9 mRNA编码序列提供为SEQ ID NO:212。

[0376] 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结构域(例如,是或包含融合多肽)。

[0377] 在一些实施例中,异源功能结构域可促进RNA引导的DNA结合剂转运至细胞核中。例如,异源功能结构域可为核定位信号(NLS)。在一些实施例中,RNA引导的DNA结合剂可与1-10个NLS融合。在一些实施例中,RNA引导的DNA结合剂可与1-5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可在RNA引导的DNA结合剂序列的N-末端或C-末端连接。其亦可插入RNA引导的DNA结合剂序列中。在其它实施例中,RNA引导的DNA结合剂可与一个以上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可为相同的(例如,两个SV40 NLS)或不同的。在一些实施例中,RNA引导的DNA结合剂与在羧基末端连接的两个SV40 NLS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,一个在N-末端连接且一个在C-末端连接。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可为单分序列,例如SV40 NLS、PKKKRKV(SEQ ID NO:274)或PKKKRRV(SEQ ID NO:275)。在一些实施例中,NLS可为二分序列,例如核胞浆素的NLS、KRPAATKKAGQAKKKK(SEQ ID NO:276)。在具体实施例中,单一PKKKRKV(SEQ ID NO:274)NLS可在RNA引导的DNA结合剂的C-末端连接。视情况在融合位点包括一或多个连接体。

[0378] 在一些实施例中,异源功能结构域可能能够修改RNA引导的DNA结合剂的细胞内半衰期。在一些实施例中,可增加RNA引导的DNA结合剂的半衰期。在一些实施例中,可减少RNA引导的DNA结合剂的半衰期。在一些实施例中,异源功能结构域可能能够增加RNA引导的DNA结合剂的稳定性。在一些实施例中,异源功能结构域可能能够降低RNA引导的DNA结合剂的稳定性。在一些实施例中,异源功能结构域可用作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(例如蛋白酶体、溶酶体蛋白酶或卡配因蛋白酶)介导。在一些实施例中,异源功能结构域可包含PEST序列。在一些实施例中,可藉由添加泛素或多泛素链来修饰RNA引导的DNA结合剂。在一些实施例中,泛素可为泛素样蛋白(UBL)。泛素样蛋白的非限制性实例包括小泛素样修饰剂(SUMO)、泛素交叉反应蛋白(UCRP,亦称为干扰素刺激基因-15(ISG15))、泛素相关的修饰剂-1(URM1)、神经元-前体-细胞表达的发育上调的蛋白-8(NEDD8,在啤酒酵母(S.cerevisiae)中亦称为Rub1)、人类白血球抗原F相关(FAT10)、自体吞噬-8(ATG8)及自体吞噬-12(ATG12)、Fau泛素样蛋白(FUB1)、膜锚定UBL(MUB)、泛素倍数修饰剂-1(UFM1)及泛素样蛋白-5(UBL5)。

[0379] 在一些实施例中,异源功能结构域可为标记结构域。标记结构域的非限制性实例包括荧光蛋白、纯化标签、表位标识及报导基因序列。在一些实施例中,标记结构域可为荧光蛋白。适宜荧光蛋白的非限制性实例包括绿荧光蛋白(例如,GFP、GFP-2、tagGFP、turboGFP、sfGFP、EGFP、Emerald、Azami Green、Monomeric Azami Green、CopGFP、AceGFP、ZsGreen1)、黄荧光蛋白(例如,YFP、EYFP、Citrine、Venus、YPet、PhiYFP、ZsYellow1)、蓝荧光蛋白(例如,EBFP、EBFP2、Azurite、mKalamal、GFPuv、Sapphire、T-sapphire)、青荧光蛋白(例如,ECFP、Cerulean、CyPet、AmCyan1、Midoriishi-Cyan)、红荧光蛋白(例如,mKate、mKate2、mPlum、DsRed单体、mCherry、mRFP1、DsRed-Express、DsRed2、DsRed-单体、HcRed-Tandem、HcRed1、AsRed2、eqFP611、mRasberry、mStrawberry、Jred)及橙荧光蛋白(mOrange、mKO、Kusabira-Orange、Monomeric Kusabira-Orange、mTangerine、tdTomato)或任何其它适宜荧光蛋白。在其它实施例中,标记结构域可为纯化标签和/或表位标识。非限制性实例性标签包括谷胱甘肽-S-转移酶(GST)、几丁质结合蛋白(CBP)、麦芽糖结合蛋白(MBP)、硫氧还蛋白(TRX)、聚(NANP)、串联亲和性纯化(TAP)标签、myc、AcV5、AU1、AU5、E、ECS、E2、FLAG、HA、nus、Softag 1、Softag 3、Strep、SBP、Glu-Glu、HSV、KT3、S、S1、T7、V5、VSV-G、6xHis、8xHis、生物素羧基载体蛋白(BCCP)、聚-His及携钙蛋白。非限制性实例性报导基因包括谷胱甘肽-S-转移酶(GST)、辣根过氧化物酶(HRP)、氯霉素乙酰基转移酶(CAT)、β-半乳糖苷酶、β-葡萄糖醛酸苷酶、荧光素酶或荧光蛋白。

[0380] 在额外实施例中,异源功能结构域可将RNA引导的DNA结合剂靶向特定细胞器、细

胞类型、组织或器官。在一些实施例中，异源功能结构域可将RNA引导的DNA结合剂靶向线粒体。

[0381] 在其它实施例中，异源功能结构域可为效应结构域。当RNA引导的DNA结合剂引导至其靶序列时，例如当Cas核酸酶由gRNA引导至靶序列时，效应结构域可修饰或影响靶序列。在一些实施例中，效应结构域可选自核酸结合结构域、核酸酶结构域(例如，非Cas核酸酶结构域)、后生修饰结构域、转录活化结构域或转录阻遏结构域。在一些实施例中，异源功能结构域是核酸酶，例如FokI核酸酶。参见，例如，美国专利第9,023,649号。在一些实施例中，异源功能结构域是转录活化剂或阻遏剂。参见(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.Methods 10:973-6(2013);Mali等人，“CAS9 transcriptional 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结合剂基本上变成转录因子，其可使用引导RNA引导至结合期望靶序列。

[0382] B.经修饰gRNA及mRNA

[0383] 在一些实施例中，gRNA经化学修饰。包含一或多个经修饰核苷或核苷酸的gRNA称为“经修饰”gRNA或“经化学修饰”gRNA，以产生一或多种代替典型A、G、C及U残基使用或除典型A、G、C及U残基之外使用的非天然和/或天然组分或构形的存在。在一些实施例中，利用非典型核苷或核苷酸合成经修饰gRNA，在此称为“经修饰”。经修饰核苷及核苷酸可包括以下中的一项或多项：(i)改变、例如置换非连接磷酸酯氧中的一个或两个和/或磷酸二酯主链链接中的连接磷酸酯氧中的一个或多个(实例性主链修饰)；(ii)改变、例如置换核糖的成分，例如核糖上的2′羟基(实例性糖修饰)；(iii)用“去磷酸化”连接体批量置换磷酸酯部分(实例性主链修饰)；(iv)包括用非典型核碱基修饰或置换天然核碱基(实例性碱基修饰)；(v)修饰或置换核糖-磷酸酯主链(实例性主链修饰)；(vi)修饰寡核苷酸的3′端或5′端，例如移除、修饰或置换末端磷酸酯基团或结合部分、帽或连接体(该3′或5′帽修饰可包含糖和/或主链修饰)；及(vii)修饰或置换糖(实例性糖修饰)。

[0384] 如上所述，在一些实施例中，本文揭示的组合物或调配物包含含有编码RNA引导的DNA结合剂(例如如本文所述Cas核酸酶)的开放阅读框(ORF)的mRNA。在一些实施例中，提供、使用或施用包含编码RNA引导的DNA结合剂(例如Cas核酸酶)的ORF的mRNA。在一些实施例中，编码RNA引导的DNA核酸酶的ORF是“经修饰RNA引导的DNA结合剂ORF”或简称为“经修饰ORF”，其用作速记以指示，ORF以下列方式中的一项或多项经修饰：(1)经修饰ORF的尿苷含量范围为自其最小尿苷含量至最小尿苷含量的150％；(2)经修饰ORF的尿苷二核苷酸含量范围为自其最小尿苷二核苷酸含量至最小尿苷二核苷酸含量的150％；(3)经修饰ORF与SEQ ID NO:201、204、210、214、215、223、224、250、252、254、265或266中的任一种具有至少90％一致性；(4)经修饰ORF由一组密码子组成，其中至少75％的密码子是最小尿苷密码子的表3A中列出的密码子；或(5)经修饰ORF包含至少一个经修饰尿苷。在一些实施例中，经修饰ORF以至少两种、三种或四种前述方式经修饰。在一些实施例中，经修饰ORF包含至少一个

经修饰尿苷且以上述(1)-(4)中的至少一种、二种、三种或全部经修饰。

[0385] 最小尿苷密码子的表3A

[0386]

	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

[0387]

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
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

[0388] 在上述实施例的任一个中，经修饰ORF可由一组密码子组成，其中至少75％、80％、85％、90％、95％、98％、99％或100％的密码子是显示最小尿苷密码子的表3A中列出的密码子。

[0389] 在上述实施例的任一个中，经修饰ORF可包含与SEQ ID NO:201、204、210、214、215、223、224、250、252、254、265或266中的任一种具有至少90％、95％、98％、99％或100％一致性的序列。

[0390] 在上述实施例的任一个中，经修饰ORF的尿苷含量可在其最小尿苷含量至最小尿

苷含量的150％、145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％的范围内。

[0391] 在上述实施例的任一个中，经修饰ORF的尿苷二核苷酸含量可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％、145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％的范围内。

[0392] 在上述实施例的任一个中，经修饰ORF可包含至少在一个、复数个或全部尿苷位置的经修饰尿苷。在一些实施例中，经修饰尿苷是在5位经(例如)卤素、甲基或乙基修饰的尿苷。在一些实施例中，经修饰尿苷是在1位经(例如)卤素、甲基或乙基修饰的假尿苷。经修饰尿苷可为(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，经修饰尿苷是5-甲氧基尿苷。在一些实施例中，经修饰尿苷是5-碘尿苷。在一些实施例中，经修饰尿苷是假尿苷。在一些实施例中，经修饰尿苷是N1-甲基-假尿苷。在一些实施例中，经修饰尿苷是假尿苷与N1-甲基-假尿苷的组合。在一些实施例中，经修饰尿苷是假尿苷与5-甲氧基尿苷的组合。在一些实施例中，经修饰尿苷是N1-甲基假尿苷与5-甲氧基尿苷的组合。在一些实施例中，经修饰尿苷是5-碘尿苷与N1-甲基-假尿苷的组合。在一些实施例中，经修饰尿苷是假尿苷与5-碘尿苷的组合。在一些实施例中，经修饰尿苷是5-碘尿苷与5-甲氧基尿苷的组合。

[0393] 在一些实施例中，根据本揭示内容的mRNA中的至少10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％、98％、99％或100％的尿苷位置是经修饰尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是经修饰尿苷，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，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是5-甲氧基尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是假尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是N1-甲基假尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是5-碘尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是5-甲氧基尿苷，且其余是N1-甲基假尿苷。在一些实施例中，根据本揭示内容的mRNA中的10％-25％、15-25％、25-35％、35-45％、45-55％、55-65％、65-75％、75-85％、85-95％或90-100％的尿苷位置是5-碘尿苷，且其余是N1-甲基假尿苷。

[0394] 在一些实施例中，mRNA包含来自表达的哺乳动物mRNA(例如组成型表达的mRNA)的一个UTR。若mRNA在健康成年哺乳动物的至少一个组织中连续转录，则认为该mRNA在哺乳动物中组成型表达。在一些实施例中，mRNA包含来自表达的哺乳动物RNA(例如组成型表达的哺乳动物mRNA)的5’UTR、3’UTR或5’及3’UTR。肌动蛋白mRNA是组成型表达的mRNA的实例。

[0395] 在一些实施例中，mRNA包含来自羟基类固醇17-β去氢酶4(HSD17B4或HSD)的一个UTR(例如HSD的5’UTR)。在一些实施例中，mRNA包含来自珠蛋白mRNA(例如人类α珠蛋白

(HBA)mRNA、人类β珠蛋白(HBB)mRNA或非洲爪蟾β珠蛋白(XBG)mRNA)的至少一个UTR。在一些实施例中,mRNA包含来自珠蛋白mRNA(例如HBA、HBB或XBG)的5'UTR、3'UTR或5'及3'UTR。在一些实施例中,mRNA包含来自牛生长激素、巨细胞病毒(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、巨细胞病毒、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、巨细胞病毒、小鼠Hba-a1、HSD、白蛋白基因、HBA、HBB、XBG、热休克蛋白90(Hsp90)、3-磷酸甘油醛去氢酶(GAPDH)、β-肌动蛋白、α-微管蛋白、肿瘤蛋白(p53)或表皮生长因子受体(EGFR)的5'及3'UTR。

[0396] 在一些实施例中,mRNA包含来自相同来源(例如,组成型表达的mRNA,例如肌动蛋白、白蛋白或诸如HBA、HBB或XBG等珠蛋白)的5'及3'UTR。

[0397] 在一些实施例中,mRNA不包含5'UTR,例如,在5'帽与起始密码子之间无额外核苷酸。在一些实施例中,mRNA在5'帽与起始密码子之间包含Kozak序列(如下所述),但不含任何额外5'UTR。在一些实施例中,mRNA不包含3'UTR,例如,在终止密码子与多-A尾之间无额外核苷酸。

[0398] 在一些实施例中,mRNA包含Kozak序列。Kozak序列可影响转译起始及自mRNA转译的多肽的总产率。Kozak序列包括可用作起始密码子的甲硫氨酸密码子。最小Kozak序列是NNNRUGN,其中以下中的至少一项为真:第一个N是A或G,且第二个N是G。在核苷酸序列的上下文中,R意指嘌呤(A或G)。在一些实施例中,Kozak序列是RNNRUGN、NNNRUGG、RNNRUGG、RNNAUGN、NNNAUGG或RNNAUGG。在一些实施例中,Kozak序列是与以小写字母表示的位置具有零错配或最多一个或两个错配的rccRUGg。在一些实施例中,Kozak序列是与以小写字母表示的位置具有零错配或最多一个或两个错配的rccAUGg。在一些实施例中,Kozak序列是与以小写字母表示的位置具有零错配或最多一个、两个或三个错配的gccRccAUGG(SEQ ID NO:277)。在一些实施例中,Kozak序列是与以小写字母表示的位置具有零错配或最多一个、两个、三个或四个错配的gccAccAUG。在一些实施例中,Kozak序列是GCCACCAUG。在一些实施例中,Kozak序列是与以小写字母表示的位置具有零错配或最多一个、两个、三个或四个错配的gccgccRccAUGG(SEQ ID NO:278)。

[0399] 在一些实施例中,编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:1具有至少90%一致性的序列,视情况其中SEQ ID NO:1的ORF(亦即,SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0400] 在一些实施例中,编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:244具有至少90%一致性的序列,视情况其中SEQ ID NO:244的ORF(亦即,SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0401] 在一些实施例中,编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:256具有至少90%一致性的序列,视情况其中SEQ ID NO:256的ORF(亦即,SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0402] 在一些实施例中,编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:257具有至少90%一致性的序列,视情况其中SEQ ID NO:257的ORF(亦即,SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0403] 在一些实施例中,编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:257具

有至少90％一致性的序列，视情况其中SEQ ID NO:258的ORF(亦即，SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0404] 在一些实施例中，编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:259具有至少90％一致性的序列，视情况其中SEQ ID NO:259的ORF(亦即，SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

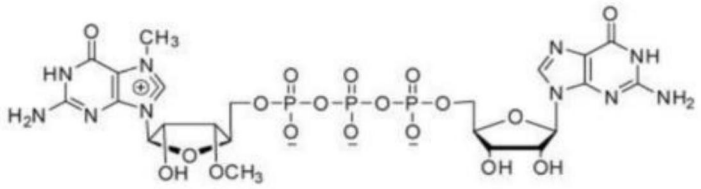
[0405] 在一些实施例中，编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:260具有至少90％一致性的序列，视情况其中SEQ ID NO:260的ORF(亦即，SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

[0406] 在一些实施例中，编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:261具有至少90％一致性的序列，视情况其中SEQ ID NO:261的ORF(亦即，SEQ ID NO:204)经SEQ ID NO:210、214、215、223、224、250、252、254、265或266中的任一种的替代ORF取代。

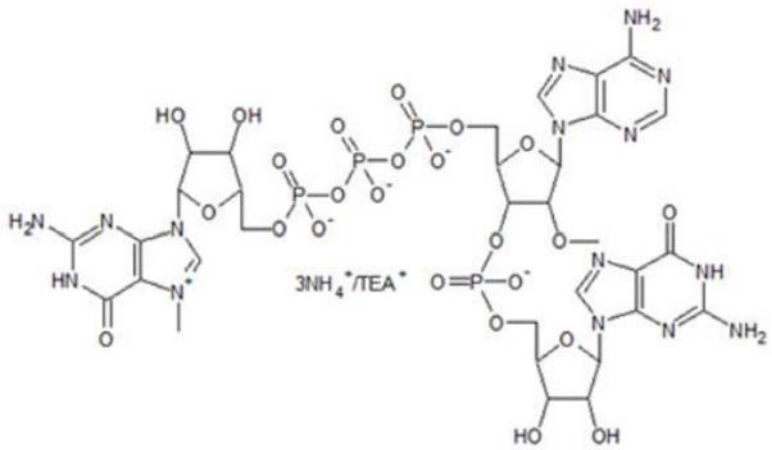
[0407] 在一些实施例中，与SEQ ID NO 243、244或256-261的视情况经取代的序列的一致性程度是95％。在一些实施例中，与SEQ ID NO 243、244或256-261的视情况经取代的序列的一致性程度是98％。在一些实施例中，与SEQ ID NO 243、244或256-261的视情况经取代的序列的一致性程度是99％。在一些实施例中，与SEQ ID NO 243、244或256-261的视情况经取代的序列的一致性程度是100％。

[0408] 在一些实施例中，本文揭示的mRNA包含5’帽，例如Cap0、Cap1或Cap2。5’帽通常是经由5’-三磷酸酯至mRNA的5’-至-3’链的第一核苷酸(亦即，第一帽近端核苷酸)的5’部分连接的7-甲基鸟嘌呤核糖核苷酸(其可进一步经修饰，如下文所论述，例如关于ARCA)。在Cap0中，mRNA的第一及第二帽近端核苷酸的核糖二者皆包含2’-羟基。在Cap1中，mRNA的第一及第二转录的核苷酸的核糖分别包含2’-甲氧基及2’-羟基。在Cap2中，mRNA的第一及第二帽近端核苷酸的核糖二者皆包含2’-甲氧基。例如，参见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。大部分内源性高等真核mRNA(包括哺乳动物mRNA，例如人类mRNA)包含Cap1或Cap2。与Cap1及Cap2不同的Cap0及其它帽结构在哺乳动物(例如人类)中可能是免疫性的，此乃因被先天免疫系统的组分(例如IFIT-1及IFIT-5)被识别“非自我”，此可导致升高的细胞介素含量，包括I型干扰素。先天免疫系统的组分(例如IFIT-1及IFIT-5)亦可与eIF4E竞争mRNA与Cap1或Cap2以外的帽的结合，从而潜在地抑制mRNA的转译。

[0409] 帽可包括共转录。例如，ARCA(抗反转帽类似物;Thermo Fisher Scientific目录号AM8045)是包含与鸟嘌呤核糖核苷酸的5’位置连接的7-甲基鸟嘌呤3’-甲氧基-5’-三磷酸酯的帽类似物，该鸟嘌呤核糖核苷酸可在起始时活体外纳入的转录物中。ARCA产生Cap0帽，其中第一帽近端核苷酸的2’位置是羟基。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结构如下所示。

[0410] 

[0411] 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)可用于提供共转录Cap1结构。CleanCap™ AG及CleanCap™ GG的3'-O-甲基化型式亦可分别以目录号N-7413及N-7433自TriLink Biotechnologies获得。CleanCap™ AG结构如下所示。

[0412] 

[0413] 或者,可在转录后将RNA添加至RNA中。例如,牛痘加帽酶有市售(New England Biolabs目录号M2080S)具有其D1亚单位提供的RNA三磷酸酶及鸟苷酰基转移酶活性以及由其D12亚单位提供的鸟嘌呤甲基转移酶。因此,其可向RNA中添加7-甲基鸟嘌呤,以便在S-腺苷甲硫胺及GTP存在下产生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。关于帽及加帽方法的其它论述,参见(例如)WO2017/053297及Ishikawa等人,Nucl.Acids.Symp.Ser.(2009)第53期,129-130。

[0414] 在一些实施例中,mRNA进一步包含多腺苷酸化(多-A)尾。在一些实施例中,多-A尾包含至少20、30、40、50、60、70、80、90或100个腺嘌呤,视情况最多300个腺嘌呤。在一些实施例中,多-A尾包含95、96、97、98、99或100个腺嘌呤核苷酸。在一些情况下,多-A尾在多-A尾内的一或多个位置"杂有"一或多个非腺嘌呤核苷酸"锚"。多-A尾可包含至少8个连续腺嘌呤核苷酸,但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是实例性非腺嘌呤核苷酸。因此,本文所述mRNA上的多-A尾可包含连接腺嘌呤核苷酸,其位于编码RNA引导的DNA结合剂或所关注序列的核苷酸的3'。在一些情况下,mRNA上的多-A尾包含位于编码RNA引导的DNA结合剂或所关注序列的核苷酸的3'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。

[0415] 在一些实施例中,一或多个非嘌呤核苷酸经定位以中断连续腺嘌呤核苷酸,使得多(A)结合蛋白可结合一段连续腺嘌呤核苷酸。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8-50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8-100个连续腺嘌呤核苷酸之后。在一些实施例中,非腺嘌呤核苷酸是在一个、两个、三个、四个、五个、六个或七个腺嘌呤核苷酸之后且之后是至少8个连续腺嘌呤核苷酸。

[0416] 多-A尾可包含连续腺嘌呤核苷酸、之后一或多个非腺嘌呤核苷酸、视情况之后额

外腺嘌呤核苷酸的一个序列。

[0417] 在一些实施例中，多-A尾包含或含有一个非腺嘌呤核苷酸或一段连续2-10个非腺嘌呤核苷酸。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8-50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。

[0418] 在一些实施例中，非腺嘌呤核苷酸是鸟嘌呤、胞嘧啶或胸腺嘧啶。在一些情况下，非腺嘌呤核苷酸是鸟嘌呤核苷酸。在一些实施例中，非腺嘌呤核苷酸是胞嘧啶核苷酸。在一些实施例中，非腺嘌呤核苷酸是胸腺嘧啶核苷酸。在一些情况下，在存在一种以上非腺嘌呤核苷酸的情况下，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实例性多-A尾提供为SEQ ID NO:4。

[0419] 在一些实施例中，mRNA进一步包含多腺苷酸化(多-A)尾。在一些情况下，多-A尾在多-A尾内的一或多个位置“杂有”一或多个非腺嘌呤核苷酸“锚”。多-A尾可包含至少8个连续腺嘌呤核苷酸，但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是实例性非腺嘌呤核苷酸。因此，本文所述mRNA上的多-A尾可包含连接腺嘌呤核苷酸，其位于编码RNA引导的DNA结合剂或所关注序列的核苷酸的3’。在一些情况下，mRNA上的多-A尾包含位于编码RNA引导的DNA结合剂或所关注序列的核苷酸的3’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。

[0420] 在一些实施例中，一或多个非嘌呤核苷酸经定位以中断连续腺嘌呤核苷酸，使得多(A)结合蛋白可结合一段连续腺嘌呤核苷酸。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8-50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8-100个连续腺嘌呤核苷酸之后。在一些实施例中，非腺嘌呤核苷酸是在一个、两个、三个、四个、五个、六个或七个腺嘌呤核苷酸之后且之后是至少8个连续腺嘌呤核苷酸。

[0421] 本发明的多-A尾可包含连续腺嘌呤核苷酸、之后一或多个非腺嘌呤核苷酸、视情况之后额外腺嘌呤核苷酸的一个序列。

[0422] 在一些实施例中，多-A尾包含或含有一个非腺嘌呤核苷酸或一段连续2-10个非腺嘌呤核苷酸。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8-50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。

[0423] 在一些实施例中，非腺嘌呤核苷酸是鸟嘌呤、胞嘧啶或胸腺嘧啶。在一些情况下，非腺嘌呤核苷酸是鸟嘌呤核苷酸。在一些实施例中，非腺嘌呤核苷酸是胞嘧啶核苷酸。在一些实施例中，非腺嘌呤核苷酸是胸腺嘧啶核苷酸。在一些情况下，在存在一种以上非腺嘌呤核苷酸的情况下，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实例性多-A尾提供为SEQ ID NO:4:AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA。

[0424] 可组合化学修饰(例如如文所列示的这些)以提供包含可具有两个、三个、四个或更多个修饰的核苷及核苷酸(统称为"残基")的经修饰gRNA和/或mRNA。例如,经修饰残基可具有经修饰糖及经修饰核碱基。在一些实施例中,gRNA的每个碱基皆经修饰,例如,所有碱基皆具有经修饰磷酸酯基团,例如硫代磷酸酯基团。在某些实施例中,gRNA分子的所有或实质上全部磷酸酯基团皆经硫代磷酸酯基团置换。在一些实施例中,经修饰gRNA包含至少一个在RNA的5′端或其附近的经修饰残基。在一些实施例中,经修饰gRNA包含至少一个在RNA的3′端或其附近的经修饰残基。

[0425] 在一些实施例中,gRNA包含一个、两个、三个或更多个经修饰残基。在一些实施例中,经修饰gRNA中的至少5%(例如,至少5%、至少10%、至少15%、至少20%、至少25%、至少30%、至少35%、至少40%、至少45%、至少50%、至少55%、至少60%、至少65%、至少70%、至少75%、至少80%、至少85%、至少90%、至少95%或100%)的位置是经修饰核苷或核苷酸。

[0426] 未经修饰核酸可易于被(例如)细胞内核酸酶或血清中发现的这些降解。例如,核酸酶可水解核酸二酯键。因此,在一个方面中,本文所述gRNA可含有一或多个经修饰核苷或核苷酸,例如,以引入对细胞内或基于血清的核酸酶的稳定性。在一些实施例中,当活体内及离体引入至细胞群体中时,本文所述的经修饰gRNA分子可展现降低的先天免疫反应。术语"先天免疫反应"包括对外源性核酸(包括单链核酸)的细胞反应,其包括细胞介素表达及释放的诱导,特别是干扰素及细胞死亡。

[0427] 在主链修饰的一些实施例中,经修饰残基的磷酸酯基团可藉由用不同取代基置换一或多个氧来修饰。此外,经修饰残基(例如,经修饰核酸中存在的经修饰残基)可包括用如本文所述的经修饰磷酸酯基团批量置换未经修饰磷酸酯部分。在一些实施例中,磷酸酯主链的主链修饰包括导致不带电连接体或带有不对称电荷分布的带电连接体的改变。

[0428] 经修饰磷酸酯基团的实例包括硫代磷酸酯、磷酸硒酸酯、硼烷基磷酸酯(boranophosphates、borano phosphate esters)、膦酸氢酯、磷酰胺酯、膦酸烷基酯或膦酸芳基酯及磷酸三酯。未经修饰磷酸酯基团中的磷原子是非手性的。然而,用上述原子或原子群中的一项置换非桥接氧中的一个可使磷原子具有手性。立体源磷原子可具有"R"构形(本文为Rp)或"S"构形(本文为Sp)。主链亦可藉由用氮(桥接磷酰胺酯)、硫(桥接硫代磷酸酯)及碳(桥接亚甲基膦酸酯)置换桥接氧(亦即,将磷酸酯连接至核苷的氧)来修饰。置换可在任一连接氧或两个连接氧处发生。

[0429] 在某些主链修饰中,磷酸酯基团可由不合磷的连接基团置换。在一些实施例中,带电的磷酸酯基团可由天然部分置换。可置换磷酸酯基团的部分的实例可包括(但不限于)例如膦酸甲基酯、羟基胺基、硅氧烷、碳酸酯、羧基甲基、胺基甲酸酯、酰胺、硫醚、环氧乙烷连接体、磺酸酯、磺酰胺、硫代甲缩醛、甲缩醛、肟、亚甲基亚胺基、亚甲基甲基亚胺基、亚甲基伸肼基、亚甲基二甲基伸肼基及亚甲基氧基甲基亚胺基。

[0430] 可模拟核酸的支架亦可经构筑,其中磷酸酯连接体及核糖由核酸酶抗性核苷或核苷酸代用品置换。该修饰可包括主链及糖修饰。在一些实施例中,核碱基可由代用品主链束

缚。实例可包括(但不限于)吗啉基、环丁基、吡咯啶及肽核酸(PNA)核苷代用品。

[0431] 经修饰核苷及经修饰核苷酸可包括对糖基团的一或多种修饰,亦即糖修饰。例如,2′羟基(OH)可由许多不同“氧基”或“脱氧”取代基修饰,例如置换。在一些实施例中,对2′羟基的修饰可增强核酸的稳定性,此乃因羟基不再被去质子化以形成2′-烷氧离子。

[0432] 2′羟基修饰的实例可包括烷氧基或芳基氧基(OR,其中“R”可为例如烷基、环烷基、芳基、芳烷基、杂芳基或糖);聚乙二醇(PEG)、O(CH2CH2O)nCH2CH2OR,其中R可为例如H或视情况经取代烷基,且n可为0至20(例如,0至4、0至8、0至10、0至16、1至4、1至8、1至10、1至16、1至20、2至4、2至8、2至10、2至16、2至20、4至8、4至10、4至16及4至20)的整数。在一些实施例中,2′羟基修饰可为2′-O-Me。在一些实施例中,2′羟基修饰可为2′-氟修饰,其用氟化物置换2′羟基。在一些实施例中,2′羟基修饰可包括“锁定”核酸(LNA),其中2′羟基可藉由(例如)C1-6伸烷基或C1-6伸杂烷基桥连接至相同核糖的4′碳,其中实例性桥可包括亚甲基、伸丙基、醚或胺基桥;O-胺基(其中胺基可为例如NH2;烷基胺基、二烷基胺基、杂环基、芳基胺基、二芳基胺基、杂芳基胺基或二杂芳基胺基、乙二胺或多胺基)及胺基烷氧基、O(CH2)n-胺基(其中胺基可为例如NH2;烷基胺基、二烷基胺基、杂环基、芳基胺基、二芳基胺基、杂芳基胺基或二杂芳基胺基、乙二胺或多胺基)。在一些实施例中,2′羟基修饰可包括“解锁”核酸(UNA),其中核糖环无C2′-C3′键。在一些实施例中,2′羟基修饰可包括甲氧基乙基(MOE)、(OCH2CH2OCH3,例如,PEG衍生物)。

[0433] “脱氧”2′修饰可包括氢(亦即例如在部分dsRNA的悬突部分的脱氧核糖);卤基(例如,溴、氯、氟或碘);胺基(其中胺基可为例如NH2;烷基胺基、二烷基胺基、杂环基、芳基胺基、二芳基胺基、杂芳基胺基、二杂芳基胺基或氨基酸);NH(CH2CH2NH)nCH2CH2-胺基(其中胺基可例如如本文所述)、-NHC(O)R(其中R可为例如烷基、环烷基、芳基、芳烷基、杂芳基或糖)、氰基;巯基;烷基-硫基-烷基;硫烷氧基;及烷基、环烷基、芳基、烯基及炔基,其可视情况经(例如)如本文所述的胺基取代。

[0434] 糖修饰可包含糖基团,其亦可含有一或多个具有与核糖中相应碳相反的立体化学构形的碳。因此,经修饰核酸可包括含有例如阿拉伯糖作为糖的核苷酸。经修饰核酸亦可包括无碱基糖。该无碱基糖亦可在一或多个构成糖原子进一步经修饰。经修饰核酸亦可包括一种或多种呈L形式(例如L-核苷酸)的糖。

[0435] 可纳入经修饰核酸中的本文所述的经修饰核苷及经修饰核苷酸可包括经修饰碱基,亦称为核碱基。核碱基的实例包括(但不限于)腺嘌呤(A)、鸟嘌呤(G)、胞嘧啶(C)及尿嘧啶(U)。该核碱基可经修饰或完全经置换,以提供可纳入经修饰核酸中的经修饰残基。核苷酸的核碱基可独立地选自嘌呤、嘧啶、嘌呤类似物或嘧啶类似物。在一些实施例中,核碱基可包括例如碱基的天然及合成衍生物。

[0436] 在采用双重引导RNA的实施例中,crRNA及tracr RNA中的每一种可含有修饰。该修饰可在crRNA和/或tracr RNA的一端或两端。在包含sgRNA的实施例中,可化学修饰sgRNA的一端或两端的一或多个残基,或可化学修饰整个sgRNA。某些实施例包含5′端修饰。某些实施例包含3′端修饰。在某些实施例中,引导RNA分子的单链悬突中的一或多个或全部核苷酸是脱氧核苷酸。

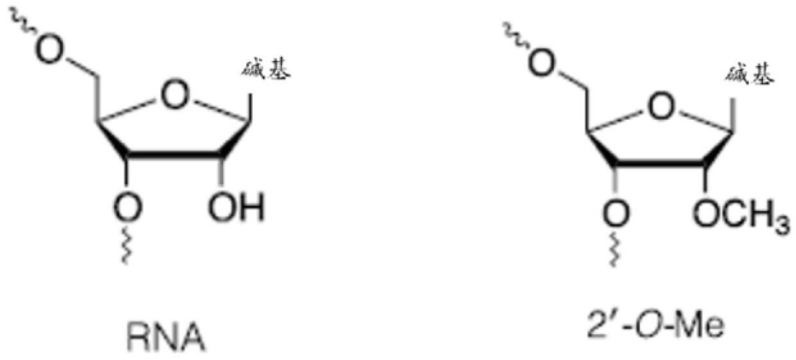
[0437] 在一些实施例中,本文揭示的引导RNA包含于2016年12月8日提出申请的标题为“Chemically Modified Guide RNAs”的US 62/431,756中揭示的修饰模式之一,其内容为

以全文引用方式并入本文中。

[0438] 在一些实施例中，本发明包含含有一或多个修饰的gRNA。在一些实施例中，修饰包含2′-O-甲基(2′-O-Me)修饰的核苷酸。在一些实施例中，修饰包括核苷酸之间的硫代磷酸酯(PS)键。

[0439] 术语“mA”、“mC”、“mU”或“mG”可用于表示已用2’-O-Me修饰的核苷酸。

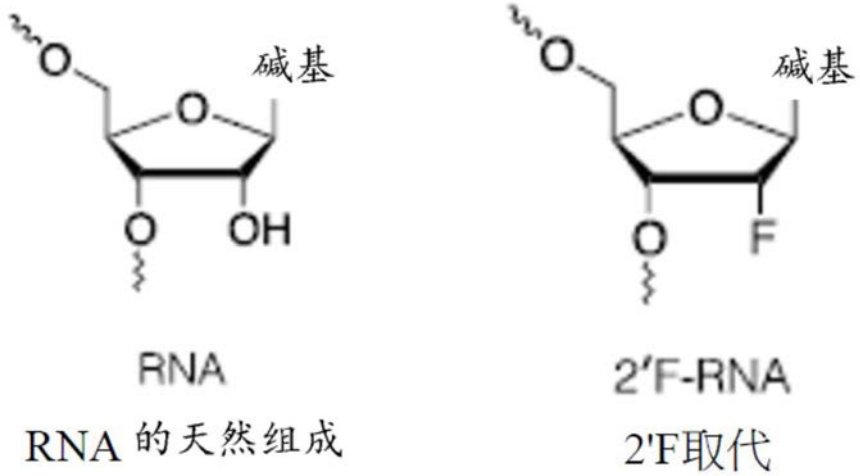
[0440] 2’-O-甲基的修饰可绘示如下：

[0441] 

[0442] 已显示影响核苷酸糖环的另一化学修饰是卤素取代。例如，核苷酸糖环上的2’-氟(2’-F)取代可增加寡核苷酸结合亲和性及核酸酶稳定性。

[0443] 在本申请案中，术语“fA”、“fC”、“fU”或“fG”可用于表示经2’-F取代的核苷酸。

[0444] 2’-F的取代可绘示如下：

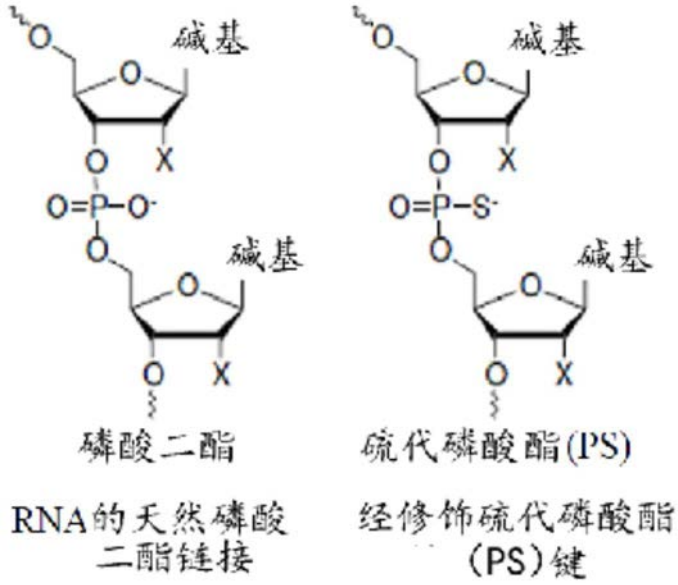
[0445] 

[0446] 硫代磷酸酯(PS)链接或键是指其中将硫取代为磷酸二酯链接中(例如核苷酸碱基之间的键中)的一个非桥接磷酸酯氧。当使用硫代磷酸酯以生成寡核苷酸时，经修饰寡核苷酸亦可称为S-寡核苷酸。

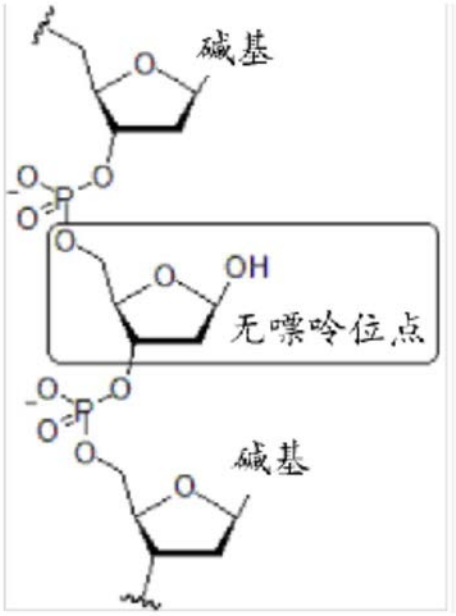
[0447] “*”可用于绘示PS修饰。在本申请案中，术语A*、C*、U*或G*可用于表示利用PS键连接至下一(例如，3’)核苷酸的核苷酸。

[0448] 在本申请案中，术语“mA*”、“mC*”、“mU*”或“mG*”可用于表示经2’-O-Me取代且利用PS键连接至下一(例如，3’)核苷酸的核苷酸。

[0449] 下图显示S-取代成非桥接磷酸酯氧，从而生成PS键代替磷酸二酯键：

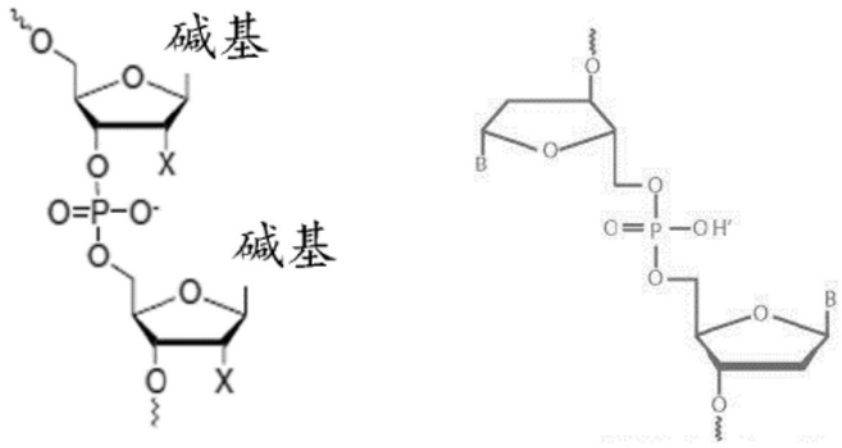
[0450] 

[0451] 无碱基核苷酸是指无含氮碱基的这些。下图绘示具有缺乏碱基的无碱基(亦称为无嘌呤)位点的寡核苷酸：

[0452] 

[0453] 倒置碱基是指具有自正常5’至3’链接倒置的链接(亦即,5’至5’链接或3’至3’链接)的这些。例如：

[0454]



[0455] 无碱基核苷酸可利用倒置链接连接。例如,无碱基核苷酸可经由5'至5'链接连接至末端5'核苷酸,或无碱基核苷酸可经由3'至3'链接连接至末端3'核苷酸。末端5'或3'核苷酸处的倒置无碱基核苷酸亦可称作倒置无碱基端帽。

[0456] 在一些实施例中,在5′末端之前三个、四个或五个核苷酸中的一项或多项以及在3′末端的最后三个、四个或五个核苷酸中的一项或多项经修饰。在一些实施例中,修饰是2'-O-Me、2'-F、倒置无碱基核苷酸、PS键或业内众所周知可增加稳定性和/或性能的其它核苷酸修饰。

[0457] 在一些实施例中,在5′末端之前四个核苷酸以及在3′末端的最后四个核苷酸利用硫代磷酸酯(PS)键连接。

[0458] 在一些实施例中,在5′末端之前三个核苷酸以及在3′末端的最后三个核苷酸包含2′-O-甲基(2′-O-Me)修饰的核苷酸。在一些实施例中,在5′末端之前三个核苷酸以及在3′末端的最后三个核苷酸包含2′-氟(2′-F)修饰的核苷酸。在一些实施例中,在5′末端之前三个核苷酸以及在3′末端的最后三个核苷酸包含倒置无碱基核苷酸。

[0459] 在一些实施例中,引导RNA包含经修饰sgRNA。在一些实施例中,sgRNA包含SEQ ID No:3中所示的修饰模式,其中N是任何天然或非天然核苷酸,且其中全体N包含将核酸酶引导至靶序列的引导序列。

[0460] 在一些实施例中,引导RNA包含SEQ ID No:87-124的任一种中所示的sgRNA。在一些实施例中,引导RNA包含含有SEQ ID No:5-82的引导序列及SEQ ID No:125的核苷酸中的任一种的sgRNA,其中SEQ ID No:125的核苷酸在引导序列的3'端上,且其中引导序列可如SEQ ID No:3中所示经修饰。

[0461] C.核糖核蛋白复合物

[0462] 在一些实施例中,涵盖包含表1的一或多个引导序列的一或多个gRNA或表2的一或多个sgRNA以及RNA引导的DNA结合剂(例如,核酸酶,例如Cas核酸酶,例如Cas9)的组合物。在一些实施例中,编码的RNA引导的DNA结合剂具有裂解酶活性,其亦可称为双链内核酸酶活性。在一些实施例中,RNA引导的DNA结合剂包含Cas核酸酶。Cas9核酸酶的实例包括酿脓链球菌、金黄色葡萄球菌及其它原核生物(例如,参见下一段中的清单)及其修饰(例如,工程化或突变体)型式的II型CRISPR系统的这些。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亚单位;及I型CRISPR系统的级联复合物或其Cas3亚单位。在一些实施例中,Cas核酸酶可来自IIA型、IIB型或IIC型系统。关于各种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)。

[0463] Cas核酸酶可源自的非限制实例性物种包括酿脓链球菌、嗜热链球菌、链球菌属、金黄色葡萄球菌、无害李斯特菌、加氏乳杆菌、新凶手弗朗西斯氏菌、琥珀酸弧菌、华德萨特菌、Y-变形杆菌、脑膜炎双球菌、空肠弯曲杆菌、多杀巴斯德菌、产琥珀酸丝状杆菌、红红螺菌、达松维尔类诺卡菌、始旋链霉菌、产绿色链霉菌、产绿色链霉菌、玫瑰链孢囊菌、玫瑰链孢囊菌、酸热脂环酸芽孢杆菌、假真菌样芽孢杆菌、还原硒酸盐芽孢杆菌、西伯利亚微小杆菌、戴白氏乳杆菌、唾液乳酸杆菌、布氏乳杆菌、齿垢密螺旋体、海洋微颤菌、伯克霍尔德氏菌、食萘极单胞菌、极单胞菌属、海洋固氮菌、蓝杆藻属、铜绿微囊藻、聚球藻属、阿拉伯糖醋杆菌、制氨菌、热角军纤维素菌、金矿菌、肉毒梭菌、难养芽胞梭菌、大芬戈尔德菌、嗜热盐碱厌氧菌、丙酸互营细菌、喜温嗜酸硫杆菌、嗜酸氧化亚铁硫杆菌、红色硫黄细菌、海洋杆菌属、嗜盐亚硝化球菌、瓦氏亚硝化球菌、游海假交替单胞菌、消旋纤线杆菌、调查甲烷盐菌、多变鱼腥藻、泡沫节球藻、念珠藻属、极大节旋藻、纯顶节旋藻、节旋藻属、鞘丝藻属、原形微鞘藻、颤藻属、运动石袍菌、非洲栖热腔菌、巴氏链球菌、灰色奈瑟菌、红嘴鸥弯曲杆菌、食清洁剂细小棒菌、白喉棒状杆菌、氨基酸球菌属、毛螺科菌ND2006及海洋无核绿藻。

[0464] 在一些实施例中,Cas核酸酶是来自酿脓链球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自嗜热链球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自脑膜炎双球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自金黄色葡萄球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶是来自新凶手弗朗西斯氏菌的Cpf1核酸酶。在一些实施例中,Cas核酸酶是来自氨基酸球菌属的Cpf1核酸酶。在一些实施例中,Cas核酸酶是来自毛螺科菌ND2006的Cpf1核酸酶。在其它实施例中,Cas核酸酶是来自以下的Cpf1核酸酶:土伦病弗朗西斯氏菌、毛螺科菌、亨氏丁酸弧菌、异域门菌、Parcubacteria菌、密斯氏菌属、氨基酸球菌属、产甲烷古菌念珠菌、挑剔真杆菌、牛眼莫拉氏菌、稻田氏钩端螺旋体、狗口腔红棕色单胞菌、解糖胨普雷沃菌或猕猴卟啉单胞菌。在某些实施例中,Cas核酸酶是来自氨基酸球菌属或毛螺菌科的Cpf1核酸酶。

[0465] 在一些实施例中,gRNA与RNA引导的DNA结合剂一起称作核糖核蛋白复合物(RNP)。在一些实施例中,RNA引导的DNA结合剂是Cas核酸酶。在一些实施例中,gRNA与Cas核酸酶一起称作Cas RNP。在一些实施例中,RNP包含I型、II型或III型组分。在一些实施例中,Cas核酸酶是来自II型CRISPR/Cas系统的Cas9蛋白。在一些实施例中,gRNA与Cas9一起称作Cas9 RNP。

[0466] 野生型Cas9具有两个核酸酶结构域:RuvC及HNH。RuvC结构域切割非靶DNA链,且HNH结构域切割DNA的靶链。在一些实施例中,Cas9蛋白包含一个以上RuvC结构域和/或一个以上HNH结构域。在一些实施例中,Cas9蛋白是野生型Cas9。在组合物、用途及方法实施例中的每一个中,Cas诱导靶DNA中的双链断裂。

[0467] 野生型Cas9具有两个核酸酶结构域:RuvC及HNH。RuvC结构域切割非靶DNA链,且HNH结构域切割DNA的靶链。在一些实施例中,Cas9核酸酶包含一个以上RuvC结构域和/或一

个以上HNH结构域。在一些实施例中，Cas9核酸酶是野生型Cas9。在一些实施例中，Cas9能诱导靶DNA中的双链断裂。在某些实施例中，Cas核酸酶可切割dsDNA，其可切割dsDNA的一条链，或其可无DNA裂解酶或切口酶活性。实例性Cas9氨基酸序列提供为SEQ ID NO:203。包括起始及终止密码子的实例性Cas9mRNA ORF序列提供为SEQ ID NO:204。适于包括于融合蛋白中的实例性Cas9 mRNA编码序列提供为SEQ ID NO:210。

[0468] 在一些实施例中，使用嵌合Cas核酸酶，其中蛋白质的一个结构域或区域由不同蛋白质的一部分置换。在一些实施例中，Cas核酸酶结构域可经来自不同核酸酶(例如Fok1)的结构域置换。在一些实施例中，Cas核酸酶可为经修饰核酸酶。

[0469] 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。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。

[0470] 在一些实施例中，RNA引导的DNA结合剂具有单链切口酶活性，亦即可切割一条DNA链以产生单链断裂，亦称为“切口”。在一些实施例中，RNA引导的DNA结合剂包含Cas切口酶。切口酶是一种在dsDNA中产生切口、亦即切割DNA双螺旋的一条链但非另一链的酶。在一些实施例中，Cas切口酶是Cas核酸酶的一种形式(例如，如上所论述的Cas核酸酶)，其中内核核溶解活性位点因(例如)催化结构域的一或多个变化(例如点突变)而不活化。关于Cas切口酶及实例性催化结构域变化的论述，参见(例如)美国专利第8,889,356号。在一些实施例中，Cas切口酶(例如Cas9切口酶、)具有不活化的RuvC或HNH结构域。实例性Cas9切口酶氨基酸序列提供为SEQ ID NO:206。包括起始及终止密码子的实例性Cas9切口酶mRNA ORF序列提供为SEQ ID NO:207。适于包括于融合蛋白中的实例性Cas9切口酶mRNA编码序列提供为SEQ ID NO:211。

[0471] 在一些实施例中，RNA引导的DNA结合剂经修饰以仅含有一个功能性核酸酶结构域。例如，试剂蛋白可经修饰，使得核酸酶结构域中的一个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结构域的切口酶。在一些实施例中，使用具有无活性RuvC结构域的切口酶。在一些实施例中，使用具有活性降低的HNH结构域的切口酶。在一些实施例中，使用具有无活性HNH结构域的切口酶。

[0472] 在一些实施例中，Cas蛋白核酸酶结构域内的保守胺基经取代以降低或改变核酸酶活性。在一些实施例中，Cas核酸酶可包含RuvC或RuvC样核酸酶结构域中的氨基酸取代。RuvC或RuvC样核酸酶结构域中的实例性氨基酸取代包括D10A(基于酿脓链球菌Cas9蛋白)。例如，参见Zetsche等人(2015)Cell Oct 22:163(3):759-771。在一些实施例中，Cas核酸酶可包含HNH或HNH样核酸酶结构域中的氨基酸取代。HNH或HNH样核酸酶结构域中的实例性氨基酸取代包括E762A、H840A、N863A、H983A及D986A(基于酿脓链球菌Cas9蛋白)。例如，参见Zetsche等人(2015)。其它实例性氨基酸取代包括D917A、E1006A及D1255A(基于新凶手弗朗西斯氏菌U112 Cpf1(FnCpf1)序列(UniProtKB-A0Q7Q2(CPF1_FRATN))。

[0473] 在一些实施例中，编码切口酶的mRNA与一对分别与靶序列的正义链及反义链互补的引导RNA组合提供。在此实施例中，引导RNA将切口酶引导至靶序列并藉由在靶序列的相对链上产生切口引入DSB(亦即，双切口形成)。在一些实施例中，使用双切口形成可提高特异性并减少脱靶效应。在一些实施例中，切口酶与两个靶向DNA的相反链的单独引导RNA一

起使用以在靶DNA中产生双切口。在一些实施例中，切口酶与经选择以紧密靠近的两个单独的引导RNA一起使用以在靶DNA中产生双切口。

[0474] 在一些实施例中，RNA引导的DNA结合剂无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，而基本上无催化(裂解酶/切口酶)活性。在一些实施例中，dCas多肽是dCas9多肽。在一些实施例中，无裂解酶及切口酶活性或dCas DNA结合多肽的RNA引导的DNA结合剂是Cas核酸酶的一种形式(例如，上文论述的Cas核酸酶)，其中其内核溶解活性位点因其催化结构域的一或多个变化(例如，点突变)不活化。例如，参见US 2014/0186958 A1;US 2015/0166980 A1。实例是dCas9氨基酸序列提供为SEQ ID NO:208。包括起始及终止密码子的实例性Cas9 mRNA ORF序列提供为SEQ ID NO:209。适于包括于融合蛋白中的实例性Cas9 mRNA编码序列提供为SEQ ID NO:212。

[0475] 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结构域(例如，是或包含融合多肽)。

[0476] 在一些实施例中，异源功能结构域可促进RNA引导的DNA结合剂转运至细胞核中。例如，异源功能结构域可为核定位信号(NLS)。在一些实施例中，RNA引导的DNA结合剂可与1-10个NLS融合。在一些实施例中，RNA引导的DNA结合剂可与1-5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可在RNA引导的DNA结合剂序列的N-末端或C-末端连接。在一些实施例中，RNA引导的DNA结合剂可与一个NLS在C-末端融合。NLS亦可插入RNA引导的DNA结合剂序列中。在其它实施例中，RNA引导的DNA结合剂可与一个以上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可为相同的(例如，两个SV40 NLS)或不同的。在一些实施例中，RNA引导的DNA结合剂与在羧基末端连接的两个SV40 NLS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，一个在N-末端连接且一个在C-末端连接。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可为单分序列，例如SV40 NLS、PKKKRKV(SEQ ID NO:274)或PKKKRRV(SEQ ID NO:275)。在一些实施例中，NLS可为二分序列，例如核胞浆素的NLS、KRPAATKKAGQAKKKK(SEQ ID NO:276)。在具体实施例中，单一PKKKRKV(SEQ ID NO:274)NLS可在RNA引导的DNA结合剂的C-末端连接。视情况在融合位点包括一或多个连接体。在一些实施例中，根据上述实施例的一或多个NLS与一或多个额外异源功能结构域(例如下文所述异源功能结构域中的任一种)组合存于RNA引导的DNA结合剂中。

[0477] 在一些实施例中，异源功能结构域可能能够修改RNA引导的DNA结合剂的细胞内半衰期。在一些实施例中，可增加RNA引导的DNA结合剂的半衰期。在一些实施例中，可减少RNA引导的DNA结合剂的半衰期。在一些实施例中，异源功能结构域可能能够增加RNA引导的DNA结合剂的稳定性。在一些实施例中，异源功能结构域可能能够降低RNA引导的DNA结合剂的稳定性。在一些实施例中，异源功能结构域可用作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(例如蛋白酶体、溶酶体蛋白酶或卡配因蛋白酶)介导。在一些实施例中，异源功能结构域可包含PEST序列。在一些实施例中，可藉由添加泛素或多泛素链来修饰RNA引导的DNA结合剂。在一些实施例中，泛素可为泛素样蛋白(UBL)。泛素样蛋白的

非限制性实例包括小泛素样修饰剂(SUMO)、泛素交叉反应蛋白(UCRP,亦称为干扰素刺激基因-15(ISG15))、泛素相关的修饰剂-1(URM1)、神经元-前体-细胞表达的发育上调的蛋白-8(NEDD8,在啤酒酵母(S.cerevisiae)中亦称为Rub1)、人类白血球抗原F相关(FAT10)、自体吞噬-8(ATG8)及自体吞噬-12(ATG12)、Fau泛素样蛋白(FUB1)、膜锚定UBL(MUB)、泛素倍数修饰剂-1(UFM1)及泛素样蛋白-5(UBL5)。

[0478] 在一些实施例中,异源功能结构域可为标记结构域。标记结构域的非限制性实例包括荧光蛋白、纯化标签、表位标识及报导基因序列。在一些实施例中,标记结构域可为荧光蛋白。适宜荧光蛋白的非限制性实例包括绿荧光蛋白(例如,GFP、GFP-2、tagGFP、turboGFP、sfGFP、EGFP、Emerald、Azami Green、Monomeric Azami Green、CopGFP、AceGFP、ZsGreen1)、黄荧光蛋白(例如,YFP、EYFP、Citrine、Venus、YPet、PhiYFP、ZsYellow1)、蓝荧光蛋白(例如,EBFP、EBFP2、Azurite、mKalama1、GFPuv、Sapphire、T-sapphire)、青荧光蛋白(例如,ECFP、Cerulean、CyPet、AmCyan1、Midoriishi-Cyan)、红荧光蛋白(例如,mKate、mKate2、mPlum、DsRed单体、mCherry、mRFP1、DsRed-Express、DsRed2、DsRed-单体、HcRed-Tandem、HcRed1、AsRed2、eqFP611、mRasberry、mStrawberry、Jred)及橙荧光蛋白(mOrange、mKO、Kusabira-Orange、Monomeric Kusabira-Orange、mTangerine、tdTomato)或任何其它适宜荧光蛋白。在其它实施例中,标记结构域可为纯化标签和/或表位标识。非限制性实例性标签包括谷胱甘肽-S-转移酶(GST)、几丁质结合蛋白(CBP)、麦芽糖结合蛋白(MBP)、硫氧还蛋白(TRX)、聚(NANP)、串联亲和性纯化(TAP)标签、myc、AcV5、AU1、AU5、E、ECS、E2、FLAG、HA、nus、Softag 1、Softag 3、Strep、SBP、Glu-Glu、HSV、KT3、S、S1、T7、V5、VSV-G、6xHis、8xHis、生物素羧基载体蛋白(BCCP)、聚-His及携钙蛋白。非限制性实例性报导基因包括谷胱甘肽-S-转移酶(GST)、辣根过氧化物酶(HRP)、氯霉素乙酰基转移酶(CAT)、β-半乳糖苷酶、β-葡萄糖醛酸苷酶、荧光素酶或荧光蛋白。

[0479] 在额外实施例中,异源功能结构域可将RNA引导的DNA结合剂靶向特定细胞器、细胞类型、组织或器官。在一些实施例中,异源功能结构域可将RNA引导的DNA结合剂靶向线粒体。

[0480] 在其它实施例中,异源功能结构域可为效应结构域。当RNA引导的DNA结合剂引导至其靶序列时,例如当Cas核酸酶由gRNA引导至靶序列时,效应结构域可修饰或影响靶序列。在一些实施例中,效应结构域可选自核酸结合结构域、核酸酶结构域(例如,非Cas核酸酶结构域)、后生修饰结构域、转录活化结构域或转录阻遏结构域。在一些实施例中,异源功能结构域是核酸酶,例如FokI核酸酶。参见,例如,美国专利第9,023,649号。在一些实施例中,异源功能结构域是转录活化剂或阻遏剂。参见(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.Methods 10:973-6(2013);Mali等人,"CAS9 transcriptional 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结合剂基本上变成转录因子,其可使用引导RNA引导至结合期望靶序列。

[0481] D.gRNA的效能的测定

[0482] 在一些实施例中，当与形成RNP的其它组分一起递送或表达时，测定gRNA的效能。在一些实施例中，gRNA与RNA引导的DNA核酸酶(例如Cas蛋白)一起表达。在一些实施例中，gRNA递送至细胞系或在细胞系中表达，该细胞系已经稳定地表达RNA引导的DNA核酸酶(例如Cas蛋白)。在一些实施例中，gRNA作为RNP的一部分递送至细胞。在一些实施例中，将gRNA与编码RNA引导的DNA核酸酶(例如Cas核酸酶)的mRNA一起递送至细胞。

[0483] 如本文所述，使用本文揭示的RNA引导的DNA核酸酶及引导RNA可导致DNA中的双链断裂，在藉由细胞机制修复时，其可以插入/缺失(插入或缺失)突变的形式产生误差。由于插入或缺失导致的许多突变改变阅读框或引入过早终止密码子，且因此产生非功能性蛋白。

[0484] 在一些实施例中，基于活体外模型测定特定gRNA的效能。在一些实施例中，活体外模型是稳定表达Cas9的HEK293细胞(HEK293_Cas9)。在一些实施例中，活体外模型是HUH7人类肝癌细胞。在一些实施例中，活体外模型是HepG2细胞。在一些实施例中，活体外模型是原代人类肝细胞。在一些实施例中，活体外模型是原代食蟹猴肝细胞。关于使用原代人类肝细胞，可使用市售原代人类肝细胞以提供实验之间更大一致性。在一些实施例中，藉由(例如)分析经Cas9 mRNA及引导RNA活体外转染的原代人类肝细胞的基因组DNA测定在活体外模型中(例如，在原代人类肝细胞中)发生缺失或插入的脱靶位点的数目。在一些实施例中，该测定包含分析经Cas9 mRNA、引导RNA及供体寡核苷酸活体外转染的原代人类肝细胞的基因组DNA。该测定的实例性程序提供于以下工作实例中。

[0485] 在一些实施例中，针对gRNA选择过程，跨多个活体外细胞模型测定特定gRNA的效能。在一些实施例中，实施具有所选gRNA的数据的细胞系比较。在一些实施例中，实施多个细胞模型的交叉筛选。

[0486] 在一些实施例中，基于活体内模型测定特定gRNA的效能。在一些实施例中，活体内模型是啮齿动物模型。在一些实施例中，啮齿动物模型是表达人类TTR基因(其可为突变人类TTR基因)的小鼠。在一些实施例中，活体内模型是非人类灵长类动物，例如食蟹猴。

[0487] 在一些实施例中，藉由TTR的编辑百分比测量引导RNA的效能。在一个实施例中，将TTR的编辑百分比与(例如)在活体外模型的情形下在细胞培养基中或在活体内模型的情形下在血清或组织中实现TTR蛋白的减弱所必需的编辑百分比进行比较。

[0488] 在一些实施例中，藉由在靶细胞类型的基因组内的脱靶序列处的插入或缺失的数目和/或频率测量引导RNA的效能。在一些实施例中，提供有效引导RNA，其在细胞群体中和/或相对于靶位点处插入或缺失产生的频率以极低频率(例如，＜5％)在脱靶位点处产生插入或缺失。因此，本揭示内容提供引导RNA，其在靶细胞类型(例如，肝细胞)中不展现脱靶插入或缺失形成，或在细胞群体中和/或相对于靶位点处插入或缺失产生的频率产生＜5％的脱靶插入或缺失频率。在一些实施例中，本揭示内容提供引导RNA，其在靶细胞类型(例如，肝细胞)中不展现任何脱靶插入或缺失形成。在一些实施例中，提供引导RNA，其在小于5个脱靶位点产生插入或缺失，例如如藉由本文所述的一或多种方法所评估。在一些实施例中，提供引导RNA，其在小于或等于4、3、2或1个脱靶位点产生插入或缺失，例如如藉由本文所述的一或多种方法所评估。在一些实施例中，脱靶位点不出现在靶细胞(例如，肝细胞)基因组中的蛋白质编码区中。

[0489] 在一些实施例中，检测基因编辑事件，例如在靶DNA中形成插入/缺失(“插入或缺失”)突变及同源性定向修复(HDR)事件利用带有标记引物的线性扩增且分离标记的扩增产物(下文中称为“LAM-PCR”或“线性扩增(LA)”方法)。

[0490] 在一些实施例中，该方法包含自细胞分离细胞DNA，该细胞已经诱导以具有双链断裂(DSB)且视情况其已提供有HDR模板以修复DSB；实施带有标记的引物的DNA的线性扩增的至少一个循环；分离包含标签的线性扩增产物，从而丢弃用未标记的引物扩增的任何扩增产物；视情况进一步扩增分离的产物；及分析线性扩增产物或进一步扩增的产物，以确定存在或不存在编辑事件，例如靶DNA中的双链断裂、插入、缺失或HDR模板序列。在一些情况下，编辑事件可量化。如本文所用的量化及诸如此类(包括在HDR及非HDR编辑事件(例如插入或缺失)的上下文中)包括检测群体中的编辑事件的频率和/或类型。

[0491] 在一些实施例中，仅执行一个线性扩增循环。

[0492] 在一些情况下，标记的引物包含分子条形码。在一些实施例中，标记的引物包含分子条形码，且仅执行一个线性扩增循环。

[0493] 在一些实施例中，分析步骤包含对线性扩增产物或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，包括将线性扩增产物或进一步扩增的产物次世代测序及克隆至质粒中并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进一步扩增的产物实施数字PCR(dPCR)或液滴数字PCR(ddPCR)。在其它情况下，分析步骤包含使线性扩增产物或进一步扩增的产物与经设计以鉴别包含HDR模板序列的DNA的核酸探针接触并检测与线性扩增产物或进一步扩增的产物结合的探针。在一些实施例中，该方法进一步包含确定靶DNA中HDR模板的位置。

[0494] 在某些实施例中，该方法进一步包括测定靶DNA中插入位点的序列，其中插入位点是HDR模板纳入靶DNA中的位置，且其中插入位点可包括一些靶DNA序列及一些HDR模板序列。

[0495] 在一些实施例中，实施带有标记的引物的靶DNA的线性扩增1-50个循环、1-60个循环、1-70个循环、1-80个循环、1-90个循环或1-100个循环。

[0496] 在一些实施例中，带有标记的因子的靶DNA的线性扩增包含变性步骤以分离DNA双链体、退火步骤以容许因子结合，及伸长步骤。在一些实施例中，线性扩增是等温的(不需要温度改变)。在一些实施例中，等温线性扩增是环介导等温扩增(LAMP)、链置换扩增(SDA)、解旋酶依赖性扩增或切口酶扩增反应。

[0497] 在一些实施例中，标记的引物退火至靶DNA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个核苷酸远离预计编辑事件位置(例如，插入、缺失或模板插入位点)。

[0498] 在一些实施例中，标记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。

[0499] 在一些实施例中，引物上的标签是生物素、链霉抗生物素蛋白、长叶毛地黄配质、DNA序列或荧光黄异硫氰酸盐(FITC)。

[0500] 在一些实施例中，使用对引物上的标签具有特异性的捕获试剂分离线性扩增产

物。在一些实施例中，捕获试剂是在珠粒、固体支持物、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获试剂接触。在一些实施例中，捕获试剂是生物素、链霉抗生物素蛋白、长叶毛地黄配质、DNA序列或荧光黄异硫氰酸盐(FITC)。

[0501] 在一些实施例中，标签是生物素，且捕获试剂是链霉抗生物素蛋白。在一些实施例中，标签是链霉抗生物素蛋白，且捕获试剂是生物素。在一些实施例中，标签是在引物的5'末端、引物的3'末端或引物内部。在一些实施例中，在分离步骤之后移除标签和/或捕获试剂。在一些实施例中，不移除标签和/或捕获试剂，且在标签和/或捕获存在下实施进一步扩增及分析步骤。

[0502] 在一些实施例中，进一步扩增是非线性的。在一些实施例中，进一步扩增是数位PCR、qPCR或RT-PCR。在一些实施例中，测序是次世代测序(NGS)。

[0503] 在一些实施例中，靶DNA是基因组或线粒体。在一些实施例中，靶DNA是原核或真核细胞的基因组DNA。在一些实施例中，靶DNA是哺乳动物。靶DNA可来自非分裂细胞或分裂细胞。在一些实施例中，靶DNA可来自原代细胞。在一些实施例中，靶DNA来自复制细胞。

[0504] 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，剪切的DNA的平均大小在0.5kb与20kb之间。在一些情况下，将细胞DNA剪切至平均大小为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。在一些情况下，将细胞DNA剪切至平均大小为约1.5kb。

[0505] 在一些实施例中，藉由TTR的分泌测量引导RNA的效能。在一些实施例中，使用利用细胞培养基或血清的酶联免疫吸附分析(ELISA)分析测量TTR的分泌。在一些实施例中，在用于测量编辑的相同活体或活体内系统或模型中测量TTR的分泌。在一些实施例中，在原代人类肝细胞中测量TTR的分泌。在一些实施例中，在HUH7细胞中测量TTR的分泌。在一些实施例中，在HepG2细胞中测量TTR的分泌。

[0506] ELISA分析法通常为熟习此项技术者所知，且可设计用于测定血清TTR含量。在一个实例性实施例中，收集血液并分离血清。可使用小鼠前白蛋白(甲状腺素运载蛋白)ELISA试剂盒(Aviva Systems Biology，目录OKIA00111)或用于测量人类TTR的类似试剂盒测定总TTR血清含量。若没有试剂盒可用，则可使用预先涂布有特定针对所测量TTR的捕获抗体的分析盘发展成ELISA。随后在洗涤之前，先让分析盘于室温下培育一段时间。添加酶-抗TTR抗体结合物并培育。移除未结合的抗体结合物，并在添加与酶反应的产色底物溶液之前先洗涤分析盘。在适当分析盘读数仪上，在针对所用酶及底物特有的吸光度下读取分析盘。

[0507] 在一些实施例中，由细胞(包括这些来自组织的细胞)中TTR的量测量gRNA的效能。在一些实施例中，使用西方墨点测量细胞中TTR的量。在一些实施例中，所用细胞是HUH7细胞。在一些实施例中，所用细胞是原代人类肝细胞。在一些实施例中，所用细胞是自动物获得的原代细胞。在一些实施例中，将TTR的量与甘油醛3-磷酸酯去氢酶GAPDH(管家基因)的量进行比较，以控制细胞数的变化。

[0508] III.LNP调配物及ATTR的治疗

[0509] 在一些实施例中,提供一种诱导TTR基因内的双链断裂(DSB)的方法,其包括施用一种组合物,其包含含有SEQ ID No:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的引导RNA。在一些实施例中,施用包含SEQ ID No:5-82中任一项或多项引导序列的gRNA,以诱导TTR基因中的DSB。引导RNA可与RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))或编码RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))的mRNA或载体一起施用。

[0510] 在一些实施例中,提供一种修饰TTR基因的方法,其包括施用一种组合物,其包含含有SEQ ID No:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的引导RNA。在一些实施例中,施用包含SEQ ID No:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的gRNA,以修饰TTR基因。引导RNA可与RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))或编码RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))的mRNA或载体一起施用。

[0511] 在一些实施例中,提供治疗ATTR的方法,其包括施用包含含有SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的引导RNA的组合物。在一些实施例中,施用包含SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的gRNA以治疗ATTR。引导RNA可与RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))或编码RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))的mRNA或载体一起施用。

[0512] 在一些实施例中,提供减少TTR血清浓度的方法,其包含施用包含含有SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的引导RNA的组合物。在一些实施例中,施用包含SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的gRNA以减少或预防TTR在淀粉样蛋白或淀粉样原纤维中累积。gRNA可与RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))或编码RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))的mRNA或载体一起施用。

[0513] 在一些实施例中,提供减少或预防TTR在个体的淀粉样蛋白或淀粉样原纤维中的累积的方法,其包含施用包含含有SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的引导RNA的组合物。在一些实施例中,提供减少或预防TTR在个体的淀粉样蛋白或淀粉样原纤维中累积的方法,其包含施用包含SEQ ID No:87-113中任一项或多项sgRNA的组合物。在一些实施例中,施用包含SEQ ID NO:5-82中任一项或多项引导序列或SEQ ID No:87-124中任一项或多项sgRNA的gRNA以减少或预防TTR在淀粉样蛋白或淀粉样原纤维中的累积。gRNA可与RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))或编码RNA引导的DNA核酸酶(例如Cas核酸酶(例如Cas9))的mRNA或载体一起施用。

[0514] 在一些实施例中,包含表1的引导序列的gRNA或来自表2的一或多个sgRNA与RNA引导的DNA核酸酶(例如Cas核酸酶)一起诱导DSB,且修复期间的非同源末端接合(NHEJ)导致TTR基因突变。在一些实施例中,NHEJ导致核苷酸的缺失或插入,其诱导TTR基因中的框移或无义突变。

[0515] 在一些实施例中,施用本发明的引导RNA(例如,在本文提供的组合物中)降低个体中TTR的含量(例如,血清含量),且因此防止TTR在淀粉样蛋白或淀粉样原纤维中的累积及

聚集。

[0516] 在一些实施例中，减少或预防TTR在个体的淀粉样蛋白或淀粉样原纤维中的累积包含减少或预防个体的一或多个组织(例如胃、结肠或神经组织)中的TTR沉积。在一些实施例中，神经组织包含坐骨神经或背根神经节。在一些实施例中，在胃、结肠、背根神经节及坐骨神经中的两项、三项或四项中，TTR沉积减少。可使用生检样本、例如使用免疫染色测定给定组织中的沉积程度。在一些实施例中，减少或预防TTR在个体的淀粉样蛋白或淀粉样原纤维中的累积和/或减少或预防TTR沉积是基于降低血清TTR含量达一段时间来推断。如实例中所论述，已发现，根据本文提供的方法及用途的减少血清TTR含量可导致自组织(例如上文及实例中论述的这些)清除沉积的TTR，例如如施用组合物后8周所测量。

[0517] 在一些实施例中，个体是哺乳动物。在一些实施例中，个体是人类。在一些实施例中，个体是牛、猪、猴、羊、狗、猫、鱼或家禽。

[0518] 在一些实施例中，提供包含表1中任一项或多项引导序列或表2的一或多种sgRNA的引导RNA(例如，在本文提供的组合物中)的用途，其用于制备用于治疗患有ATTR的人类个体的药剂。

[0519] 在一些实施例中，静脉内施用引导RNA、组合物及调配物。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。

[0520] 在一些实施例中，包含本文提供的引导RNA的组合物的单一施用足以敲低突变蛋白的表达。在一些实施例中，包含本文提供的引导RNA的组合物的单一施用足以剔除细胞群体中突变蛋白的表达。在其它实施例中，包含本文提供的引导RNA的组合物的一次以上施用可经由累积效应最大化编辑。例如，本文提供的组合物可施用2、3、4、5或更多次，例如2次。施用可分开以下范围内的时间段：例如1天至2年，例如1至7天、7至14天、14天至30天、30天至60天、60天至120天、120天至183天、183天至274天、274天至366天或366天至2年。

[0521] 在一些实施例中，组合物是以如下范围内的有效量施用：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，或0.1、0.2、0.3、0.5、1、2、3、5或10mpk。

[0522] 在一些实施例中，在递送后1年、2年、3年、4年、5年或10年观察到本发明的组合物的治疗效能。在一些实施例中，藉由测量治疗之前及之后TTR的血清含量来评估本发明的组合物的治疗效能。在1周、2周、3周、4周、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月时或在11个月时观察到经由TTR的血清含量降低评估的组合物的治疗效能。

[0523] 在一些实施例中，治疗减慢或停止疾病进展。

[0524] 在一些实施例中，治疗减慢或停止FAP的进展。在一些实施例中，治疗导致感觉运动神经病变或自主神经病变的症状改良、稳定或减慢其变化。

[0525] 在一些实施例中，治疗导致FAC的症状改良、稳定或减慢其变化。在一些实施例中，治疗导致限制性心肌病或郁血性心脏衰竭的症状改良、稳定或减慢其变化。

[0526] 在一些实施例中，藉由个体增加的存活时间来测量治疗效能。

[0527] 在一些实施例中，藉由感觉动作或自主神经病变的症状的改良或减慢其进展来测量治疗效能。在一些实施例中，藉由移动身体区域或在身体任何区域中有感觉的能力的增加或减慢该能力降低来测量治疗效能。在一些实施例中，藉由吞咽、呼吸、使用手臂、手、腿或脚或走路的能力的改良或减慢该能力降低来测量治疗效能。在一些实施例中，藉由神经

痛的改良或减慢其进展来测量治疗效能。在一些实施例中，神经痛的特征在于疼痛、灼热、麻刺感或异常感觉。在一些实施例中，藉由体位性低血压、眩晕、胃肠运动障碍、膀胱功能障碍或性功能障碍的改良或减慢其增加来测量治疗效能。在一些实施例中，藉由虚弱的改良或减慢其进展来测量治疗效能。在一些实施例中，使用肌电图、神经传导测试或患者报告的结果来测量治疗效能。

[0528] 在一些实施例中，藉由郁血性心脏衰竭或CHF的改良或减慢其进展来测量治疗效能。在一些实施例中，藉由呼吸短促、呼吸困难、疲劳或踝、足、腿、腹部或颈部静脉肿胀的减少或减慢其增加来测量治疗效能。在一些实施例中，藉由体内流体积聚的改良或减慢其进展来测量治疗效能，该体内流体积聚可藉由诸如增重、尿频或夜间咳嗽等量度来评估。在一些实施例中，使用心脏生物标记测试(例如B型利钠肽[BNP]或N-末端前b型利钠肽[NT-proBNP])、肺功能测试、胸部x射线或心电图来测量治疗效能。

[0529] A.组合疗法

[0530] 在一些实施例中，本发明包含组合疗法，该组合疗法包含含有表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)以及适于缓和ATTR的症状的额外疗法。

[0531] 在一些实施例中，ATTR的额外疗法是感觉动作或自主神经病变的治疗。在一些实施例中，感觉动作或自主神经病变的治疗是非类固醇消炎药、抗抑郁药、抗痉挛药、抗心律失常药或麻醉剂。在一些实施例中，抗抑郁药是三环剂或血清素-去甲肾上腺素再摄取抑制剂。在一些实施例中，抗抑郁药是阿米替林(amitriptyline)、度洛西汀(duloxetine)或万拉法辛(venlafaxine)。在一些实施例中，抗痉挛剂是加巴喷丁(gabapentin)、普瑞巴林(pregabalin)、托吡酯(topiramate)或卡巴马平(carbamazepine)。在一些实施例中，感觉运动神经病变的额外疗法是透皮电神经刺激。

[0532] 在一些实施例中，ATTR的额外疗法是限制性心肌病或郁血性心脏衰竭(CHF)的治疗。在一些实施例中，CHF的治疗是ACE抑制剂、醛固酮拮抗剂、血管收缩肽受体阻断剂、β阻断剂、地高辛(digoxin)、利尿剂或硝酸异山梨酯/盐酸肼苯哒嗪。在一些实施例中，ACE抑制剂是依那普利(enalapril)、卡托普利(captopril)、雷米普利(ramipril)、培哚普利(perindopril)、咪达普利(imidapril)或喹那普利(quinapril)。在一些实施例中，醛固酮拮抗剂是依普利酮(eplerenone)或螺内酯(spironolactone)。在一些实施例中，血管收缩肽受体阻断剂是阿齐沙坦(azilsartan)、坎地沙坦(cadesartan)、依普罗沙坦(eprosartan)、厄贝沙坦(irbesartan)、氯沙坦(losartan)、奥美沙坦(olmesartan)、替米沙坦(telmisartan)或缬沙坦(valsartan)。在一些实施例中，β阻断剂是醋丁洛尔(acebutolol)、阿替洛尔(atenolol)、比索洛尔(bisoprolol)、美托洛尔(metoprolol)、纳多洛尔(nadolol)、奈必洛尔(nebivolol)或普萘洛尔(propranolol)。在一些实施例中，利尿剂是氯噻嗪(chlorothiazide)、氯噻酮(chlorthalidone)、氢氯噻嗪(hydrochlorothiazide)、吲达帕胺(indapamide)、美托拉宗(metolazone)、布美他尼(bumetanide)、呋塞米(furosemide)、托塞米(torsemide)、阿米洛利(amiloride)或胺苯蝶啶(triameterene)。

[0533] 在一些实施例中，组合疗法包含含有表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)以及靶向TTR或突变TTR的

siRNA。在一些实施例中，siRNA是能够进一步减少或消除野生型或突变TTR表达的任何siRNA。在一些实施例中，siRNA是药物帕替司兰(Patisiran)(ALN-TTR02)或ALN-TTRsc02。在一些实施例中，在包含表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)之后施用siRNA。在一些实施例中，在用本文提供的任何gRNA组合物治疗后定期施用siRNA。

[0534] 在一些实施例中，组合疗法包含含有表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)以及靶向TTR或突变TTR的反义核苷酸。在一些实施例中，反义核苷酸是任何能够进一步减少或消除野生型或突变TTR的表达的反义核苷酸。在一些实施例中，反义核苷酸是药物Inotersen(IONS-TTR_{Rx})。在一些实施例中，在包含表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)之后施用反义核苷酸。在一些实施例中，在用本文提供的任何gRNA组合物治疗后定期施用反义核苷酸。

[0535] 在一些实施例中，组合疗法包含含有表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)以及促进TTR的正确折叠的四聚体形式的动力学稳定的小分子稳定剂。在一些实施例中，小分子稳定剂是药物他发米帝司(tafamidis)(Vyndaqel®)或二氟尼柳(diflunisal)。在一些实施例中，在包含表1中揭示的任一项或多项引导序列或表2中任一项或多项sgRNA的任一gRNA(例如，在本文提供的组合物中)之后施用小分子稳定剂。在一些实施例中，在用本文提供的任何gRNA组合物治疗后定期施用小分子稳定剂。

[0536] B.gRNA组合物的递送

[0537] 在一些实施例中，单独或在一或多个载体上编码的本文所述引导RNA组合物调配于脂质纳米颗粒中或经由脂质纳米颗粒施用；参见(例如)于2017年3月30日提出申请的标题为“LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS”的PCT/US2017/024973，其全部内容以引用方式并入本文中。熟习此项技术者已知能将核苷酸递送至个体的任何脂质纳米颗粒(LNP)可与本文所述引导RNA以及编码RNA引导的DNA核酸酶(例如Cas或Cas9)或RNA引导的DNA核酸酶(例如Cas或Cas9蛋白自身)的mRNA一起使用。

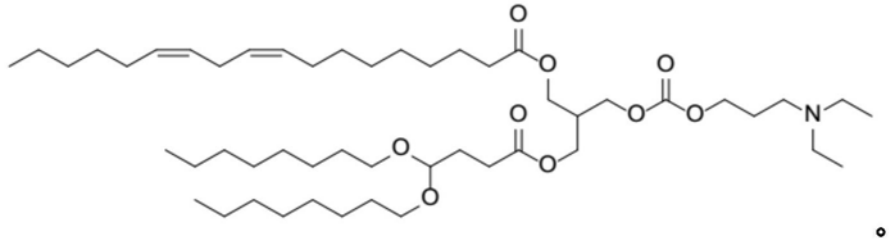
[0538] 本文揭示RNA的LNP调配物的各个实施例，包括CRISPR/Cas运送子。该LNP调配物可包括(i)CCD脂质，例如胺脂质，(ii)中性脂质，(iii)辅助脂质，及(iv)隐密脂质，例如PEG脂质。LNP调配物的一些实施例包括“胺脂质”，以及辅助脂质、中性脂质及隐密脂质(例如PEG脂质)。“脂质纳米颗粒”意指包含复数个(即一个以上)藉由分子间力彼此物理缔合的脂质分子的粒子。

[0539] CCD脂质

[0540] 用于递送CRISPR/Cas mRNA及引导RNA组分至肝细胞的脂质组合物包含CCD脂质。

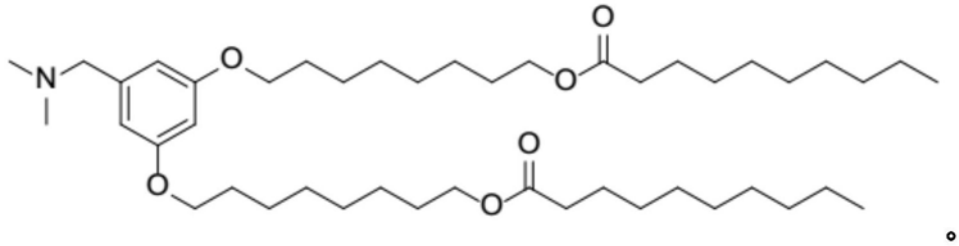
[0541] 在一些实施例中，CCD脂质是脂质A，其是(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛基氧基)丁酰基)氧基)-2-((((3-(二乙基胺基)丙氧基)羰基)氧基)甲基)丙基酯((9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate)，亦称作(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛基氧基)丁酰基)氧基)-2-((((3-(二乙基胺基)丙氧基)羰基)氧基)甲基)丙基酯(3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)

carbonyl)oxy)methyl)propyl(9Z,12Z)-octadeca-9,12-dienoate)。脂质A可绘示为：

[0542] 

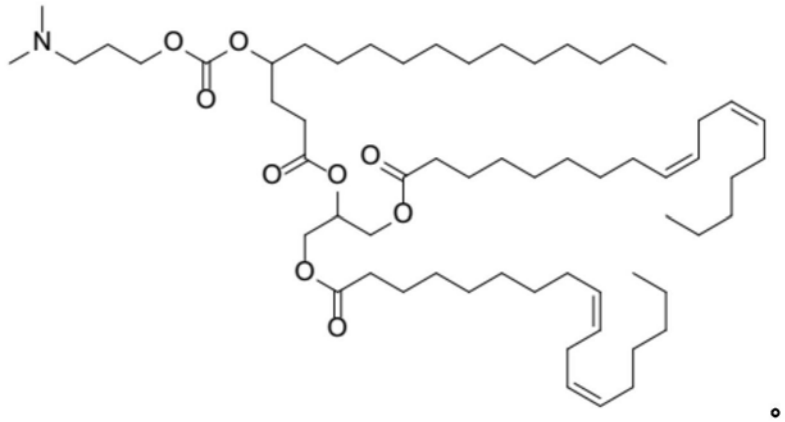
[0543] 脂质A可根据WO2015/095340(例如,第84-86页)合成。

[0544] 在一些实施例中,CCD脂质是脂质B,其是双(癸酸)((5-((二甲基胺基)甲基)-1,3-伸苯基)双(氧基))双(辛烷-8,1-二基)酯(((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate)),亦称作双(癸酸)((5-((二甲基胺基)甲基)-1,3-伸苯基)双(氧基))双(辛烷-8,1-二基)酯(((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate))。脂质B可绘示为：

[0545] 

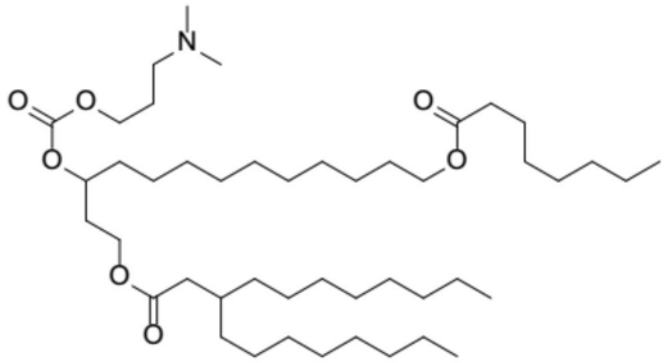
[0546] 脂质B可根据WO2014/136086(例如,第107-09页)合成。

[0547] 在一些实施例中,CCD脂质是脂质C,其是(9Z,9′Z,12Z,12′Z)-双(十八-9,12-二烯酸)2-((4-(((3-(二甲基胺基)丙氧基)羰基)氧基)十六酰基)氧基)丙烷-1,3-二基酯。脂质C可绘示为：

[0548] 

[0549] 在一些实施例中,CCD脂质是脂质D,其是3-辛基十一酸3-(((3-(二甲基胺基)丙氧基)羰基)氧基)-13-(辛酰基氧基)十三烷基酯。

[0550] 脂质D可绘示为：

[0551] 

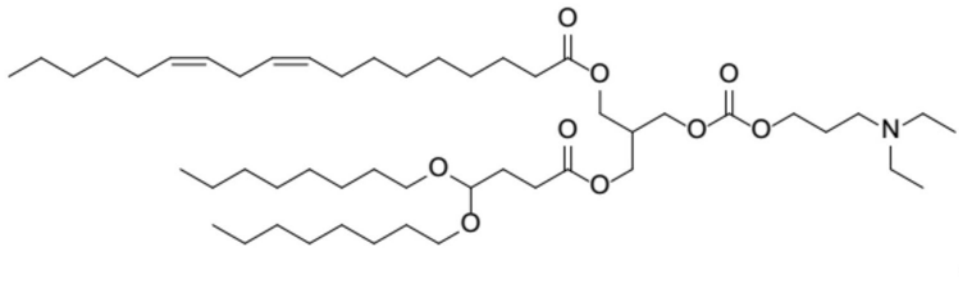
[0552] 脂质C及脂质D可根据WO2015/095340合成。

[0553] 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脂质是脂质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。

[0554] 胺脂质

[0555] 在一些实施例中，用于递送生物活性剂的LNP组合物包含“胺脂质”，其定义为脂质A、脂质B、脂质C、脂质D或脂质A的等效物(包括脂质A的缩醛类似物)、脂质B的等效物、脂质C的等效物及脂质D的等效物。

[0556] 在一些实施例中，胺脂质是脂质A，其是(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛基氧基)丁酰基)氧基)-2-((((3-(二乙基胺基)丙氧基)羰基)氧基)甲基)丙基酯((9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate)，亦称作(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛基氧基)丁酰基)氧基)-2-((((3-(二乙基胺基)丙氧基)羰基)氧基)甲基)丙基酯(3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl(9Z,12Z)-octadeca-9,12-dienoate)。脂质A可绘示为：

[0557] 

[0558] 脂质A可根据WO2015/095340(例如第84-86页)合成。在某些实施例中，胺脂质是脂质A的等效物。

[0559] 在某些实施例中，胺脂质是脂质A的类似物。在某些实施例中，脂质A类似物是脂质A的缩醛类似物。在特定LNP组合物中，缩醛类似物是C4-C12缩醛类似物。在一些实施例中，缩醛类似物是C5-C12缩醛类似物。在额外实施例中，缩醛类似物是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。

[0560] 适用于本文所述LNP的胺脂质在活体内生物可降解。胺脂质具有低毒性(例如，在大于或等于10mg/kg的量下，在动物模型中耐受，而无不良效应)。在某些实施例中，包含胺脂质的LNP包括其中在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％的胺脂质的这些。在某些实施例中，包含胺脂质的LNP包括其中在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的这些。在某些实施例中，包含胺脂质的LNP包括其中(例如)藉由测量脂质(例如胺脂质)、RNA(例如mRNA)或其它组分在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的这些。在某些实施例中，测量LNP的脂质囊封对游离脂质、RNA或核酸组分。

[0561] 可如文献中所述测量脂质清除率。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，向六至八周龄的雄性C57Bl/6小鼠经由侧尾静脉藉由静脉内浓注注射施用0.3mg/kg的含有靶向荧光素酶的siRNA的LNP-siRNA系统。在投药后0.083、0.25、0.5、1、2、4、8、24、48、96及168小时收集血液、肝及脾样本。在组织收集之前用盐水灌注小鼠并处理血液样本以获得血浆。所有样本皆经处理且藉由LC-MS分析。此外，Maier阐述在施用LNP-siRNA调配物后评估毒性的程序。例如，经由单次静脉内浓注注射以5mL/kg的剂量体积向雄性Sprague-Dawley大鼠以0、1、3、5及10mg/kg(5只动物/组)施用靶向荧光素酶的siRNA。24小时后，自有意识动物的颈静脉获得约1mL血液，且分离血清。在投药后72小时，对所有动物进行安全死进行验尸。评估临床体征、体重、血清化学、器官重量及组织病理学。尽管Maier阐述用于评估siRNA-LNP调配物的方法，但该方法可应用于评估施用本发明的LNP组合物的清除率、药物动力学及毒性。

[0562] 胺脂质导致清除率增加。在一些实施例中，清除率是脂质清除率，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，清除率是RNA清除率，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，清除率是自血液、血清或血浆清除LNP的速率。在一些实施例中，清除率是自组织(例如肝组织或脾组织)清除LNP的速率。在某些实施例中，高清除率导致安全性概况而无显著不良效应。胺脂质减少循环及组织中的LNP累积。在一些实施例中，循环及组织中LNP累积的减少导致安全性概况，而无显著不良效应。

[0563] 本发明的胺脂质可为可电离的，此取决于其所处的介质的pH。例如，在微酸性介质中，胺脂质可经质子化并因此带正电荷。相反，在略微碱性的介质(例如pH约为7.35的血液)中，胺脂质可未经质子化，且因此不带电荷。在一些实施例中，本发明的胺脂质可在至少约9的pH下质子化。在一些实施例中，本发明的胺脂质可在至少约10的pH下质子化。

[0564] 胺脂质带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约5.8至约6.2的范围内的pKa。例如，本发明的胺脂质可各自独立地具有约5.8至约6.5的范围内的pKa。此可为有利的，此乃因已发现，具有约5.1至约7.4范围的pKa的阳离子脂质对于将运送子活体内递送(例如)肝是有效的。此外，已发现，具有约5.3至约6.4范围内的pKa的阳离子脂质对于活体内递送至(例如)肿瘤是有效的。例如，参见WO2014/136086。

[0565] 额外脂质

[0566] 适用于本揭示内容的脂质组合物中的“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)5-十七烷基苯-1，3-二醇(间苯二酚)、二棕榈酰基磷脂酰胆碱(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)、二肉豆蔻酰基磷脂酰胆碱(DMPC)、1-肉豆蔻酰基-2-棕榈酰基磷脂酰胆碱(MPPC)、1-棕榈酰基-2-肉豆蔻酰基磷脂酰胆碱(PMPC)、1-棕榈酰基-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-棕榈酰基磷脂酰胆碱(SPPC)、1,2-二(二十烯酰基)-sn-甘油-3-磷酸胆碱(DEPC)、棕榈酰基油酰磷脂酰胆碱(POPC)、溶血磷脂酰胆碱、二油酰磷脂酰乙醇胺(DOPE)、二亚麻油酰氧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)、二棕榈酰基磷脂酰乙醇胺(DPPE)、棕榈酰基油酰磷脂酰乙醇胺(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)组成的群。在另一实施例中,天然磷脂可为二硬脂酰基磷酯酰胆碱(DSPC)。

[0567] “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、5-十七烷基间苯二酚及半琥珀酸胆固醇酯。在一个实施例中,辅助脂质可为胆固醇。在一个实施例中,辅助脂质可为半琥珀酸胆固醇酯。

[0568] “隐密脂质”是改变纳米颗粒可于活体内(例如在血液中)存在的时间长度的脂质。隐密脂质可藉由(例如)减少粒子聚集及控制粒径有助于调配过程。本文所用隐密脂质可调节LNP的药物动力学性质。适用于本揭示内容的脂质组合物中的隐密脂质包括(但不限于)具有连接至脂质部分的亲水头基的隐密脂质。适用于本发明的脂质组合物的隐密脂质及关于该脂质的生物化学的信息可参见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。额外适宜PEG脂质揭示于(例如)WO 2006/007712中。

[0569] 在一个实施例中,隐密脂质的亲水首基包含选自基于PEG的聚合物的聚合物部分。隐密脂质可包含脂质部分。在一些实施例中,隐密脂质是PEG脂质。

[0570] 在一个实施例中,适宜隐密脂质包含选自以下的聚合物部分:基于PEG(有时称为聚(环氧乙烷)的聚合物、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-羟基丙基)甲基丙烯酰胺]。

[0571] 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。

[0572] 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咪唑双酰胺,包括包含具有独立地包含约C4至约C40饱和或不饱和碳原子的烷基链长度的二烷基甘油或二烷基咪唑双酰胺基团的这些,其中链可包含一或多个官能基,例如酰胺或酯。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团可进一步包含一或多个经取代烷基。链长度可为对称的或不对称的。

[0573] 除非另外指明,否则如本文所使用的术语“PEG”意指任何聚乙二醇或其它聚伸烷基醚聚合物。在一个实施例中,PEG是乙二醇或环氧乙烷的视情况经取代的直链或具支链聚合物。在一个实施例中,PEG未经取代。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,该术语包括诸如PEG-聚胺基甲酸酯或PEG-聚丙烯等PEG共聚物(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,在子实施例中约150至约30,000,在子实施例中约150至约20,000,在子实施例中约150至约15,000,在子实施例中约150至约10,000,在子实施例中约150至约6,000,在子实施例中约150至约5,000,在子实施例中约150至约4,000,在子实施例中约150至约3,000,在子实施例中约300至约3,000,在子实施例中约1,000至约3,000,且在子实施例中约1,500至约2,500。

[0574] 在某些实施例中,PEG(例如,结合至脂质部分或脂质,例如隐密脂质)是“PEG-2K”,亦称为“PEG 2000”,其平均分子量为约2,000道尔顿。PEG-2K在本文中由下式(I)表示,其中n为45,此意味着数量平均聚合度包含约45个亚单位。然而,可使用业内已知的其它PEG实施例,包括例如数量平均聚合度包含约23个亚单位(n=23)和/或68个亚单位(n=68)的这些。在一些实施例中,n可在约30至约60的范围内。在一些实施例中,n可在约35至约55的范围内。在一些实施例中,n可在约40至约50的范围内。在一些实施例中,n可在约42至约48的范围内。在一些实施例中,n可为45。在一些实施例中,R可选自H、经取代烷基及未经取代烷基。在一些实施例中,R可为未经取代烷基。在一些实施例中,R可为甲基。

[0575] 在本文所述实施例中的任一个中,PEG脂质可选自PEG-二月桂酰基甘油、PEG-二肉豆蔻酰基甘油(PEG-DMG)(目录号GM-020,来自NOF,Tokyo,Japan)、PEG-二棕榈酰基甘油、PEG-二硬脂酰基甘油(PEG-DSPE)(目录号DSPE-020CN,NOF,Tokyo,Japan)、PEG-二月桂基咪唑双酰胺、PEG-二肉豆蔻基咪唑双酰胺、PEG-二棕榈酰基咪唑双酰胺、及PEG-二硬脂酰基咪唑双酰胺、PEG-胆固醇(1-[8′-(胆固-5-烯-3[β]-氧基)甲酰胺基-3′,6′-二氧杂辛烷基]胺甲酰基-[ω]-甲基-聚(乙二醇)、PEG-DMB(3,4-二-十四氧基-[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Avanti Polar Lipids,Alabaster,Alabama,USA)、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Avanti Polar Lipids,Alabaster,Alabama,USA)、1,2-二硬脂酰基-sn-甘油甲氧基聚乙二醇(PEG2k-DSG;GS-020,NOF Tokyo,Japan)、聚(乙二醇)-2000-二甲基丙烯酸酯(PEG2k-DMA)及1,2-二硬脂酰基氧基丙基-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WO2016/010840(第至段)中揭示的化合物S027。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。

[0576] LNP调配物

[0577] LNP可含有(i)用于囊封及用于胞内体逃逸的胺脂质,(ii)用于稳定的中性脂质,(iii)亦用于稳定的辅助脂质及(iv)隐密脂质,例如PEG脂质。

[0578] 在一些实施例中,LNP组合物可包含RNA组分,其包括RNA引导的DNA结合剂、Cas核酸酶mRNA、2类Cas核酸酶mRNA、Cas9 mRNA及gRNA中的一项或多项。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、中性脂质及隐密脂质。在某些LNP组合物中,辅助脂质是胆固醇。在其它组合物中,中性脂质是DSPC。在额外实施例中,隐密脂质是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;隐密脂质;及引导RNA。在某些组合物中,胺脂质是脂质A。在某些组合物中,胺脂质是脂质A或其缩醛类似物;辅助脂质是胆固醇;中性脂质是DSPC;且隐密脂质是PEG2k-DMG。

[0579] 在某些实施例中,根据调配物中组分脂质的各自摩尔比阐述脂质组合物。本发明的实施例提供,根据调配物中组分脂质的各自摩尔比阐述的脂质组合物。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-%将为靶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-%将为靶mol-%的±4mol-%、±3mol-%、±2mol-%、±1.5mol-%、±1mol-%、±0.5mol-%或±0.25mol-%。所有mol-%数皆随LNP组合物的脂质组分变化给出。在某些实施例中,胺脂质mol-%的LNP批间可变性将小于15%、小于10%或小于5%。

[0580] 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-%将为靶中性脂质mol-%的±30%、±25%、±20%、±15%、±10%、±5%或±2.5%。在某些实施例中,LNP批间可变性将小于15%、小于10%或小于5%。

[0581] 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脂质浓度调节辅助脂质的mol-%以使脂质组分达到100mol-%。在一些实施例中,LNP批料的辅助mol-%将为靶mol-%的±30%、±25%、±20%、±15%、±10%、±5%或±2.5%。在某些实施例中,LNP批间可变性将小于15%、小于10%或小于5%。

[0582] 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-%将为靶PEG脂质mol-%的±30%、±25%、±20%、±15%、±10%、±5%或±2.5%。在某些实施例中,LNP批间可变性将小于15%、小于10%或小于5%。

[0583] 在某些实施例中,运送子包括编码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与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,胺脂质是脂质A。在一些方面中,胺脂质是脂质A等效物,例如脂质A的类似物。在某些方面中,胺脂质是脂质A的缩醛类似物。在各个实施例中,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,辅助脂质是胆固醇。在某些实施例中,中性脂质是DSPC。在具体实施例中,PEG脂质是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组合物包含含有DMG的PEG脂质。在某些实施例中，胺脂质是选自脂质A及脂质A的等效物，包括脂质A的缩醛类似物。在额外实施例中，LNP组合物包含脂质A、胆固醇、DSPC及PEG2k-DMG。

[0584] 本发明的实施例亦提供根据胺脂质的带正电的胺基(N)与欲囊封的核酸的带负电的磷酸酯基团(P)之间的摩尔比阐述的脂质组合物。此可由公式N/P以数学方式表示。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、中性脂质及辅助脂质；及核酸组分，其中N/P比率是约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、中性脂质及辅助脂质；及RNA组分，其中N/P比率是约3至10。在一个实施例中，N/P比率可为约5-7。在一个实施例中，N/P比率可为约4.5-8。在一个实施例中，N/P比率可为约6。在一个实施例中，N/P比率可为6±1。在一个实施例中，N/P比率可为约6±0.5。在一些实施例中，N/P比率将为靶N/P比率的±30%、±25%、±20%、±15%、±10%、±5%或±2.5%。在某些实施例中，LNP批间可变性将小于15%、小于10%或小于5%。

[0585] 在一些实施例中，RNA组分可包含mRNA，例如本文揭示的例如编码Cas核酸酶的mRNA。在一个实施例中，RNA组分可包含Cas9 mRNA。在包含编码Cas核酸酶的mRNA的一些组合物中，LNP进一步包含gRNA核酸，例如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。

[0586] 在某些实施例中，LNP组合物可包含本文揭示的例如编码Cas核酸酶(例如2类Cas核酸酶)的mRNA、胺脂质、辅助脂质、中性脂质及PEG脂质。在包含编码Cas核酸酶(例如2类Cas核酸酶)的mRNA的某些LNP组合物中，辅助脂质是胆固醇。在包含编码Cas核酸酶(例如2类Cas核酸酶)的mRNA的其它组合物中，中性脂质是DSPC。在包含编码Cas核酸酶(例如2类Cas核酸酶)的mRNA的额外实施例中，PEG脂质是PEG2k-DMG或PEG2k-C11。在包含编码Cas核酸酶(例如2类Cas核酸酶)的mRNA的具体组合物中，胺脂质是选自脂质A及其等效物，例如脂质A的缩醛类似物。

[0587] 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。在包含gRNA的某些LNP组合物中，辅助脂质是胆固醇。在包含gRNA的一些组合物中，中性脂质是DSPC。在包含gRNA的额外实施例中，PEG脂质是PEG2k-DMG或PEG2k-C11。在某些实施例中，胺脂质是选自脂质A及其等效物，例如脂质A的缩醛类似物。

[0588] 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。在包含sgRNA的一些组合物中，LNP包括胺脂质、辅助脂质、中性脂质及PEG脂质。在包含sgRNA的某些组合物中，辅助脂质是胆固醇。在包含sgRNA的其它组合物中，中性脂质是DSPC。在包含sgRNA的额外实施例中，PEG脂质是PEG2k-DMG或PEG2k-C11。在某些实施例中，胺脂质是选自脂质A及其等效物，例如脂质A的缩醛类似物。

[0589] 在某些实施例中，LNP组合物包含编码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。在包含编码Cas核酸酶的mRNA及gRNA的某些组合物中，辅助脂质是胆固醇。在包含编码Cas核酸酶的mRNA及gRNA的一些组合物中，中性脂质是DSPC。在包含编码Cas核酸酶的mRNA及gRNA的额外实施例中，PEG脂质是PEG2k-DMG或PEG2k-C11。在某些实施例中，胺脂

质是选自脂质A及其等效物，例如脂质A的缩醛类似物。

[0590] 在某些实施例中，LNP组合物包括Cas核酸酶mRNA(例如2类Cas mRNA)及至少一种gRNA。在某些实施例中，LNP组合物包括约25:1至约1:25的gRNA对Cas核酸酶mRNA(例如2类Cas核酸酶mRNA)的比率。在某些实施例中，LNP调配物包括约10:1至约1:10的gRNA对Cas核酸酶mRNA(例如2类Cas核酸酶mRNA)的比率。在某些实施例中，LNP调配物包括约8:1至约1:8的gRNA对Cas核酸酶mRNA(例如2类Cas核酸酶mRNA)的比率。如本文所测量，比率是以重量计。在一些实施例中，LNP调配物包括约5:1至约1:5的gRNA对Cas核酸酶mRNA(例如2类Cas mRNA)的比率。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对mRNA比率是约3:1或约2:1。在一些实施例中，gRNA对Cas核酸酶mRNA(例如2类Cas核酸酶)的比率是约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。

[0591] 本文揭示的LNP组合物可包括模板核酸。模板核酸可与编码Cas核酸酶的mRNA(例如2类Cas核酸酶mRNA)共调配。在一些实施例中，模板核酸可与引导RNA共调配。在一些实施例中，模板核酸可与编码Cas核酸酶的mRNA及引导RNA二者共调配。在一些实施例中，模板核酸可与编码Cas核酸酶的mRNA或引导RNA分开调配。模板核酸可与LNP组合物一起递送或分开递送。在一些实施例中，视期望修复机制而定，模板核酸可为单链或双链。模板可具有与靶DNA或与毗邻靶DNA的序列同源的区域。

[0592] 在一些实施例中，藉由将RNA水溶液与基于有机溶剂的脂质溶液(例如，100％乙醇)混合形成LNP。适宜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。可使用(例如)用于活体内施用LNP的医药上可接受的缓冲液。在某些实施例中，缓冲液用于将包含LNP的组合物的pH维持在pH 6.5或以上。在某些实施例中，缓冲液用于将包含LNP的组合物的pH维持在pH 7.0或以上。在某些实施例中，组合物的pH范围为约7.2至约7.7。在额外实施例中，组合物的pH范围为约7.3至约7.7或约7.4至约7.6。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。可用微pH探针测量组合物的pH。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。实例性组合物可包含高达10％的低温保护剂，例如蔗糖。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％的低温保护剂。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％的蔗糖。在一些实施例中，LNP组合物可包括缓冲液。在一些实施例中，缓冲液可包含磷酸盐缓冲液(PBS)、Tris缓冲液、柠檬酸盐缓冲液及其混合物。在某些实例性实施例中，缓冲液包含NaCl。在某些实施例中，省略NaCl。NaCl的实例性量可在约20mM至约45mM的范围内。NaCl的实例性量可在约40mM至约50mM的范围内。在一些实施例中，NaCl的量约为45mM。在一些实施例中，缓冲液是Tris缓冲液。Tris的实例性量可在约20mM至约60mM的范围内。Tris的实例性量可在约40mM至约60mM的范围内。在一些实施例中，Tris的量约为50mM。在一些实施例中，缓冲液包含NaCl及Tris。LNP组合物的某些实例性实施例在Tris缓冲液中含有5％蔗糖及45mM NaCl。在其它实例性实施例中，组合物含有约5％w/v的量的蔗糖、约45mM NaCl及约50mM Tris(pH 7.5)。可改变盐、缓冲液及低温保护剂的量，使得维持整个调配物的渗透重量莫耳浓度。例如，最终渗透重量莫耳浓度可维持于小于450mOsm/L。在其它实施例中，渗透重量莫耳浓度介于350mOsm/L与250mOsm/L之间。某

些实施例具有300+/-20mOsm/L的最终渗透重量莫耳浓度。

[0593] 在一些实施例中，使用微流体混合、T-混合或交叉混合。在某些方面中，可改变流速、结大小、结几何学、结形状、管直径、溶液和/或RNA及脂质浓度。可经由(例如)透析、切向流过滤或层析浓缩或纯化LNP或LNP组合物。例如，LNP可以悬浮液、乳液或冻干粉末形式储存。在一些实施例中，将LNP组合物储存于2-8℃，在某些方面中，将LNP组合物储存于室温下。在额外实施例中，将LNP组合物于例如-20℃或-80℃下冷冻储存。在其它实施例中，将LNP组合物于约0℃至约-80℃范围内的温度下储存。冷冻的LNP组合物可在使用前例如在冰上、于4℃下、于室温下或于25℃下解冻。冷冻的LNP组合物可维持在各种温度下，例如在冰上、于4℃下、于室温下、于25℃下或于37℃下。

[0594] 在一些实施例中，LNP组合物具有大于约80％的囊封。在一些实施例中，LNP组合物的粒径小于约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，存在该特征中的至少两个。在一些实施例中，存在该三个特征中的每一个。用于测定该参数的分析方法在下文的一般试剂及方法部分中论述。

[0595] 在一些实施例中，使用微流体混合、T-混合或交叉混合。在某些方面中，可改变流速、结大小、结几何学、结形状、管直径、溶液和/或RNA及脂质浓度。可经由(例如)透析或层析浓缩或纯化LNP或LNP组合物。例如，LNP可以悬浮液、乳液或冻干粉末形式储存。在一些实施例中，将LNP组合物储存于2-8℃，在某些方面中，将LNP组合物储存于室温下。在额外实施例中，将LNP组合物于(例如)-20℃或-80℃下冷冻储存。在其它实施例中，将LNP组合物储存于0℃至-80℃范围内的温度。冷冻的LNP组合物可在使用前(例如)在冰上、于室温下或于25℃下解冻。

[0596] 动态光散射(“DLS”)可用于表征本发明的LNP的多分散性指数(“pdi”)及大小。DLS测量由于使样本经受光源而产生的光散射。如自DLS测量测定的PDI表示群体中粒径(在平均粒径附近)的分布，其中完全均匀的群体的PDI为零。在一些实施例中，pdi可在0.005至0.75的范围内。在一些实施例中，pdi可在0.01至0.5的范围内。在一些实施例中，pdi可在0.02至0.4的范围内。在一些实施例中，pdi可在0.03至0.35的范围内。在一些实施例中，pdi可在0.1至0.35的范围内。

[0597] 在一些实施例中，本文揭示的LNP的大小为1nm至250nm。在一些实施例中，LNP的大小为10nm至200nm。在其它实施例中，LNP的大小为20nm至150nm。在一些实施例中，LNP的大小为50nm至150nm。在一些实施例中，LNP的大小为50nm至100nm。在一些实施例中，LNP的大小为50nm至120nm。在一些实施例中，LNP的大小为75nm至150nm。在一些实施例中，LNP的大小为30nm至200nm。除非另外指明，否则本文中提及的所有大小皆是完全形成的纳米颗粒的平均大小(直径)，如在Malvern Zetasizer上藉由动态光散射所测量。将纳米颗粒样本稀释于磷酸盐缓冲盐水(PBS)中以使计数率为约200-400kct。数据呈现为强度测量的加权平均值。在一些实施例中，形成LNP，且平均囊封效率在50％至100％范围内。在一些实施例中，形成LNP，且平均囊封效率在50％至70％范围内。在一些实施例中，形成LNP，且平均囊封效率在70％至90％范围内。在一些实施例中，形成LNP，且平均囊封效率在90％至100％范围内。在一些实施例中，形成LNP，且平均囊封效率在75％至95％范围内。

[0598] 在一些实施例中，与本文揭示的gRNA相关的LNP用于制备用于治疗ATTR的药剂。在一些实施例中，与本文揭示的gRNA相关的LNP用于制备用于在患有ATTR的个体中减少或预

防TTR在淀粉样蛋白或淀粉样原纤维中累积及聚集的药剂。在一些实施例中，与本文揭示的gRNA相关的LNP用于制备用于降低血清TTR浓度的药剂。在一些实施例中，与本文揭示的gRNA相关的LNP用于治疗个体(例如哺乳动物，例如灵长类动物，例如人类)的ATTR。在一些实施例中，与本文揭示的gRNA相关的LNP用于在患有ATTR的个体(例如哺乳动物，例如灵长类动物，例如人类)中减少或预防TTR在淀粉样蛋白或淀粉样原纤维中累积及聚集。在一些实施例中，与本文揭示的gRNA相关的LNP用于降低个体(例如哺乳动物，例如灵长类动物，例如人类)的血清TTR浓度。

[0599] 电穿孔亦是用于递送运送子的熟知方式，且任何电穿孔方法可用于递送本文揭示的gRNA中的任一种。在一些实施例中，电穿孔可用于递送本文揭示的gRNA中的任一种及RNA引导的DNA核酸酶(例如Cas9)或编码RNA引导的DNA核酸酶(例如Cas9)的mRNA。

[0600] 在一些实施例中，本发明包含用于将本文揭示的gRNA中的任一种递送至离体细胞的方法，其中gRNA与LNP相关或不与LNP相关。在一些实施例中，gRNA/LNP或gRNA亦与RNA引导的DNA核酸酶(例如Cas9)或编码RNA引导的DNA核酸酶(例如Cas9)的mRNA相关。

[0601] 在某些实施例中，本发明包含编码包含本文所述引导序列中的任一项或多项的任何引导RNA的DNA或RNA载体。在一些实施例中，除了引导RNA序列之外，载体进一步包含不编码引导RNA的核酸。不编码引导RNA的核酸包括(但不限于)启动子、增强子、调节序列及编码RNA引导的DNA核酸酶(其可为核酸酶，例如Cas9)的核酸。在一些实施例中，载体包含一或多个编码crRNA、trRNA或crRNA及trRNA的核苷酸序列。在一些实施例中，载体包含一或多个编码sgRNA及mRNA的核苷酸序列，该mRNA编码RNA引导的DNA核酸酶，其可为Cas核酸酶，例如Cas9或Cpf1。在一些实施例中，载体包含一或多个编码crRNA、trRNA及mRNA的核苷酸序列，该mRNA编码RNA引导的DNA核酸酶，其可为Cas蛋白，例如Cas9。在一个实施例中，Cas9是来自酿脓链球菌(亦即，Spy Cas9)。在一些实施例中，编码crRNA、trRNA、或crRNA及trRNA(其可为sgRNA)的核苷酸序列包含侧接天然CRISPR/Cas系统的重复序列的全部或一部分的引导序列或由该引导序列组成。包含crRNA、trRNA或crRNA及trRNA或由其组成的核酸可进一步包含载体序列，其中载体序列包含并非与crRNA、trRNA或crRNA及trRNA一起天然发现的核酸或由该核酸组成。

[0602] 在一些实施例中，crRNA及trRNA由一个载体内的非邻接核酸编码。在其它实施例中，crRNA及trRNA可由邻接核酸编码。在一些实施例中，crRNA及trRNA由单一核酸的相反链编码。在其它实施例中，crRNA及trRNA由单一核酸的相同链编码。

[0603] 在一些实施例中，载体可为环形的。在其它实施例中，载体可为线性的。在一些实施例中，载体可包封在脂质纳米颗粒、非脂质纳米颗粒或病毒衣壳中。非限制性实例性载体包括质粒、噬粒、黏粒、人工染色体、微型染色体、转位子、病毒载体及表达载体。

[0604] 在一些实施例中，载体可为病毒载体。在一些实施例中，病毒载体可自其野生型对应体遗传修饰。例如，病毒载体可包含一或多个核苷酸的插入、缺失或取代，以促进克隆或使得载体的一或多种性质发生变化。该性质可包括包装能力、转导效率、免疫原性、基因组整合、复制、转录及转译。在一些实施例中，病毒基因组的一部分可缺失，使得病毒能包装大小更大之外源序列。在一些实施例中，病毒载体可具有增强的转导效率。在一些实施例中，可减少病毒在宿主中诱导的免疫反应。在一些实施例中，促进病毒序列整合至宿主基因组中的病毒基因(例如，整合酶)可突变，使得病毒变得非整合。在一些实施例中，病毒载体可

为复制缺陷的。在一些实施例中，病毒载体可包含用于驱动编码序列在载体上表达之外源转录或转译控制序列。在一些实施例中，病毒可为辅助依赖的。例如，病毒可能需要一或多种辅助病毒以供应扩增并将载体包装至病毒颗粒中所需的病毒组分(例如，病毒蛋白)。在这种情况下，一或多种辅助组分(包括一或多个编码病毒组分的载体)可与本文所述的载体系统一起引入宿主细胞中。在其它实施例中，该病毒可为无辅助的。例如，病毒可在无任何辅助病毒的情况下扩增及包装载体。在一些实施例中，本文所述的载体系统亦可编码病毒扩增及包装所需的病毒组分。

[0605] 非限制性实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、辅助依赖性腺病毒载体(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。在一些实施例中，病毒载体是AAV2、AAV3、AAV3B、AAV5、AAV6、AAV6.2、AAV7、AAVrh.64R1、AAVhu.37、AAVrh.8、AAVrh.32.33、AAV8、AAV9、AAVrh10或AAVLK03。在其它实施例中，病毒载体可为慢病毒载体。

[0606] 在一些实施例中，慢病毒可为非整合的。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，腺病毒可为高克隆能力或“无病毒基因的”腺病毒，其中自病毒缺失除了5′及3′倒置末端重复(ITR)及包装信号(“I”)的所有编码病毒区域，以增加其包装能力。在其它实施例中，病毒载体可为HSV-1载体。在一些实施例中，基于HSV-1的载体是辅助依赖的，且在其它实施例中，其是辅助独立的。例如，仅保留包装序列的扩增子载体需要具有用于包装的结构组分的辅助病毒，而移除非必需病毒功能的30kb缺失的HSV-1载体不需要辅助病毒。在额外实施例中，病毒载体可为噬菌体T4。在一些实施例中，当病毒的头部被清空时，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能力的AAV或慢病毒载体的实施例中，可能需要使用一个以上载体来递送如本文揭示的载体系统的所有组分。例如，一个AAV载体可含有编码RNA引导的DNA核酸酶(例如Cas核酸酶)的序列，而第二AAV载体可含有一或多个引导序列。

[0607] 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的表达。在一些实施例中，细胞可为原核细胞，例如细菌细胞。在一些实施例中，细胞可为真核细胞，例如酵母、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。适于驱动不同类型细胞中表达的启动子为业内已知。在一些实施例中，启动子可为野生型。在其它实施例中，可修改启动子以便更高效或更有效地表达。在其它实施例中，启动子可经截短但仍保留其功能。例如，启动子可具有适于正常大小或将载体恰当包装至病毒中的减小大小。

[0608] 在一些实施例中，载体可包含编码RNA引导的DNA核酸酶(例如本文所述核酸酶)的核苷酸序列。在一些实施例中，由载体编码的核酸酶可为Cas蛋白。在一些实施例中，载体系统可包含编码核酸酶的核苷酸序列的一个拷贝。在其它实施例中，载体系统可包含编码核酸酶的核苷酸序列的一个以上拷贝。在一些实施例中，编码核酸酶的核苷酸序列可操作连接至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操作连接至至少一个启动子。

[0609] 在一些实施例中，启动子可为组成型、诱导型或组织特定性的。在一些实施例中，启动子可为组成型启动子。非限制性实例性组成型启动子包括巨细胞病毒立即早期启动子

(CMV)、猿猴病毒(SV40)启动子、腺病毒主要晚期(MLP)启动子、劳斯肉瘤病毒(Rous sarcoma virus,RSV)启动子、小鼠乳房肿瘤病毒(MMTV)启动子、磷酸甘油酸盐激酶(PGK)启动子、伸长因子-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、其功能片段或上述中的任一种的组合。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,启动子可为诱导型启动子。非限制性实例性诱导型启动子包括可由热休克、光、化学品、肽、金属、类固醇、抗生素或酒精诱导的这些。在一些实施例中,诱导型启动子可为具有低基础(非诱导)表达程度者,例如Tet-On®启动子(Clontech)。

[0610] 在一些实施例中,启动子可为组织特异性启动子,例如对肝中的表达具有特异性的启动子。

[0611] 载体可进一步包含编码本文所述引导RNA的核苷酸序列。在一些实施例中,载体包含引导RNA的一个拷贝。在其它实施例中,载体包含引导RNA的一个以上拷贝。在具有一个以上引导RNA的实施例中,引导RNA可为不相同的,使得其靶向不同靶序列,或可为相同的,使得其靶向相同靶序列。在载体包含一个以上引导RNA的一些实施例中,每一引导RNA可在与RNA引导的DNA核酸酶的复合物(例如Cas RNP复合物)中具有其它不同性质,例如活性或稳定性。在一些实施例中,编码引导RNA的核苷酸序列可操作连接至至少一个转录或转译控制序列,例如启动子、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.2015 21:1683-9;Scherer等人,NucleicAcids Res.200735:2620-2628。在一些实施例中,启动子可由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的核苷酸序列可操作连接至小鼠或人类U6启动子。在其它实施例中,编码引导RNA的核苷酸序列可操作连接至小鼠或人类H1启动子。在具有一个以上引导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的核苷酸可提供于同一载体上。在一些实施例中,编码crRNA的核苷酸及编码trRNA的核苷酸可由相同启动子驱动。在一些实施例中,crRNA及trRNA可转录成单一转录物。例如,可自单一转录物处理crRNA及trRNA以形成双分子引导RNA。或者,crRNA及trRNA可转录成单一分子引导RNA(sgRNA)。在其它实施例中,crRNA及trRNA可由同一载体上的其相应启动子驱动。在其它实施例中,crRNA及trRNA可由不同载体编码。

[0612] 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上,该载体包含编码RNA引导的DNA核酸酶(例如Cas核酸酶)的核苷酸序列。在一些实施例中,引导RNA的表达及RNA引导的DNA核酸酶(例如Cas蛋白)的表达可由其自身相应的启动子驱动。在一些实施例中,引导RNA的表达可由驱动RNA引导的DNA核酸酶(例如Cas蛋白)的表达的相同启动子驱动。在一些实施例中,引导RNA及RNA引导的DNA核酸酶(例如Cas蛋白转录物)可包含于单一转录物中。例如,引导RNA可在RNA引导的DNA核酸酶(例如Cas蛋白转录物)的非转译区(UTR)内。在一些实施例中,引导RNA可在转录物的5′UTR内。在其它实施例中,引导RNA可在转录物的3′UTR内。在一些实施例中,转录物的细胞内半衰期可藉由在其3′UTR内含有引导RNA且从而缩短其3′UTR的长度来减少。在额外实施例中,引导RNA可在转录物的内含子内。在一些实施例中,可在引导RNA所位于的内含子添加适宜剪接位点,使得引导RNA自转录物中恰当剪接出。在一些实施例中,来自相同载体的RNA引导的DNA核酸酶(例如Cas蛋白)及引导RNA的

紧密时间接近的表达可促进更有效地形成CRISPR RNP复合物。

[0613] 在一些实施例中，组合物包含载体系统。在一些实施例中，载体系统可包含单一载体。在其它实施例中，载体系统可包含两个载体。在额外实施例中，载体系统可包含三个载体。当使用不同引导RNA进行多任务化时或当使用引导RNA的多个拷贝时，载体系统可包含三个以上载体。

[0614] 在一些实施例中，载体系统可包含诱导型启动子以仅在其递送至靶细胞后开始表达。非限制性实例性诱导型启动子包括可由热休克、光、化学品、肽、金属、类固醇、抗生素或酒精诱导的这些。在一些实施例中，诱导型启动子可为具有低基础(非诱导)表达程度者，例如Tet-On®启动子(Clontech)。

[0615] 在额外实施例中，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之后开始表达。

[0616] 载体可由脂质粒、纳米颗粒、外来体或微囊泡递送。载体亦可由脂质纳米颗粒(LNP)递送；参见(例如)于2016年12月12日提出申请的标题为“LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS”的U.S.S.N.62/433,228，其全部内容以引用方式并入本文中。本文所述LNP及LNP调配物中的任一种适于单独递送引导或与cas核酸酶或编码cas核酸酶的mRNA一起。在一些实施例中，涵盖包含以下的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、辅助脂质及隐密脂质；及其中N/P比率是约1-10。

[0617] 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比率为约1-10。在一些实施例中，脂质组分包含：约40-60mol-％胺脂质；约5-15mol-％中性脂质；及约1.5-10mol-％PEG脂质，其中脂质组分的其余部分是辅助脂质，其中LNP组合物的N/P比率是约3-10。在一些实施例中，脂质组分包含约50-60mol-％胺脂质；约8-10mol-％中性脂质；及约2.5-4mol-％PEG脂质，其中脂质组分的其余部分是辅助脂质，其中LNP组合物的N/P比率是约3-8。在一些情况下，脂质组分包含：约50-60mol-％胺脂质；约5-15mol-％DSPC；及约2.5-4mol-％PEG脂质，其中脂质组分的其余部分是胆固醇，且其中LNP组合物的N/P比率是约3-8。在一些情况下，脂质组分包含：48-53mol-％脂质A；约8-10mol-％DSPC；及1.5-10mol-％PEG脂质，其中脂质组分的其余部分是胆固醇，且其中LNP组合物的N/P比率为3-8±0.2。

[0618] 在一些实施例中，载体可全身地递送。在一些实施例中，载体可递送至肝循环中。

[0619] 本说明书及实例性实施例不应视为具有限制性。出于本说明书及所附申请专利范围的目的，除非另有说明，否则在说明书及申请专利范围中使用的表示数量、百分比或比例的所有数字及其它数值应理解为在所有情况下皆由术语“约”修饰至其尚未经如此修饰的程度。因此，除非指示相反情况，否则以下说明书及所附申请专利范围中所阐明的数字参数皆为可根据寻求获得的期望性质而变化的近似值。最低限度地，且并非试图限制申请专利范围的等效项的原则的应用，每一数字参数均应至少根据所报告有效位的数量且藉由施用普通舍入技术来解释。

[0620] 应注意，除非特别地且明确地限于一个指示物，否则如在本说明书及所附申请专利范围中所使用的单数形式“一(a、an)”及“该”及任何词语的任何单数使用包括复数个指示物。如本文所用术语“包括”及其语法变体意欲非限制性，使得清单中的项目的列举不排

除可取代或添加至列出的项目的其它类似项目。

[0621] 实例

[0622] 提供以下实例以阐释某些揭示的实施例,而不应解释为以任何方式限制本揭示内容的范围。

[0623] 实例1.材料及方法

[0624] 核酸酶mRNA的活体外转录(“IVT”)

[0625] 藉由使用线性化质粒DNA模板及T7 RNA聚合酶活体外转录产生含有N1-甲基假-U的封端及腺苷酸化酿脓链球菌(“Spy”)Cas9 mRNA。藉由利用以下条件在37℃下与XbaI一起培育2小时线性化含有T7启动子(即根据SEQ ID NO:1或2的转录序列)及100nt多(A/T)区的质粒DNA:200ng/μL质粒、2U/μL XbaI(NEB)及1×反应缓冲液。藉由在65℃加热反应20min使XbaI不活化。使用二氧化硅maxi旋转管柱(Epoch Life Sciences)自酶及缓冲液盐纯化线性化质粒,且藉由琼脂糖凝胶分析以确认线性化。将产生Cas9修饰的mRNA的IVT反应在37℃下在以下条件下培育4小时:50ng/μL线性化质粒;2mM各GTP、ATP、CTP及N1-甲基假-UTP(Trilink);10mM ARCA(Trilink);5U/μL T7 RNA聚合酶(NEB);1U/μL鼠类RNase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。4小时培育后,添加TURBO DNase(ThermoFisher)至最终浓度为0.01U/μL,且将反应再培育30分钟以移除DNA模板。根据制造商的方案(ThermoFisher)使用MegaClear转录清除试剂盒自酶及核苷酸纯化Cas9mRNA。或者,经由沉淀方案纯化mRNA,在一些情形下,之后是基于HPLC的纯化。简言之,在DNase消化后,藉由添加0.21×vol的7.5M LiCl溶液并混合沉淀mRNA,并藉由离心使沉淀的mRNA成团块。移除上清液后,将mRNA重构于水中。使用乙酸铵及乙醇再次沉淀mRNA。将5M乙酸铵添加至mRNA溶液中,最终浓度为2M,同时添加2×体积的100%EtOH。将溶液混合并在-20℃下培育15min。藉由离心再次使沉淀的mRNA成团块,移除上清液,并将mRNA重构于水中。作为最后步骤,使用乙酸钠及乙醇沉淀mRNA。将1/10体积的3M乙酸钠(pH 5.5)与2x体积的100%EtOH一起添加至溶液中。将溶液混合并在-20℃下培育15min。藉由离心再次使沉淀的mRNA成团块,移除上清液,用70%冷乙醇洗涤团块并使其风干。将mRNA重构于水中。对于HPLC纯化的mRNA,在LiCl沉淀及重构后,藉由RP-IP HPLC纯化mRNA(例如,参见Kariko等人Nucleic Acids Research,2011,第39卷,第21期,e142)。合并选择用于汇集的部分且如上所述藉由乙酸钠/乙醇沉淀脱盐。藉由测量260nm的吸光度(Nanodrop)测定转录物浓度,并藉由Bioanlayzer(Agilent)的毛细管电泳分析转录物。

[0626] 当下文关于RNA提及SEQ ID NO:1及2时,应理解,Ts应用Us置换(其为如上所述的N1-甲基假尿苷)。实例中使用的Cas9 mRNA包括5’帽及3’多-A尾,例如,至多100nt,且由SEQ ID NO鉴别。

[0627] SEQ ID NO:1:用于转录的Cas9序列1。

[0628]

GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGT

GTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGAC

AAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTC

GGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAG

AAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAA

GAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA

CAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGA

TACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC

TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCAC

AGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCA

CGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGC

ATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAA

GCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTA

CCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCT

GATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACA

AGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCG

AAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCA

ATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAA

CCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGT

TCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACT

TCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAG

CTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTG

GCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCA

AAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGA

GTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCAT

GATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCT

GAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGG

AAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACA

TCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCA

AGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTG

GTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAAC

[0629] ATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGA

ACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT

CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATT

CAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG

CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCAC

ACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG

CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACC

TGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACG

AATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG

TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAA

CAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAG

AAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGA

AGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAG

ACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGA

AGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC

GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTC

GAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGC

ACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAA

GAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATC

AACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGA

CTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA

GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCA

GAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAAC

[0630] ACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGA

ATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTC

ATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCA

AGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCA

GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTG

GGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACA

GCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGG

AAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACT

GAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCT

GAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCG

ACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAA

GTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAAC

GCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAA

GGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGAT

TCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAG

CACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTAC

GACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC

ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCC

AGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCAC

ACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCA

AGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACT

ACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAAC

AGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCA

[0631] ACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACG

GAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAA

ACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAAC

AGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAA

GAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCA

TCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAG

AAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCC

GACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAA

GGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGG

GAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGA

TCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAG

GACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTG

GAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACT

GCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCA

ACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAA

GCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAG

CACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA

TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAG

GTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGA

GAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAAC

CTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATC

[0632]

GACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGC

AACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAG

AATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCC

CGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAA

GCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGA

TCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGC

CAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCC

TCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTC

GAG

[0633] SEQ ID NO:2:用于转录的Cas9序列2。

GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGT

GTGTGTGTCGTTGCAGGCCTTATTCGGATCCATGGATAAGAAG

[0634]

TACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGG

CAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCA

AGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAAAATC

TCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAG

CGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGC

GGAAGAATCGCATCTGCTATCTGCAAGAGATCTTTTCGAACGA

AATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGA

ATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCC

TATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGAAAA

GTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCA

ACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCC

ATATGATCAAATTCCGCGGACACTTCCTGATCGAAGGCGATCT

GAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTG

[0635] GTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAAT

GCTAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTG

TCGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGCTGCCG

GGAGAGAAAAAGAACGGACTTTTCGGCAACTTGATCGCTCTC

TCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGG

CCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGAC

GACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTAC

GCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCT

TGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAG

CGCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCA

CCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCAACT

GCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAA

TGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGA

GTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGA

ACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTC

CGGAAACAGAGAACCTTTGACAACGGATCCATTCCCCACCAG

ATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAG

GACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAG

AAAATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGG

CGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATCAG

AGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATA

AGGGAGCTTCGGCACAAAGCTTCATCGAACGAATGACCAACT

TCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACA

[0636] GCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAA

AGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCT

GTCCGGAGAACAGAAGAAAGCAATTGTCGATCTGCTGTTCAA

GACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACT

ACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCG

GGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATG

ATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACG

AGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTTGA

CCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGA

CCTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAA

GCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCT

GATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTG

GATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGC

AATTGATCCACGACGACAGCCTGACCTTTAAGGAGGACATCC

AAAAAGCACAAGTGTCCGGACAGGGAGACTCACTCCATGAA

CACATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGA

ATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTGAAGGTC

ATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCC

CGAGAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCG

CGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGG

GCAGCCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAG

CTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAATGGA

CGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTG

[0637] TCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGA

AGGATGACTCGATCGATAACAAGGTGTTGACTCGCAGCGACA

AGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCG

TGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGA

AGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCG

AGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCA

AACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGG

CGCAGATCTTGGACTCCCGCATGAACACTAAATACGACGAGA

ACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAA

GCAAACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACA

AAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCAT

ACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACC

CTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTA

CGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAATCGG

GAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAAC

TTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGG

AAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAAT

CGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGT

GCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGT

GCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAG

AAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCC

GAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTC

CGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAA

[0638] AGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGG

AACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGG

CGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAAC

TCCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGC

GCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGC

TGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTC

GCACTACGAAAAACTCAAAGGGTCACCGGAAGATAACGAAC

AGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATG

AAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCT

CGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAA

GCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTAT

CCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTT

CAAGTACTTCGATACTACTATCGATCGCAAAAGATACACGTCC

ACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATC

ACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGT

GGCGATGGCGGTGGATCTCCGAAAAAGAAGAGAAAGGTGTAA

[0639] TGAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAG

AATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTT

TTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAAC

ATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT

AATAAAAAATGGAAAGAACCTCGAG

[0640] 人类TTR引导设计及具有食蟹猴同源性引导设计的人类TTR

[0641] 使用人类参考基因组(例如,hg38)及用户定义的所关注基因组区域(例如,编码外显子的TTR蛋白),计算机仿真实施初始引导选择,用于鉴别所关注区域中的PAM。对于每一鉴别的PAM,实施分析并报告统计值。进一步选择gRNA分子并基于许多准则(例如,GC含量、预测靶上活性及潜在脱靶活性)进行等级次序化。

[0642] 针对靶向外显子1、2、3及4内的蛋白质编码区的TTR(ENSG00000118271)设计总共68个引导RNA。在总共68个引导中,33个在食蟹猴(cynomolgus monkey,cyno)中100%同源。此外,对于10个在食蟹猴中并非完全同源的人类TTR引导,平行设计及制造“代用”引导以与相应食蟹猴靶序列完美匹配。可在食蟹猴中筛选该“代用”或“工具”引导以例如近似同源人类引导序列的活性及功能。提供引导序列及相应基因组坐标(表1)。将所有引导RNA制成双重导向RNA,并将导向序列的亚组制成经修饰的单引导RNA(表2)。提供双重引导RNA(dgRNA)ID、经修饰单引导RNA(sgRNA)ID、与食蟹猴基因组的错配数量以及食蟹猴精确匹配的ID间的引导ID比对(表3)。在dgRNA用于实例中详述的实验的情况下,使用SEQ ID NO:270。

[0643] 活体外Cas9 mRNA及引导RNA递送

[0644] HEK293_Cas9细胞系.在补充有10%胎牛血清及500μg/ml G418的DMEM培养基中培养组成型表达Spy Cas9的人类胚肾癌细胞系HEK293(“HEK293_Cas9”)。在转染之前24小时,将细胞以10,000个细胞/孔的密度平铺于96孔板中。根据制造商的方案用Lipofectamine RNAiMAX(ThermoFisher,目录13778150)转染细胞。用含有个别crRNA(25nM)、trRNA(25nM)、Lipofectamine RNAiMAX(0.3μL/孔)及OptiMem的脂复合物转染细胞。

[0645] HUH7细胞系.在补充有10%胎牛血清的DMEM培养基中培养人类肝细胞癌细胞系HUH7(Japanese Collection of Research Bioresources Cell Bank,目录JCRB0403)。在转染之前20小时,将细胞以15,000个细胞/孔的密度平铺于96孔板中。根据制造商的方案用Lipofectamine MessengerMAX(ThermoFisher,目录LMRNA003)转染细胞。依序用含有Spy Cas9 mRNA(100ng)、MessengerMAX(0.3μL/well)及OptiMem的脂复合物、之后含有个别crRNA(25nM)、示踪RNA(25nM)、MessengerMAX(0.3μL/孔)及OptiMem的单独脂复合物转染细

胞。

[0646] HepG2细胞系.在补充有10％胎牛血清的DMEM培养基中培养人类肝细胞癌细胞系HepG2(美国模式培养物保藏所(American Type Culture Collection),目录HB-8065)。计数细胞,并在转染前24小时,以10,000个细胞/孔的密度平铺于96孔板中的生物涂层胶原I涂布的96孔板(ThermoFisher,目录877272)上。根据制造商的方案用Lipofectamine 2000(ThermoFisher,目录11668019)转染细胞。依序用含有Spy Cas9 mRNA(100ng)、Lipofectamine 2000(0.2μL/孔)及OptiMem的脂复合物、之后含有个别crRNA(25nM)、示踪RNA(25nM)、Lipofectamine 2000(0.2μL/孔)及OptiMem的单独脂复合物转染细胞。

[0647] 原代肝细胞根据制造商的方案(Invitrogen,方案11.28.2012)培养原代人类肝细胞(PHH)及原代食蟹猴肝细胞(PCH)(Gibco)。简言之,将细胞解冻并重新悬浮于具有补充物的肝细胞解冻培养基(Gibco,目录CM7000)中,之后对于人类以100g离心10分钟且对于食蟹猴以80g离心4分钟。弃去上清液,将成团块的细胞重新悬浮于加补充包的肝细胞平板培养基(Invitrogen,目录A1217601及CM3000)中。计数细胞,并对于人类以33,000个细胞/孔或对于食蟹猴以60,000个细胞/孔(或当分析对TTR蛋白的效应时(下文进一步阐述),为65,000个细胞/孔)的密度平铺于生物涂层胶原I涂布的96孔板(ThermoFisher,目录877272)上。使平铺的细胞在37℃及5％CO_2气氛下在组织培养培育器中沉降并黏附6或24小时。在检查培育细胞单层形成后,将培养基替换为含有无血清补充包(Invitrogen,目录A1217601及CM4000)的肝细胞培养基。

[0648] 根据制造商的方案执行基于Lipofectamine RNAiMax(ThermoFisher,目录13778150)的转染。依序用含有Spy Cas9 mRNA(100ng)、Lipofectamine RNAiMax(0.4μL/孔)及OptiMem的脂复合物、之后含有crRNA(25nM)及示踪RNA(25nM)或sgRNA(25nM)、Lipofectamine RNAiMax(0.4μL/孔)及OptiMem的单独脂复合物转染细胞。

[0649] 在装载有引导RNA(RNP)的Spy Cas9蛋白的电穿孔或转染至细胞上之前实施核糖核苷酸形成。对于双重引导(dgRNA),藉由混合等效量的试剂并在95℃下培育2min并冷却至室温来预处理个别crRNA及trRNA。将单引导(sgRNA)在95℃下煮沸2min并冷却至室温。将煮沸的dgRNA或sgRNA于室温下与Optimem中的Spy Cas9蛋白一起培育10分钟以形成核糖核蛋白(RNP)复合物。

[0650] 对于RNP电穿孔进入原代人类及食蟹猴肝细胞,将细胞解冻并重新悬浮于Lonza电穿孔原代细胞P3缓冲液中,浓度为对于人类肝细胞2500个细胞/μL,且对于食蟹猴肝细胞3500个细胞/μL。每个引导一起混合20μL体积的重新悬浮的细胞及5μL RNP。将20μL混合物置于Lonza电穿孔板中。使用具有预设方案EX-147的Lonza nucleofector对细胞进行电穿孔。电穿孔后,将细胞转移至含有预热的维持培养基的Biocoat板中,并置于37℃及5％CO_2的组织培养培育器中。

[0651] 对于RNP脂复合物转染,根据制造商的方案用Lipofectamine RNAiMAX(ThermoFisher,目录13778150)转染细胞。用含有Spy Cas9(10nM)、个别引导(10nM)、示踪RNA(10nM)、Lipofectamine RNAiMAX(1.0μL/孔)及OptiMem的RNP转染细胞。如上所述实施RNP形成。

[0652] 根据制造商的方案藉由使用Precision Nanosystems NanoAssemblr™台式仪器微流体混合脂质及RNA溶液或交叉流混合来形成LNP。

[0653] LNP调配物-NanoAssemblr

[0654] 通常,将脂质纳米颗粒组分溶解于100%乙醇中,其中脂质组分具有各种摩尔比。将RNA运送子溶解于25mM柠檬酸盐、100mM NaCl(pH 5.0)中,从而产生约0.45mg/mL的RNA运送子浓度。用脂质胺对RNA磷酸酯(N:P)摩尔比为约4.5或约6调配LNP,其中mRNA对gRNA的重量比为1:1。

[0655] 根据制造商的方案藉由使用Precision Nanosystems NanoAssemblr™台式仪器微流体混合脂质及RNA溶液来形成LNP。在混合期间使用不同的流速维持2:1的水溶液对有机溶剂的比率。混合后,收集LNP,稀释于水(约1:1 v/v)中,于室温下保持1小时,并在最终缓冲液更换之前用水(约1:1 v/v)进一步稀释。利用PD-10脱盐管柱(GE)完成至50mM Tris、45mM NaCl、5%(w/v)蔗糖(pH 7.5)(TSS)的最终缓冲液更换。若需要,藉由用Amicon 100kDa离心过滤器(Millipore)离心浓缩调配物。然后使用0.2μm无菌过滤器过滤所得混合物。将最终LNP储存于-80℃直至进一步使用。

[0656] LNP调配物-交叉流

[0657] 对于使用交叉流技术制备的LNP,藉由将乙醇中的脂质与两体积的RNA溶液及一体积的水进行喷射混合来形成LNP。乙醇中的脂质经由混合交叉与两体积的RNA溶液混合。第四水流经由线内T形管与十字架的出口流混合。(参见WO2016010840图2)。将LNP于室温下保持1小时,并进一步用水(约1:1 v/v)稀释。使用切向流过滤在平板盒(Sartorius,100kD MWCO)上浓缩稀释的LNP,且然后藉由渗滤至50mM Tris、45mM NaCl、5%(w/v)蔗糖(pH 7.5)(TSS)中更换缓冲液。或者,用PD-10脱盐管柱(GE)完成至TSS的最终缓冲液更换。若需要,藉由用Amicon 100kDa离心过滤器(Millipore)离心浓缩调配物。然后使用0.2μm无菌过滤器过滤所得混合物。将最终LNP储存于4℃或-80℃直至进一步使用。

[0658] 调配物分析

[0659] 动态光散射(“DLS”)用于表征本发明的LNP的多分散性指数(“pdi”)及大小。DLS测量由于使样本经受光源而产生的光散射。如自DLS测量测定的PDI表示群体中粒径(在平均粒径附近)的分布,其中完全均匀的群体的PDI为零。使用Malvern Zetasizer DLS仪器藉由动态光散射(DLS)测量平均粒径及多分散性。在藉由DLS测量之前,将LNP样本在PBS中稀释30×。报告Z-平均直径(其是基于强度的平均粒径测量)以及数目平均直径及pdi。Malvern Zetasizer仪器亦用于测量LNP的ζ电位。在测量之前,将样本在0.1X PBS(pH 7.4)中1:17稀释(50uL稀释成800uL)。

[0660] 基于荧光的分析(Ribogreen®,ThermoFisher Scientific)用于测定总RNA浓度及游离RNA。囊封效率计算为(总RNA-游离RNA)/总RNA。将LNP样本用含有0.2%Triton-X 100的1x TE缓冲液适当稀释以测定总RNA或用1x TE缓冲液适当稀释以测定游离RNA。藉由利用用于制备调配物且稀释于1x TE缓冲液+/-0.2%Triton-X 100中的起始RNA溶液来制备标准曲线。然后将稀释的RiboGreen®染料(根据制造商的说明书)添加至标样及样本中的每一中且于室温下在不存在光下培育约10分钟。使用SpectraMax M5微量读数板(Molecular Devices)读取样本,其中激发、自动截止及发射波长设定为488nm、515nm及525nm。自适当标准曲线测定总RNA及游离RNA。

[0661] 囊封效率计算为(总RNA-游离RNA)/总RNA。可使用相同程序来测定基于DNA的运送子组分的囊封效率。对于单链DNA,可使用Oligreen染料,且对于双链DNA,可使用Picogreen

染料。

[0662] 通常,当制备LNP时,囊封>80%,粒径<120nm,且pdi<0.2。

[0663] 活体内LNP递送

[0664] 除非另有说明,否则在每一研究中使用6-10周龄范围内的CD-1雌性小鼠。对动物进行称重并根据体重分组,以基于组平均重量制备投用溶液。以0.2mL/动物的体积(每公斤体重约10mL)经由侧尾静脉投用LNP。在投药后约6小时观察动物的不良效应。在投药后24小时测量体重,且藉由在异氟醚麻醉下经由心脏穿刺驱血在不同时间点使动物安乐死。如本文所述,将血液收集至血清分离管中或含有用于血浆的缓冲柠檬酸钠的管中。对于涉及活体内编辑的研究,自每只动物的中间叶或三个独立叶(例如,右中值、左中值及左侧叶)收集肝组织用于DNA提取及分析。

[0665] 动物研究中所用的甲状腺素运载蛋白(TTR)ELISA分析

[0666] 收集血液并如所指示分离血清。使用小鼠前白蛋白(甲状腺素运载蛋白)ELISA试剂盒(Aviva Systems Biology,目录OKIA00111)测定小鼠TTR血清总含量;使用大鼠特异性ELISA试剂盒(Aviva Systems Biology,目录号OKIA00159)测量大鼠TTR血清含量;使用人类特异性ELISA试剂盒(Aviva Systems Biology,目录号OKIA00081)测量人类TTR血清含量;各自根据制造商的方案。简言之,当测量小鼠血清中的人类TTR时,用试剂盒样本稀释剂将血清连续稀释至10,000倍或5,000倍的最终稀释度。将100ul制备的标准曲线或稀释的血清样本添加至ELISA板中,于室温下培育30分钟,且然后用提供的洗涤缓冲液洗涤3次。然后向每一孔中添加100uL检测抗体,且于室温下培育20分钟,之后洗涤3次。添加100uL底物,然后于室温下培育10分钟,之后添加100uL停止溶液。在Spectramax M5分析盘读数仪上使用SoftmaxPro 7.0版软件进行分析,测量内容物的吸光度。使用4参数逻辑拟合将血清TTR含量根据标准曲线定量,并表示为ug/mL血清或相对于对照(媒剂处理的)动物的减弱百分比。

[0667] 基因组DNA分离

[0668] 在转化染后24、48或72小时收获转染细胞。根据制造商的方案使用50μL/孔BuccalAmp DNA提取溶液(Epicenter,目录QE09050)自96孔板的每一孔提取基因组DNA。如本文所述,使所有DNA样本经受PCR及随后的NGS分析。

[0669] 次世代测序(“NGS”)分析

[0670] 为了定量地测定基因组中靶位置的编辑效率,利用测序来鉴别由基因编辑引入的插入及缺失的存在。

[0671] 引物设计在所关注基因(例如TTR)内的靶位点周围,且扩增所关注基因组区域。

[0672] 根据制造商的方案(Illumina)实施额外PCR以添加用于测序的化学。在Illumina MiSeq仪器上对扩增子进行测序。在消除具有低质量评分的这些读数之后,将读数与参考基因组(例如,人类参考基因组(hg38)、食蟹猴参考基因组(mf5)、大鼠参考基因组(rn6)或小鼠参考基因组(mm10))进行比对。将含有读数的所得文件映射至参考基因组(BAM文件),其中选择与所关注靶区重迭的读数,且计算野生型读数的数量与含有插入、取代或缺失的读数的数量。

[0673] 编辑百分数(例如,“编辑效率”或“编辑百分比”或“插入或缺失频率”)定义为相对于序列读段(包括野生型)的总数的具有插入/缺失(“插入或缺失”)或取代的序列读段的总数。

[0674] 藉由西方墨点分析分泌的甲状腺素运载蛋白(“TTR”)蛋白

[0675] 使用蛋白质印迹法测定培养基中TTR蛋白的分泌含量。如前所述,用表1的选择引导转染HepG2细胞。在转染后每3天实施一次培养基更换。转染后6天,移除培养基,且用不合胎牛血清(FBS)的培养基洗涤细胞一次。将无血清的培养基添加至细胞中并在37℃下培育。4hr后,移除培养基并离心以使任何碎片成团块;基于自对剩余细胞的CTG分析获得的值来估计每一孔的细胞数且与板平均值进行比较。离心后,将培养基转移至新板并储存于-20℃下。实施培养基的丙酮沉淀以沉淀已分泌至培养基中的任何蛋白质。将四体积的冰冷丙酮添加至一体积的培养基中。将溶液充分混合并在-20℃下保持90min。将丙酮:培养基混合物以15,000xg且于4℃下离心15min。弃去上清液,且将保留的沉淀风干以消除任何残余丙酮。将团块重新悬浮于15μL RIPA缓冲液(Boston Bio Products,目录BP-115)及新鲜添加的蛋白酶抑制剂混合物中,该蛋白酶抑制剂混合物由全蛋白酶抑制剂混合剂(Sigma,目录11697498001)及1mM DTT组成。将溶解物与Laemmli缓冲液混合,且在95℃下变性10分钟。使用NuPage系统在12%Bis-Tris凝胶(ThermoFisher)上根据制造商的方案运行蛋白质印迹法,之后湿转移至0.45μm硝化纤维素膜(Bio-Rad,目录1620115)上。于室温下在实验室游戏杆上使用TBS中的5%奶粉封闭墨点30分钟。将墨点用TBST冲洗,并用TBST中1:1000的兔α-TTR单株抗体(Abcam,目录Ab75815)探测。使用α-1抗胰蛋白酶作为TBST中的1:1000的上样对照(Sigma,目录HPA001292),并与TTR一级抗体同时培育。将墨点密封于袋中并在4℃下在实验室游戏杆上保持过夜。培育后,将墨点在TBST中冲洗3次,每次5min,并于室温下用TBST中1:25,000的兔的二次抗体(ThermoFisher,目录PISA535571)探测30min。培育后,将墨点在TBST中冲洗3次,每次5min,且用PBS冲洗2次。使用Licor Odyssey系统使墨点可视化并对其进行分析。

[0676] 藉由西方墨点分析细胞内TTR

[0677] 如前所述用表1的选择引导转染肝细胞癌细胞系HUH7。转染后6天,移除培养基并用50μL/孔RIPA缓冲液(Boston Bio Products,目录BP-115)加上新鲜添加的蛋白酶抑制剂混合物溶解细胞,该蛋白酶抑制剂混合物由全蛋白酶抑制剂混合剂(Sigma,目录11697498001)、1mM DTT及250U/ml全能核酸酶(Benzonase)(EMD Millipore,目录71206-3)组成。将细胞在冰上保持30分钟,此时添加NaCl(1M最终浓度)。将细胞溶解物充分混合并在冰上保留30分钟。将全细胞提取物(“WCE”)转移至PCR板并离心以使碎片成团块。使用Bradford分析(Bio-Rad,目录500-0001)来评估溶解物的蛋白含量。根据制造商的方案完成Bradford分析程序。使用前将提取物储存在-20℃下。实施蛋白质印迹法以评估细胞内TTR蛋白含量。将溶解物与Laemmli缓冲液混合,且在95℃下变性10min。使用NuPage系统在12%Bis-Tris凝胶(ThermoFisher)上根据制造商的方案运行蛋白质印迹法,之后湿转移至0.45μm硝化纤维素膜(Bio-Rad,目录1620115)上。转移后,用水彻底冲洗膜,并用Ponceau S溶液(Boston Bio Products,目录ST-180)染色,以确认完全且均匀转移。于室温下在实验室游戏杆上使用TBS中的5%奶粉封闭墨点30分钟。将墨点用TBST冲洗,并用TBST中1:1000的兔α-TTR单株抗体(Abcam,目录Ab75815)探测。使用TBST中1:2500的β-肌动蛋白作为上样对照(ThermoFisher,目录AM4302)且与TTR一级抗体同时培育。将墨点密封于袋中并在4℃下在实验室游戏杆上保持过夜。培育后,将墨点在TBST中冲洗3次,每次5分钟,并于室温下用TBST中1:25,000的小鼠及兔的二次抗体(ThermoFisher,目录PI35518及PISA535571)各探

测30min。培育后，将墨点在TBST中冲洗3次，每次5min，且用PBS冲洗2次。使用Licor Odyssey系统使墨点可视化并对其进行分析。

[0678] 实例2.dgRNA序列的筛选

[0679] 多种细胞类型中TTR dgRNA的交叉筛选

[0680] 将靶向人类TTR及食蟹猴匹配序列的dgRNA格式中的引导递送至HEK293_Cas9、HUH7及HepG2细胞系以及如实例1中所述的原代人类肝细胞及原代食蟹猴肝细胞。测定包含跨每一细胞类型的每一引导序列的crRNA的编辑百分比，且然后基于最高编辑％对引导序列分等级次序。下文列示所有五种细胞系中表1中的引导序列的筛选数据(表4至11)。

[0681] 表4显示人类肾腺癌细胞系HEK293_Cas9中TTR crRNA的编辑％、插入(Ins)％及缺失(Del)％的平均及标准偏差，该人类肾腺癌细胞系组成型地过度表达Spy Cas9蛋白。

[0682] 表4:经dgRNA转染的Hek_Cas9细胞中的TTR编辑数据

[0683]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR003335	26.59	4.73	4.73	0.65	21.87	4.09
CR003336	29.09	4.57	3.31	0.24	25.78	4.32
CR003337	42.72	1.72	5.24	1.62	37.48	0.70
CR003338	52.42	3.28	4.76	0.03	47.66	3.30
CR003339	56.37	4.13	49.39	3.23	6.98	0.91
CR003340	42.38	8.43	27.88	4.31	14.50	4.13
CR003341	20.04	5.26	6.73	1.86	13.31	3.41

[0684]

CR003342	36.57	5.80	1.19	0.22	35.38	5.59
CR003343	24.36	1.51	4.82	0.43	19.53	1.39
CR003344	33.87	2.93	4.32	0.58	29.54	2.37
CR003345	35.02	7.05	19.00	3.58	16.01	3.48
CR003346	48.33	5.81	33.03	3.12	15.30	2.72
CR003347	21.45	5.57	0.95	0.33	20.50	5.26
CR003348	35.53	5.81	22.32	3.79	13.21	2.03
CR003349	13.19	4.46	8.03	2.81	5.16	1.66
CR003350	22.31	4.25	5.54	0.74	16.77	3.51
CR003351	49.67	3.77	28.42	1.69	21.24	2.22
CR003352	27.90	7.55	4.91	1.35	22.99	6.26
CR003353	25.03	5.16	3.71	0.75	21.32	4.42
CR003354	18.46	2.02	2.56	0.21	15.90	1.89
CR003355	30.60	2.53	6.99	0.80	23.61	1.75
CR003356	32.21	4.71	10.03	1.39	22.19	3.36
CR003357	43.23	6.71	5.38	0.87	37.85	5.88
CR003358	5.44	0.86	1.29	0.16	4.14	0.84
CR003359	37.75	7.50	18.35	3.73	19.40	3.78
CR003360	22.68	3.16	2.70	0.56	19.98	2.60
CR003361	34.45	8.97	8.66	1.66	25.78	7.32
CR003362	9.90	2.66	1.48	0.33	8.41	2.33
CR003363	31.03	10.74	14.77	4.21	16.26	6.54
CR003364	35.65	7.90	19.17	4.24	16.48	3.76
CR003365	36.43	6.20	11.83	1.88	24.61	4.45
CR003366	47.36	6.59	10.10	1.28	37.26	5.32
CR003367	47.11	15.43	28.44	9.11	18.67	6.33
CR003368	40.35	10.13	3.73	0.96	36.61	9.17
CR003369	33.10	7.26	9.06	1.12	24.04	6.16
CR003370	34.22	5.69	4.49	0.67	29.73	5.06
CR003371	25.60	8.33	3.84	1.41	21.76	6.92
CR003372	15.24	7.92	3.25	1.61	11.99	6.31
CR003373	13.55	2.40	1.31	0.21	12.25	2.19
CR003374	10.91	0.88	0.81	0.10	10.10	0.81
CR003375	11.63	3.18	0.78	0.17	10.85	3.05
CR003376	28.16	4.49	1.35	0.18	26.81	4.52
CR003377	24.70	4.44	2.71	0.54	21.99	3.91
CR003378	20.97	2.67	4.49	0.49	16.48	2.18
CR003379	26.32	2.91	5.34	0.61	20.98	2.30
CR003380	47.64	5.74	3.64	0.24	44.00	5.52
CR003381	22.04	5.74	3.82	1.26	18.23	4.64
CR003382	29.95	3.13	4.46	0.45	25.49	2.73

[0685]

CR003383	40.47	0.64	25.12	0.45	15.35	0.66
CR003384	17.45	1.32	1.45	0.23	16.00	1.42
CR003385	26.19	5.62	7.36	1.57	18.82	4.06
CR003386	33.12	10.65	2.94	0.63	30.18	10.03
CR003387	24.68	5.93	7.75	1.99	16.92	3.94
CR003388	19.23	4.41	1.41	0.39	17.82	4.07
CR003389	34.18	5.09	10.30	2.12	23.87	3.02
CR003390	28.02	3.77	4.31	0.25	23.71	3.61
CR003391	44.81	4.67	0.61	0.07	44.19	4.63
CR003392	21.67	7.52	0.85	0.26	20.82	7.27

[0686] 表5显示人类肝细胞癌细胞系HUH7中经Spy Cas9 mRNA(SEQ ID NO:2)共转染的测试TTR crRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0687] 表5:经Spy Cas9 mRNA及dgRNA转染的HUH7细胞中的TTR编辑数据

[0688]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR003335	31.95	4.50	4.62	0.83	27.57	4.08
CR003336	30.05	4.25	4.14	1.07	26.56	3.55
CR003337	55.72	3.12	8.34	0.93	48.95	2.24
CR003338	75.64	2.03	10.22	1.42	67.06	2.79
CR003339	79.97	4.73	60.55	3.94	20.13	1.02
CR003340	46.93	7.12	33.33	6.01	14.23	1.65
CR003341	20.58	5.98	7.78	1.64	13.20	4.44
CR003342	45.14	7.16	1.23	0.91	44.66	7.68
CR003343	76.13	7.04	9.58	3.49	66.97	6.10
CR003344	64.02	3.33	10.76	1.35	54.40	2.71
CR003345	72.43	2.17	41.33	0.96	32.18	1.37
CR003346	18.07	1.02	13.17	1.39	6.97	3.06
CR003347	32.16	5.50	1.64	0.42	30.79	5.11
CR003348	57.14	10.98	36.08	6.97	22.71	4.42
CR003349	14.14	4.99	9.73	3.26	4.82	1.91
CR003350	52.91	7.61	13.43	2.00	41.64	6.03
CR003351	63.51	4.61	36.87	2.49	27.49	2.14
CR003352	39.68	9.53	7.62	7.42	32.79	7.37
CR003353	69.18	4.59	7.73	2.46	62.87	3.13
CR003354	12.27	3.38	1.25	0.40	11.46	3.23
CR003355	38.83	5.31	9.40	1.81	30.31	3.56

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CR003356	49.63	5.55	18.98	2.67	31.31	3.04
CR003357	36.31	5.72	6.37	1.17	30.82	4.68
CR003358	36.50	6.17	10.53	1.56	26.60	4.49
CR003359	66.75	5.84	21.73	2.30	45.97	3.93
CR003360	58.62	8.73	5.01	0.60	55.13	8.19
CR003361	28.68	6.52	6.84	1.26	22.44	5.31
CR003362	26.43	0.83	3.43	0.32	23.76	0.85
CR003363	41.01	7.16	17.83	3.32	23.78	3.97
CR003364	47.13	10.61	24.68	5.15	23.03	5.74
CR003365	60.68	5.25	17.77	1.57	43.82	3.73
CR003366	69.98	8.84	20.77	3.10	50.32	5.69
CR003367	66.29	4.48	33.62	4.14	33.48	0.51
CR003368	31.57	11.73	3.08	0.92	29.69	11.32
CR003369	24.19	6.89	7.12	2.27	17.38	4.76
CR003370	39.16	11.59	4.83	1.79	35.55	10.35
CR003371	40.47	7.68	6.07	0.89	35.65	7.01
CR003372	21.52	6.02	4.89	1.66	17.25	4.58
CR003373	27.29	4.45	3.31	0.66	25.12	4.12
CR003374	3.10	0.68	0.45	0.24	2.87	0.54
CR003375	2.38	0.22	0.26	0.14	2.25	0.12
CR003376	19.42	5.60	1.37	0.45	18.55	5.28
CR003377	34.93	5.47	5.59	0.88	29.89	4.71
CR003378	40.73	4.63	9.73	1.85	32.27	2.91
CR003379	19.18	5.17	3.38	0.77	16.48	4.32
CR003380	31.76	5.81	3.29	0.57	29.29	5.42
CR003381	99.70	0.17	1.92	0.20	99.70	0.17
CR003382	34.47	5.71	0.14	0.16	34.47	5.71
CR003383	42.89	10.14	2.14	0.56	41.19	9.67
CR003384	17.03	1.95	0.84	0.30	16.29	1.84
CR003386	69.40	19.41	0.53	0.23	69.34	19.32
CR003387	25.64	3.69	0.23	0.07	25.55	3.62
CR003388	59.48	4.29	3.88	0.68	56.45	4.45
CR003389	62.32	1.97	13.19	1.18	50.90	1.02
CR003390	18.97	4.82	3.31	0.91	16.49	3.98
CR003391	61.31	13.21	2.10	0.51	59.70	12.76
CR003392	28.37	8.58	1.93	0.73	26.98	7.94

[0690] 表6显示人类肝细胞癌细胞系HepG2中经Spy Cas9 mRNA(SEQ ID NO:2)共转染的测试TTR及对照crRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0691] 表6:经Spy Cas9 mRNA及dgRNA转染的HepG2细胞中的TTR编辑数据

[0692]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR001261 (对照)	49.16	7.45	16.46	3.46	32.71	4.06
CR001262 (对照)	63.33	5.66	59.88	4.92	3.45	0.86
CR001263 (对照)	39.19	6.98	37.59	8.01	1.60	1.92
CR001264 (对照)	57.09	12.14	47.47	9.25	9.61	2.89
CR003335	37.19	2.12	32.96	1.67	4.23	0.59
CR003336	31.31	5.47	30.48	5.10	0.83	0.75
CR003337	61.93	2.68	59.28	2.11	2.65	1.39
CR003338	68.00	6.09	65.40	6.78	2.60	1.17
CR003339	68.21	7.67	12.37	1.47	55.84	6.31
CR003340	37.76	6.01	6.12	1.95	31.65	4.07
CR003341	15.60	5.49	9.94	3.38	5.66	2.13
CR003342	11.06	6.71	10.78	6.69	0.28	0.03
CR003343	45.41	15.20	40.05	10.79	5.36	5.20
CR003344	33.43	6.11	29.81	5.09	3.62	1.13
CR003345	10.58	9.25	6.12	5.38	4.45	3.87
CR003346	0.13	0.05	0.07	0.02	0.05	0.03
CR003347	22.57	10.94	21.08	11.19	1.49	0.90
CR003348	38.44	10.45	17.04	5.04	21.40	5.89
CR003349	8.36	2.19	4.46	1.75	3.91	0.76
CR003350	29.60	5.17	25.16	4.56	4.44	0.67
CR003351	57.54	5.67	31.98	2.63	25.57	3.08
CR003352	44.28	8.71	39.51	7.10	4.77	1.79
CR003353	60.40	11.37	56.71	9.95	3.68	1.45
CR003354	5.36	3.94	4.84	3.41	0.53	0.71
CR003355	15.80	5.38	12.36	4.23	3.44	1.16
CR003356	9.39	1.82	5.67	1.03	3.72	0.92
CR003357	45.83	10.66	42.37	8.47	3.46	2.28
CR003358	35.93	7.34	28.66	7.76	7.27	1.77
CR003359	64.44	14.90	48.79	14.32	15.65	1.94
CR003360	41.31	12.23	38.94	10.60	2.38	1.78
CR003361	14.05	4.79	11.47	4.35	2.58	0.43
CR003362	17.44	4.34	16.50	4.86	0.94	0.52
CR003363	42.65	9.90	28.58	6.95	14.07	3.01

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CR003364	51.88	7.67	31.03	2.67	20.85	5.03
CR003365	46.88	15.78	35.77	13.49	11.11	2.30
CR003366	54.69	9.10	46.20	8.98	8.49	1.11
CR003367	45.55	8.19	24.28	6.57	21.27	1.62
CR003368	51.55	8.60	48.34	9.87	3.22	1.36
CR003369	22.62	4.01	17.11	4.47	5.51	2.52
CR003370	28.51	6.94	24.88	6.17	3.62	1.45
CR003371	15.91	4.17	14.07	4.02	1.84	0.22
CR003372	14.57	2.47	12.14	2.08	2.42	0.40
CR003373	17.69	8.41	15.92	6.44	1.77	1.97
CR003374	5.43	0.53	5.12	0.62	0.31	0.36
CR003375	2.06	0.04	1.96	0.06	0.10	0.03
CR003376	14.41	3.01	14.16	2.93	0.24	0.10
CR003377	16.30	2.85	15.29	2.59	1.02	0.59
CR003378	8.16	3.83	6.82	3.43	1.34	0.61
CR003379	19.74	4.24	17.70	4.30	2.04	0.33
CR003380	17.08	2.48	14.78	1.18	2.30	1.36
CR003381	6.81	3.48	6.18	3.82	0.63	0.44
CR003382	1.73	0.14	1.58	0.12	0.15	0.03
CR003383	6.35	1.67	6.19	1.68	0.16	0.04
CR003384	3.37	0.88	3.12	0.94	0.25	0.09
CR003385	53.94	9.41	46.32	10.66	7.62	1.29
CR003386	2.71	0.76	2.15	0.77	0.56	0.53
CR003387	1.39	0.15	1.27	0.17	0.12	0.02
CR003388	9.33	4.47	7.76	4.56	1.56	0.10
CR003389	31.84	6.09	27.27	5.96	4.57	1.21
CR003390	24.88	4.96	22.44	3.41	2.44	2.25
CR003391	48.78	14.41	48.28	14.44	0.50	0.52
CR003392	14.64	5.25	14.32	4.95	0.33	0.36
CR005298	42.65	10.94	21.29	8.16	21.36	2.87
CR005299	38.61	5.57	36.32	3.99	2.30	2.11
CR005300	64.34	9.55	53.20	6.59	11.15	3.33
CR005301	37.04	5.32	33.39	3.85	3.65	1.89
CR005302	33.21	2.19	30.93	2.43	2.29	0.24
CR005303	21.63	6.05	20.55	5.80	1.08	0.25
CR005304	62.82	3.28	8.07	1.22	54.75	4.27
CR005305	13.51	3.58	12.30	3.49	1.21	0.84
CR005306	24.07	5.24	21.20	5.03	2.87	1.10
CR005307	22.03	3.86	7.70	1.35	14.33	4.15

[0694] 表7显示原代人类肝细胞中经Spy Cas9蛋白(RNP)电穿孔的测试TTR dgRNA的编辑％、插入(Ins)％及缺失(Del)％的平均值及标准偏差。

[0695] 表7:经装载有dgRNA的Spy Cas9蛋白电穿孔的原代人类肝细胞中的TTR编辑数据

[0696]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR003335	72.20	4.53	69.70	4.36	2.50	0.30
CR003336	39.17	3.04	38.43	3.20	0.70	0.17
CR003337	54.27	2.70	53.23	3.05	1.30	0.26
CR003338	83.03	4.84	80.87	4.63	2.13	0.25
CR003339	43.00	2.66	8.93	1.86	34.07	1.72
CR003340	12.03	1.55	5.60	1.32	6.50	0.53
CR003341	11.43	0.71	7.03	0.50	4.40	1.21
CR003342	32.77	3.63	31.87	3.28	0.90	0.35
CR003343	77.10	2.21	75.63	2.01	1.50	0.36
CR003344	39.40	3.86	33.30	2.52	6.10	1.31
CR003345	48.07	6.24	34.53	2.95	13.57	3.74
CR003346	35.67	1.80	20.83	1.65	14.83	1.66
CR003347	82.30	5.93	81.97	5.98	0.43	0.15
CR003348	28.53	1.79	11.30	2.46	17.27	0.86
CR003349	4.10	0.17	2.33	0.46	1.87	0.25
CR003350	28.13	3.50	22.40	2.41	5.73	1.22
CR003351	51.77	5.11	30.83	3.32	20.97	2.43
CR003352	29.83	4.18	25.63	3.67	4.30	0.56
CR003353	84.83	4.68	82.23	4.05	2.63	0.74
CR003354	2.50	0.36	2.43	0.32	0.03	0.06
CR003355	12.53	1.54	10.60	2.36	1.97	1.17
CR003356	9.97	2.68	7.80	2.01	2.23	0.85
CR003357	36.23	4.02	35.47	4.11	0.77	0.61
CR003358	5.70	1.42	4.93	1.36	0.80	0.26
CR003359	63.77	7.07	56.33	5.81	7.50	1.35
CR003360	32.23	3.09	31.67	2.97	0.63	0.31
CR003361	4.10	0.36	3.73	0.42	0.37	0.06
CR003362	7.03	1.30	6.87	1.20	0.20	0.20
CR003363	9.43	8.22	7.80	6.86	1.63	1.44
CR003364	23.30	5.20	16.93	4.96	6.53	0.55
CR003365	42.37	3.88	35.57	1.88	6.83	2.00
CR003366	34.70	3.26	31.63	2.98	3.10	1.15
CR003367	39.20	5.31	22.93	4.14	16.37	1.46
CR003368	28.47	3.29	27.63	2.90	0.80	0.66
CR003369	3.67	1.16	3.30	1.06	0.40	0.20

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CR003370	15.27	1.75	14.43	1.72	0.90	0.20
CR003371	16.20	2.13	14.47	2.37	1.87	0.81
CR003372	12.17	2.69	10.47	2.63	1.77	0.12
CR003373	0.87	0.21	0.83	0.25	0.07	0.12
CR003374	0.80	0.17	0.70	0.26	0.10	0.10
CR003375	1.33	1.10	1.27	1.08	0.07	0.06
CR003376	1.90	1.06	1.87	1.00	0.03	0.06
CR003377	10.23	1.53	10.13	1.51	0.10	0.10
CR003378	4.60	1.92	3.87	1.19	0.73	0.67
CR003379	6.57	1.00	6.30	0.70	0.27	0.31
CR003380	5.37	2.57	5.27	2.54	0.10	0.10
CR003381	6.20	2.74	5.83	2.61	0.50	0.10
CR003382	8.40	2.07	8.10	1.87	0.43	0.21
CR003383	8.57	0.75	3.37	0.67	5.27	0.46
CR003384	1.87	0.67	1.73	0.57	0.23	0.12
CR003385	40.87	6.86	38.43	6.41	2.53	0.45
CR003386	4.90	1.20	4.47	1.14	0.47	0.25
CR003387	1.87	0.25	1.70	0.26	0.20	0.10
CR003388	5.70	0.40	5.47	0.40	0.27	0.12
CR003389	27.67	2.76	27.20	2.88	0.50	0.36
CR003390	15.97	3.86	15.80	3.99	0.23	0.15
CR003391	29.77	3.85	29.57	3.85	0.27	0.06
CR003392	4.13	1.21	4.00	1.15	0.17	0.06
CR005298	39.90	2.92	22.37	3.04	17.57	0.42
CR005299	8.65	0.78	8.30	0.99	0.35	0.21
CR005300	57.47	1.69	53.47	1.86	4.10	0.92
CR005301	25.37	1.65	24.00	2.26	1.60	0.82
CR005302	61.10	5.20	60.10	4.77	1.00	0.46
CR005303	53.57	8.52	53.07	8.36	0.53	0.47
CR005304	67.00	5.80	5.53	1.37	61.63	6.98
CR005305	3.83	0.78	3.53	0.61	0.40	0.17
CR005306	9.43	1.63	8.07	2.17	1.37	0.72
CR005307	8.17	1.20	5.20	0.87	3.00	0.82

[0698] 表8显示原代人类肝细胞中经Spy Cas9蛋白(RNP)转染的测试TTR及对照dgRNA的编辑％、插入(Ins)％及缺失(Del)％的平均值及标准偏差。

[0699] 表8:经装载有dgRNA的Spy Cas9转染的原代人类肝细胞中的TTR编辑数据

[0700]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR001261	32.51	1.00	12.50	0.47	20.01	0.59
CR001262	50.09	1.48	45.25	1.69	4.83	0.31
CR001263	15.25	2.41	14.83	2.37	0.42	0.10
CR001264	45.30	3.48	23.87	2.09	21.43	1.68
CR003335	51.14	4.27	49.51	4.04	1.63	0.25
CR003336	30.70	2.41	30.11	2.48	0.58	0.11
CR003337	49.43	4.75	47.54	4.49	1.88	0.47
CR003338	61.34	3.55	59.13	3.44	2.22	0.11
CR003339	45.06	9.83	8.85	1.65	36.21	8.34
CR003340	10.44	2.44	5.94	1.34	4.50	1.16
CR003341	19.66	3.67	14.64	3.31	5.02	0.37
CR003342	20.66	2.55	19.85	2.54	0.81	0.15
CR003343	43.25	4.47	41.61	4.26	1.63	0.33
CR003344	35.45	13.12	30.97	11.72	4.48	1.51
CR003345	28.90	6.33	21.00	5.23	7.91	1.81
CR003346	4.11	1.36	2.27	0.53	1.84	0.85
CR003347	66.35	4.48	66.11	4.51	0.24	0.08
CR003348	23.18	2.16	13.74	1.17	9.44	0.99
CR003349	10.83	1.57	9.00	1.41	1.83	0.32
CR003350	24.84	2.74	19.77	1.91	5.07	0.89
CR003351	40.28	1.31	23.92	0.70	16.36	0.78
CR003352	30.48	1.93	27.27	2.31	3.21	0.38
CR003353	61.54	4.13	59.38	4.04	2.16	0.11
CR003354	10.31	1.47	10.07	1.50	0.23	0.11
CR003355	19.11	0.92	17.69	0.79	1.42	0.44
CR003356	7.53	1.78	6.24	1.51	1.29	0.32
CR003357	49.35	2.53	48.45	2.54	0.90	0.13
CR003358	31.62	5.97	25.95	5.03	5.67	1.04
CR003359	59.47	6.05	50.96	5.69	8.51	0.54
CR003360	31.47	4.12	30.27	4.21	1.19	0.22
CR003361	13.08	1.48	12.52	1.45	0.56	0.18
CR003362	11.65	1.24	11.10	1.06	0.56	0.36
CR003363	27.65	2.84	21.47	2.39	6.18	0.61
CR003364	35.29	3.50	23.93	2.63	11.36	1.16
CR003365	47.78	3.67	40.24	3.12	7.54	0.72
CR003366	42.74	3.41	37.95	2.88	4.79	0.60
CR003367	31.19	4.60	16.06	2.66	15.13	1.94
CR003368	34.83	5.05	33.83	5.09	1.00	0.10
CR003369	12.98	0.26	11.67	0.21	1.31	0.11

[0701]

CR003370	20.06	1.79	18.80	1.65	1.26	0.28
CR003371	18.80	2.73	17.23	2.34	1.57	0.43
CR003372	17.56	2.26	15.74	2.16	1.81	0.10
CR003373	3.64	0.29	3.44	0.30	0.19	0.07
CR003374	2.65	0.33	2.52	0.33	0.14	0.02
CR003375	5.04	0.66	4.93	0.66	0.11	0.01
CR003376	5.00	1.10	4.86	1.10	0.14	0.03
CR003377	12.77	2.00	12.45	1.84	0.31	0.18
CR003378	8.66	1.90	8.24	1.74	0.42	0.19
CR003379	16.86	2.62	16.51	2.62	0.34	0.08
CR003380	8.17	1.42	7.71	1.47	0.46	0.10
CR003381	7.15	0.73	6.88	0.67	0.27	0.07
CR003382	2.44	0.06	2.28	0.05	0.15	0.03
CR003383	4.76	0.40	4.52	0.42	0.24	0.09
CR003384	3.56	0.26	3.39	0.26	0.17	0.01
CR003385	41.15	6.06	38.15	5.59	3.00	0.48
CR003386	3.22	0.25	2.97	0.27	0.25	0.02
CR003387	1.79	0.11	1.68	0.09	0.11	0.04
CR003388	5.43	1.03	4.38	1.00	1.05	0.25
CR003389	19.87	4.39	19.19	4.52	0.68	0.24
CR003390	16.09	2.84	15.85	2.91	0.24	0.09
CR003391	34.72	8.29	34.46	8.35	0.26	0.06
CR003392	10.07	1.06	9.93	1.02	0.14	0.04
CR005298	32.07	1.02	21.12	1.02	10.95	0.15
CR005299	19.37	0.61	18.79	0.51	0.58	0.13
CR005300	57.23	6.24	53.62	5.44	3.61	0.87
CR005301	31.37	3.02	29.53	2.88	1.84	0.15
CR005302	48.29	5.22	47.32	5.32	0.97	0.14
CR005303	36.45	4.83	36.06	4.72	0.39	0.12
CR005304	49.45	6.85	4.32	0.31	45.13	6.74
CR005305	7.07	1.43	6.73	1.30	0.34	0.17
CR005306	18.81	1.82	16.24	1.57	2.57	0.35
CR005307	18.73	1.68	10.18	0.92	8.55	0.88

[0702] 表9显示原代人类肝细胞中经Spy Cas9 mRNA(SEQ ID NO:2)共转染的测试TTR及对照dgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0703] 表9:经Spy Cas9 mRNA及dgRNA转染的原代人类肝细胞中的TTR编辑数据

[0704]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR001261	32.33	4.95	5.83	1.63	26.47	3.30
CR001262	41.50	4.71	34.43	3.31	7.13	1.42
CR001263	10.23	3.61	9.40	3.20	0.90	0.44
CR001264	42.80	0.50	11.90	1.32	30.90	1.80
CR003335	36.43	2.98	33.03	2.31	3.40	0.70
CR003336	16.93	3.78	16.20	3.41	0.80	0.44
CR003337	19.30	1.57	18.10	1.44	1.23	0.15
CR003338	36.30	9.55	33.73	9.27	2.73	0.49
CR003339	36.43	1.21	2.27	0.15	34.23	1.31
CR003340	24.97	2.78	1.83	0.23	23.17	2.66
CR003341	15.83	1.38	6.80	0.53	9.07	0.81
CR003342	22.10	1.27	20.60	0.57	1.50	0.71
CR003343	55.03	0.38	52.40	0.53	2.60	0.44
CR003344	31.50	1.30	22.40	1.31	9.20	0.10
CR003345	50.65	2.90	32.30	1.56	18.45	1.20
CR003346	19.97	1.94	5.63	0.55	14.33	1.72
CR003347	41.47	3.59	41.33	3.63	0.17	0.06
CR003348	18.00	0.87	2.30	0.66	15.80	0.61
CR003349	2.57	0.81	0.90	0.35	1.70	0.46
CR003350	26.63	4.25	16.33	2.45	10.33	1.75
CR003351	26.50	1.61	10.20	0.92	16.37	0.97
CR003352	16.80	5.03	11.73	3.86	5.07	1.14
CR003353	53.73	6.01	49.50	5.82	4.43	0.75
CR003354	2.97	0.95	2.87	0.85	0.13	0.12
CR003355	12.07	2.61	10.47	2.08	1.63	0.59
CR003356	7.27	0.72	4.70	0.53	2.67	0.21
CR003357	25.93	4.55	25.30	4.22	0.63	0.35
CR003358	3.90	0.79	2.73	0.45	1.17	0.51
CR003359	32.93	4.34	25.67	3.25	7.33	1.24
CR003360	14.90	4.85	14.13	4.66	0.90	0.52
CR003361	3.53	0.60	2.73	0.55	0.87	0.15
CR003362	6.60	1.47	6.17	1.45	0.47	0.21
CR003363	16.70	1.08	11.80	0.79	4.93	0.60
CR003364	15.63	2.45	6.73	0.81	8.93	1.70
CR003365	26.90	3.05	20.23	2.02	6.67	1.16
CR003366	24.53	1.26	20.47	1.45	4.07	0.23
CR003367	37.33	1.40	14.03	0.40	23.37	1.25
CR003368	11.10	1.91	10.53	1.90	0.60	0.10
CR003369	1.60	0.46	0.90	0.20	0.70	0.36

[0705]

CR003370	2.83	0.57	2.33	0.40	0.50	0.17
CR003371	3.40	0.80	2.67	0.75	0.73	0.15
CR003372	1.77	0.75	1.13	0.57	0.63	0.23
CR003373	1.40	0.36	1.00	0.35	0.37	0.12
CR003374	0.27	0.21	0.27	0.21	0.03	0.06
CR003375	1.27	0.64	1.23	0.58	0.03	0.06
CR003376	2.83	0.81	2.73	0.81	0.13	0.06
CR003377	17.53	6.35	16.97	6.11	0.57	0.25
CR003378	9.80	1.37	8.50	1.21	1.37	0.15
CR003379	13.20	1.18	12.00	1.05	1.27	0.15
CR003380	2.93	0.58	2.47	0.57	0.47	0.15
CR003381	4.07	1.21	3.33	0.96	0.73	0.25
CR003382	0.97	0.25	0.97	0.25	0.00	0.00
CR003383	15.70	3.22	2.07	0.35	13.70	2.82
CR003384	1.70	0.62	1.50	0.56	0.20	0.10
CR003385	36.77	0.70	33.23	0.74	3.60	0.26
CR003386	8.27	1.63	8.20	1.57	0.13	0.06
CR003387	7.87	1.58	7.80	1.64	0.03	0.06
CR003388	12.97	1.30	11.87	1.21	1.17	0.25
CR003389	44.27	1.72	41.47	1.59	2.83	0.15
CR003390	20.23	2.08	18.73	1.92	1.60	0.17
CR003391	15.47	5.87	15.20	5.72	0.30	0.10
CR003392	2.43	0.55	2.37	0.59	0.07	0.06
CR005298	15.70	2.79	4.13	0.87	11.60	2.00
CR005299	9.43	0.68	8.93	0.68	0.60	0.00
CR005300	31.53	3.44	27.60	2.77	3.97	0.76
CR005301	6.77	1.44	5.47	0.96	1.40	0.61
CR005302	34.80	7.17	33.67	7.01	1.13	0.21
CR005303	35.50	5.90	35.00	5.81	0.50	0.10
CR005304	45.27	4.71	0.83	0.15	44.47	4.57
CR005305	7.53	1.06	5.93	1.10	1.60	0.10
CR005306	9.97	0.38	7.13	0.23	2.87	0.12
CR005307	12.90	2.43	3.67	0.61	9.30	1.80

[0706] 表10显示原代食蟹猴肝细胞中经Spy Cas9蛋白(RNP)电穿孔的测试TTR dgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0707] 表10:经Spy Cas9蛋白及dgRNA电穿孔的原代食蟹猴肝细胞中的TTR编辑数据

[0708]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR003336	8.18	1.93	8.10	1.94	0.07	0.01
CR003337	24.94	5.80	24.10	4.71	0.84	1.10
CR003338	44.94	9.99	44.89	9.97	0.05	0.01
CR003339	8.95	0.89	4.93	0.64	4.02	0.25
CR003340	12.53	2.22	7.72	0.13	4.80	2.09
CR003341	8.43	10.53	7.66	9.91	0.77	0.63
CR003344	35.72	4.67	33.81	5.29	1.91	0.61
CR003345	52.92	3.26	30.74	0.78	22.19	2.48
CR003346	1.91	0.86	1.82	0.82	0.09	0.04
CR003347	72.41	0.38	72.15	0.73	0.25	0.34
CR003352	1.25	0.20	1.16	0.21	0.09	0.01
CR003353	4.75	0.43	4.67	0.47	0.08	0.04
CR003358	20.47	0.30	19.01	0.51	1.46	0.21
CR003359	46.17	1.14	40.66	2.00	5.51	0.86
CR003360	29.47	0.63	29.05	1.00	0.42	0.37
CR003361	4.53	0.14	4.46	0.18	0.08	0.04
CR003362	4.59	0.80	4.36	0.77	0.22	0.03
CR003363	15.64	1.92	13.24	2.65	2.39	0.73
CR003364	19.62	2.54	14.27	2.72	5.35	0.17
CR003365	10.31	1.81	9.33	1.80	0.97	0.01
CR003366	18.52	0.71	17.62	0.33	0.90	0.39
CR003368	18.56	3.89	18.30	3.77	0.26	0.11
CR003369	1.53	0.25	1.28	0.40	0.25	0.15
CR003370	2.52	0.64	2.40	0.63	0.12	0.01
CR003371	1.83	0.38	1.69	0.41	0.14	0.03
CR003372	2.15	0.30	1.83	0.33	0.32	0.04
CR003382	10.86	2.04	8.54	1.93	2.33	0.11
CR003383	8.86	2.30	4.31	0.69	4.55	1.61
CR003384	3.75	0.35	2.50	0.37	1.25	0.02
CR003385	30.96	1.61	26.84	2.20	4.12	0.59
CR003386	5.54	1.42	3.51	1.26	2.03	0.15
CR003387	4.72	0.03	4.55	0.08	0.17	0.11
CR003388	6.81	0.17	6.59	0.28	0.22	0.11
CR003389	18.83	4.99	18.05	4.92	0.78	0.07
CR003390	16.87	3.88	16.49	3.48	0.39	0.39
CR003391	36.44	1.09	35.73	1.37	0.71	0.28
CR003392	7.02	0.97	6.63	0.59	0.38	0.37
CR005299	13.48	2.96	13.23	2.74	0.26	0.22
CR005301	46.76	1.75	46.34	2.19	0.42	0.44

[0709]

CR005302	1.34	0.19	1.26	0.19	0.08	0.00
CR005303	59.28	1.05	58.72	1.06	0.56	0.00
CR005305	11.28	0.39	11.13	0.39	0.15	0.00
CR005307	4.56	0.71	2.01	0.49	2.55	0.21

[0710] 表11显示原代食蟹猴肝细胞上经Spy Cas9蛋白(RNP)电穿孔的测试的食蟹猴特异性TTR dgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0711] 表11:经Spy Cas9蛋白及食蟹猴特异性dgRNA电穿孔的原代食蟹猴肝细胞中的TTR编辑数据

[0712]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
CR000689	24.41	1.67	18.11	2.41	6.30	0.93
CR005364	27.70	0.74	0.58	0.29	27.11	0.60
CR005365	64.94	2.03	0.10	0.04	64.85	2.05
CR005366	77.00	1.17	0.33	0.27	76.67	0.99
CR005367	50.79	0.53	0.53	0.25	50.26	0.36
CR005368	27.60	2.07	0.33	0.45	27.27	2.32
CR005369	42.01	0.33	8.09	0.55	33.92	0.31
CR005370	63.52	3.21	0.59	0.33	62.93	2.88
CR005371	8.42	0.69	0.31	0.12	8.10	0.57
CR005372	17.98	1.39	0.83	0.77	17.16	0.71

[0713] 实例3.sgRNA序列的筛选

[0714] 多个细胞类型中TTR sgRNA的交叉筛选

[0715] 将靶向人类和/或食蟹猴TTR的经修饰sgRNA格式中的引导递送至如实例1中所述的原代人类肝细胞及原代食蟹猴肝细胞。测定包含跨每一细胞类型的每一引导序列的crRNA的编辑百分比,且然后基于最高编辑%对引导序列分等级次序。下文列示两种细胞系中表2中的引导序列的筛选数据(表12至15)。

[0716] 表12显示原代人类肝细胞中经Spy Cas9蛋白(RNP)转染的测试TTR sgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0717] 表12:经Spy Cas9蛋白及sgRNA转染的原代人类肝细胞中的TTR编辑数据

[0718]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
G000480	81.80	1.98	77.15	2.19	4.70	0.28
G000481	46.90	1.71	27.77	3.88	19.43	4.76
G000482	66.67	2.35	56.57	4.14	10.10	1.85
G000483	47.90	6.56	19.57	3.37	28.50	3.25
G000484	62.97	0.90	29.23	0.21	33.83	0.95
G000485	56.07	3.37	53.07	2.84	3.13	0.60
G000486	69.73	6.86	9.83	1.93	59.93	5.63
G000487	67.30	2.75	65.27	3.41	2.07	1.06
G000488	61.27	1.95	26.30	1.55	35.00	1.30
G000489	60.17	2.75	51.07	3.18	9.43	0.45
G000490	55.90	7.88	46.13	7.55	9.80	0.69
G000491	74.30	1.55	70.27	2.37	4.33	0.72
G000492	60.97	5.81	57.90	4.64	3.13	1.35
G000493	41.40	3.08	38.90	3.29	2.67	0.35
G000494	62.23	3.30	61.47	3.25	0.77	0.31
G000495	50.80	1.85	45.80	1.25	5.37	0.64
G000496	72.33	1.63	44.73	2.14	27.67	1.46
G000497	59.67	1.40	51.10	1.14	8.73	0.71
G000498	72.80	3.75	60.17	3.12	12.70	0.72
G000499	66.40	3.55	65.23	3.72	1.17	0.38
G000500	65.53	1.21	62.00	1.11	3.83	0.40
G000501	60.93	1.91	55.13	1.43	6.00	0.56

[0719] 表13显示原代人类肝细胞中经Spy Cas9 mRNA(SEQ ID NO:2)共转染的测试TTR sgRNA在12.5nM下的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0720] 表13:经Spy Cas9 mRNA及sgRNA转染的原代人类肝细胞中的TTR编辑数据

[0721]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
G000480	73.28	0.61	59.85	0.13	13.47	0.51
G000481	34.30	5.26	14.62	2.59	19.77	2.72
G000482	40.93	3.95	27.70	2.92	13.25	0.97
G000483	27.82	2.93	4.05	0.51	23.85	2.43

[0722]

G000484	43.37	6.79	13.98	2.61	29.48	4.15
G000485	30.82	5.76	28.87	5.50	1.97	0.28
G000486	59.13	5.62	2.82	0.86	56.37	4.92
G000487	49.57	0.99	47.38	0.89	2.27	0.24
G000488	49.40	5.05	11.98	1.40	37.48	3.68
G000489	24.25	2.82	14.17	2.01	10.28	1.38
G000490	24.72	2.35	19.38	2.04	5.38	0.41
G000491	45.93	1.22	42.42	1.06	3.60	0.33
G000492	34.65	2.21	32.45	2.01	2.22	0.25
G000493	11.55	1.35	10.65	1.58	0.97	0.30
G000494	26.22	4.03	25.17	3.89	1.07	0.15
G000495	47.77	1.88	43.40	1.91	4.45	0.17
G000496	63.30	2.60	11.08	2.10	52.25	0.67
G000497	40.33	3.32	34.48	2.71	5.85	0.61
G000498	60.02	5.42	45.20	4.34	14.90	1.08
G000499	39.30	6.04	38.58	5.86	0.77	0.12
G000500	35.50	0.61	32.47	0.49	3.10	0.18
G000501	40.32	1.50	33.82	2.04	6.62	0.55
G000567	27.28	7.59	17.35	4.72	10.02	2.94
G000568	43.75	5.83	43.00	5.81	0.80	0.18
G000570	68.42	3.64	68.08	3.61	0.35	0.00
G000571	20.47	3.41	14.47	2.72	6.13	0.78
G000572	55.42	8.13	41.62	6.48	13.85	1.60

[0723] 表14显示原代食蟹猴肝细胞上经Spy Cas9蛋白(RNP)电穿孔的测试TTR sgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。注意,引导G000480及G000488与食蟹猴具有一个错配,其可损害该引导在食蟹猴细胞中的编辑效率。

[0724] 表14:经Spy Cas9蛋白及sgRNA电穿孔的原代食蟹猴肝细胞中的TTR编辑数据

[0725]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
G000480	10.20	0.56	9.83	0.81	0.37	0.25
G000481	69.13	8.62	33.73	2.67	35.50	11.23
G000482	75.17	2.34	55.23	2.00	20.03	0.85
G000485	22.93	0.95	22.00	0.82	1.07	0.21
G000486	79.90	0.79	11.90	0.85	68.07	0.35
G000488	9.63	0.50	5.37	0.38	4.27	0.35

[0726]

G000489	67.53	1.15	53.53	1.56	14.17	0.64
G000490	61.67	0.72	54.47	1.10	7.27	1.23
G000491	66.20	1.11	64.37	0.47	1.90	0.70
G000493	50.13	0.74	48.07	1.69	2.10	0.98
G000494	81.53	0.71	79.57	0.49	2.07	0.67
G000498	91.37	1.48	68.50	1.64	22.87	1.50
G000499	83.40	0.36	82.00	0.20	1.43	0.55
G000500	45.20	3.66	42.60	3.80	2.63	0.25

[0727] 表15显示原代食蟹猴肝细胞上经Spy Cas9蛋白(RNP)电穿孔的测试的食蟹猴特异性TTR sgRNA的编辑%、插入(Ins)%及缺失(Del)%的平均值及标准偏差。

[0728] 表15:经Spy Cas9蛋白及食蟹猴特异性sgRNA(例如,具有类似人类gRNA的这些,参见表3)电穿孔的原代食蟹猴肝细胞中的TTR编辑数据

[0729]

引导ID	平均编辑%	编辑%标准偏差	平均插入%	插入%标准偏差	平均缺失%	缺失%标准偏差
G000502	95.10	0.96	13.97	1.69	81.27	2.60
G000503	58.53	2.40	52.07	1.68	6.50	2.46
G000504	77.17	0.96	69.73	1.29	7.53	0.57
G000505	95.53	1.06	95.50	1.01	0.10	0.10
G000506	89.43	1.36	86.90	1.64	3.07	0.42
G000507	71.17	3.22	67.03	2.39	4.60	1.65
G000508	45.63	3.01	41.57	2.95	4.17	0.91
G000509	93.03	0.81	43.60	1.30	49.73	1.76
G000510	90.80	0.53	89.13	0.40	1.77	0.12
G000511	62.77	1.63	60.87	1.55	2.00	0.35

[0730] 实例4.含有Spy Ca9 mRNA及sgRNA的脂质纳米颗粒(LNP)调配物的筛选

[0731] 原代人类肝细胞及原代食蟹猴肝细胞中将TTR sgRNA与Spy Cas9 mRNA调配在一起的LNP的交叉筛选。

[0732] 在剂量反应曲线中,在原代人类肝细胞及原代食蟹猴肝细胞上测试靶向人类TTR及食蟹猴匹配的sgRNA序列的经修饰sgRNA的脂质纳米颗粒调配物。如实例1中所述平铺原代人类及食蟹猴肝细胞。在用LNP处理之前,将两个细胞系在37℃、5%CO_2下培育24小时。使用Nanoassemblr™程序制备表16-19中详述的实验中使用的LNP,其各自含有指定的sgRNA及Cas9 mRNA(SEQ ID NO:2),各自具有脂质。LNP含有分别45:44:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG且N:P比率为4.5。于37℃下将LNP在含有6%食蟹猴血清的肝细胞维持培养基中培育5分钟。在培育后,以100ng mRNA开始在8点2倍剂量反应曲线上将LNP添加至原代人类或食蟹猴肝细胞上。如实例1中所述,在处理后72小时溶解细胞用于NGS分析。测定包含跨每一细胞类型的每一引导序列的crRNA的编辑百分比,且然后基于12.5ng mRNA输入及3.9nM引导浓度下的最高编辑%对引导序列分等级次序。两个细胞系中的引导序列的剂量反应曲线数据示于图4至7中。下文列示12.5ng mRNA输入及3.9nM引导浓度下的编辑%(表16至18)。

[0733] 表16显示作为剂量反应曲线的原代人类肝细胞上与Spy Cas9 mRNA调配于脂质纳米颗粒中的测试TTR sgRNA的编辑%、插入(Ins)%及缺失(Del)%于12.5ng cas9 mRNA下的平均值及标准偏差。与可为实验的人工制品的其它sgRNA相比,G000570展现了非特征性剂量反应曲线。数据在图4中以图形方式显示。

[0734] 表16:经LNP调配的Spy Cas9 mRNA(SEQ ID NO:2)及sgRNA处理的原代人类肝细胞中的TTR编辑数据

[0735]

引导ID	12.5 ng mRNA, 3.9 nM sgRNA, 平均编辑%	编辑% 标准偏差
G000480	59.33	0.73
G000481	24.37	0.37
G000482	19.10	2.64
G000483	7.37	0.67
G000484	16.67	1.23
G000485	14.23	2.36
G000486	61.33	2.59
G000487	17.37	0.95
G000488	44.80	3.00
G000489	16.85	0.06

[0736]

G000490	10.53	1.90
G000491	31.60	2.33
G000492	15.87	0.44
G000493	7.33	0.73
G000494	6.37	1.07
G000495	23.97	1.66
G000496	30.73	3.76
G000497	15.10	3.30
G000498	24.43	1.30
G000499	16.07	1.67
G000500	23.57	2.44
G000501	32.30	2.49
G000567	48.95	1.06
G000568	54.60	3.68
G000570	88.30	1.84
G000572	55.45	1.20

[0737] 表17显示作为剂量反应曲线的原代食蟹猴肝细胞上与Spy Cas9 mRNA调配于脂质纳米颗粒中的测试TTR sgRNA的编辑%、插入(Ins)%及缺失(Del)%于12.5ng mNRA及3.9nM引导浓度下的平均值及标准偏差。数据在图5中以图形方式显示。

[0738] 表17:经LNP调配的Spy Cas9 mRNA(SEQ ID NO:2)及sgRNA处理的原代食蟹猴肝细

胞中的TTR编辑数据

[0739]

引导ID	12.5 ng mRNA，3.9 nM sgRNA，平均编辑%	编辑%标准偏差
G000480	0.73	0.15
G000481	49.20	1.39
G000482	26.13	5.33
G000483	0.73	0.60
G000484	0.10	0.00
G000485	1.43	1.02
G000489	31.87	2.40
G000490	15.23	1.08
G000491	6.37	0.38
G000492	0.70	0.28
G000493	7.63	1.14

[0740]

G000494	14.30	1.06
G000495	0.73	0.06
G000497	0.23	0.06
G000498	37.90	1.42
G000499	14.63	0.70
G000500	10.47	0.32
G000501	1.37	0.31
G000567	0.10	0.00
G000568	9.25	0.21
G000570	17.30	0.85
G000571	20.20	2.26
G000572	30.60	0.42

[0741] 表18显示作为剂量反应曲线的原代食蟹猴肝细胞上与Spy Cas9 mRNA调配于脂质纳米颗粒中的测试的食蟹猴特异性TTR sgRNA的编辑％、插入(Ins)％及缺失(Del)％于12.5ng mRNA及3.9nM引导浓度下的平均值及标准偏差。数据在图6中以图形方式显示。

[0742] 表18:经LNP调配的Spy Cas9 mRNA(SEQ ID NO:2)及食蟹猴匹配的sgRNA处理的原代食蟹猴肝细胞中的TTR编辑数据

[0743]

引导ID	12.5 ng mRNA, 3.9 nM sgRNA, 编辑%	编辑% 标准偏差
G000502	80.70	0.14
G000506	60.13	0.70
G000509	74.47	7.28
G000510	61.87	2.54

[0744] 原代人类肝细胞及原代食蟹猴肝细胞中将TTR sgRNA与Spy Cas9 mRNA调配在一起的LNP的交叉筛选

[0745] 在剂量反应曲线中，在原代人类肝细胞及原代食蟹猴肝细胞上测试靶向人类TTR及食蟹猴匹配的sgRNA序列的经修饰sgRNA的脂质纳米颗粒调配物。如实例1中所述平铺原代人类及食蟹猴肝细胞。在用LNP处理之前，将两个细胞系在37℃、5％CO_2下培育24小时。使用上述交叉流程序制备表20-22中详述的实例中使用的LNP，但使用PD-10管柱(GE Healthcare Life Sciences)纯化且使用Amicon离心过滤器单元(Millipore Sigma)离心，其各自含有指定的sgRNA及Cas9 mRNA(SEQ ID NO:1)。LNP含有分别50:38:9:3摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG且N:P比率为6.0。于37℃、5％CO_2下将LNP在含有6％食蟹猴血清的肝细胞维持培养基中培育5分钟。在培育后，以300ng mRNA开始在8点3倍剂量反应曲线上将LNP添加至原代人类或食蟹猴肝细胞上。如实例1中所述，在处理后72小时溶解细胞用于NGS分析。测定包含跨每一细胞类型的每一引导序列的crRNA的编辑百分比，且然后基于EC50值及最大编辑％对引导序列分等级次序。两个细胞系中的引导序列的剂量反应曲线曲线示于图4至7中。下文列示EC50值及最大编辑％(表19至22)。

[0746] 表19显示作为剂量反应曲线的原代人类肝细胞上与U缺失的Spy Cas9 mRNA调配于脂质纳米颗粒中的测试的人类特异性TTR sgRNA的EC50及最大编辑。数据在图4中以图形方式显示。

[0747] 表19:经LNP调配的Spy Cas9 mRNA及人类特异性sgRNA处理的原代人类肝细胞中的TTR编辑数据

[0748]

引导ID	EC50	最大编辑
G000480	0.10	98.69
G000481	1.43	87.05
G000482	0.65	97.02
G000483	1.88	77.39
G000484	0.95	94.14
G000488	0.72	95.83
G000489	1.38	86.33
G000490	1.52	94.16
G000493	2.42	63.95
G000494	1.28	75.70
G000499	0.63	96.31
G000500	0.39	88.70
G000568	0.78	95.72
G000570	0.23	98.22
G000571	2.21	71.28

[0749]

G000572	0.42	97.94

[0750] 表20显示作为剂量反应曲线的原代人类肝细胞上与U缺失的Spy Cas9 mRNA调配于脂质纳米颗粒中的测试的人类特异性TTR sgRNA的EC50及最大编辑。数据在图16中以图形方式显示。

[0751] 表20:经LNP调配的Spy Cas9 mRNA及人类特异性sgRNA处理的原代食蟹猴肝细胞中的TTR编辑数据

[0752]

引导ID	EC50	最大编辑
G000480	5.28	20.32
G000481	0.93	95.07
G000482	0.89	97.47
G000483	4.40	56.52
G000484	3.47	0.22
G000488	11.56	21.63
G000489	1.79	89.21
G000490	3.09	90.76
G000493	4.97	61.15
G000494	2.77	60.84
G000499	2.00	74.94
G000500	4.42	58.04
G000567	1.76	97.06
G000568	1.87	87.93
G000570	2.00	96.73
G000571	1.55	97.03
G000572	0.79	100.31

[0753] 表21显示作为剂量反应曲线的原代人类肝细胞上与U缺失的Spy Cas9 mRNA调配于脂质纳米颗粒中的测试的食蟹猴匹配的TTR sgRNA的EC50及最大编辑。数据在图17中以图形方式显示。

[0754] 表21:经LNP调配的Spy Cas9 mRNA及食蟹猴特异性sgRNA处理的原代人类肝细胞中的TTR编辑数据

[0755]

引导ID	EC50	最大编辑
G000502	0.70	91.50
G000504	5.16	7.16

[0756]

G000505	3.57	13.48
G000506	1.26	89.49

[0757] 表22显示作为剂量反应曲线的原代食蟹猴肝细胞上与U缺失的Spy Cas9 mRNA调配于脂质纳米颗粒中的测试的食蟹猴匹配的TTR sgRNA的EC50及最大编辑。数据在图18中以图形方式显示。

[0758] 表22:经LNP调配的Spy Cas9 mRNA及食蟹猴特异性sgRNA处理的原代食蟹猴肝细胞中的TTR编辑数据

[0759]

引导ID	EC50	最大编辑
G000502	0.26	100.05
G000503	2.26	83.41
G000504	1.42	98.04
G000505	1.10	99.97
G000506	0.66	99.18

[0760] 实例5.TTR dgRNA及sgRNA的脱靶分析

[0761] TTR引导的脱靶分析

[0762] 使用基于寡核苷酸插入的分析(例如,参见Tsai等人,Nature Biotechnology 33,187-197;2015)测定由靶向TTR的Cas9切割的潜在脱靶基因组位点。在HEK293_Cas9细胞中筛选表1的45个dgRNA(及具有已知脱靶特性的两个对照引导)。在补充有10％胎牛血清及500μg/ml G418的DMEM培养基中培养组成型表达Spy Cas9的人类胚肾癌细胞系HEK293(“HEK293_Cas9”)。在转染之前24小时,将细胞以30,000个细胞/孔的密度平铺于96孔板中。根据制造商的方案用Lipofectamine RNAiMAX(ThermoFisher,目录13778150)转染细胞。用含有个别crRNA(15nM)、trRNA(15nM)、具有(10nM)Lipofectamine RNAiMAX的供体寡核苷酸(0.3μL/孔)及OptiMem的脂复合物转染细胞。转染后24小时溶解细胞,且根据制造商推荐的方案使用Zymo的Quick gDNA 96提取试剂盒(目录号D3012)提取基因组DNA。使用Qubit高灵敏度dsDNA试剂盒(Life Technologies)定量gDNA。根据Tsai等人(2015)的先前所述的方法制备文库,其中进行微小修改。在Illumina的MiSeq及HiSeq 2500上实施测序。该分析鉴别一些crRNA的潜在脱靶位点,其绘制于图2中。

[0763] 表23显示经TTR dgRNA以及双链DNA寡核苷酸供体序列转染的HekCas9细胞中检测的脱靶整合位点数。

[0764] 表23:经由基于寡核苷酸插入的分析针对TTR dgRNA检测的脱靶整合位点数

[0765]

引导ID	位点数
CR003335	0
CR003336	2
CR003337	10
CR003338	2
CR003339	3
CR003340	0
CR003342	0
CR003343	2
CR003344	0
CR003345	0
CR003346	0
CR003347	1
CR003348	3
CR003351	1
CR003352	2
CR003353	2
CR003355	1
CR003356	4
CR003357	3
CR003359	6
CR003360	0
CR003363	4
CR003365	3
CR003366	1
CR003367	1
CR003368	2
CR003369	2
CR003377	0
CR003380	0
CR003382	34
CR003383	1
CR003385	3

[0766]

CR003386	1
CR003387	6
CR003388	2
CR003389	2
CR003390	1
CR003391	0
CR003392	0
CR005298	0
CR005300	0
CR005301	0
CR005302	1
CR005303	1
CR005304	0

[0767] 另外，经由上述基于寡核苷酸的插入方法，评估引导亚组的Hek_Cas9细胞中原样修饰的sgRNA的脱靶电位。脱靶结果绘制于图4中。

[0768] 表24显示经TTR sgRNA以及双链DNA寡核苷酸供体序列转染的HekCas9细胞中检测的脱靶整合位点数。

[0769] 表24：经由插入检测方法针对TTR sgRNA检测的脱靶整合位点数

[0770]

引导ID	位点数
G000480	11
G000481	3
G000482	13
G000483	5
G000484	7
G000485	22
G000486	12
G000487	14
G000488	0
G000489	19
G000490	12
G000491	28
G000492	97
G000493	7
G000494	4
G000495	13
G000496	1
G000497	26

[0771]

G000498	82
G000499	4
G000500	46
G000501	4
G000567	9
G000568	937
G000570	19
G000571	16
G000572	15

[0772] 实例6.用于验证潜在脱靶位点的靶向测序

[0773] 实例5中用于检测潜在脱靶的HEK293_Cas9细胞组成型过度表达Cas9,与各种细胞类型中的瞬时递送范例相比,导致更高数量的潜在脱靶“命中”。此外,当递送sgRNA时(与dgRNA相反),潜在脱靶命中数可进一步增多,此乃因sgRNA分子比dgRNA更稳定(特别当经化学修饰时)。因此,可使用鉴别的潜在脱靶位点的靶向测序来验证藉由如实例5中使用的寡核苷酸插入方法鉴别的潜在脱靶位点。

[0774] 在一种方法中,用包含Cas9 mRNA及所关注sgRNA(例如,具有用于评估的潜在脱靶位点的sgRNA)的LNP处理原代肝细胞。然后溶解原代肝细胞,并将侧接潜在脱靶位点的引物用于产生用于NGS分析的扩增子。在一定程度上鉴别插入或缺失可验证潜在脱靶位点,而在潜在脱靶位点未发现插入或缺失可指示HEK293_Cas9细胞分析中的伪阳性。

[0775] 实例7.表型分析

[0776] 分泌的TTR的西方墨点分析

[0777] 如实例1中所述用表1的选择引导一式三份转染肝细胞癌细胞系HepG2。转染后2天,收获基因组DNA的一个重复,并藉由NGS测序分析编辑效率。转染后5天,一次重复更换无血清的培养基。4hr后,如前所述,藉由WB收获培养基用于分析分泌的TTR。每一引导的编辑%及细胞外TTR的减少的数据提供于图7中。

[0778] 细胞内TTR的西方墨点分析

[0779] 如实例1中所述用包含表1的引导的crRNA转染肝细胞癌细胞系HUH7。将转染的细胞池保留在组织培养中并传代用于进一步分析。转染后7天,收获细胞,且制备全细胞提取物(WCE),并如前所述藉由蛋白质印迹法经受分析。

[0780] 藉由蛋白质印迹法分析WCE的TTR蛋白减少。全长TTR蛋白具有约16kD的预测分子量。在西方墨点的对照泳道中观察到该分子量下的条带。

[0781] 使用Licor Odyssey Image Studio 5.2版软件计算TTR蛋白的减少百分比。使用GAPDH作为上样对照并与TTR同时探测。计算每一样本内GAPDH的密度测定值与涵盖TTR条带的总区域相比的比率。在比率针对对照泳道正规化后测定TTR蛋白的减少百分比。结果显示于图8中。

[0782] 实例8.至人类化TTR小鼠及具有wt(鼠类)TTR的小鼠的LNP递送

[0783] 向相对于TTR基因人类化的小鼠投用含有表25中所指示的引导的LNP调配物701-704(每个调配物5只小鼠)。对该人类化TTR小鼠进行工程改造,使得内源性鼠类TTR基因座区域缺失,并经直向同源人类TTR序列置换,以使基因座编码人类TTR蛋白。为了比较,向6只

具有鼠类TTR的小鼠投用LNP700，其含有靶向鼠类TTR的引导(G000282)。使用如上所述Nanoassemblr™程序制备具有表25中的调配物编号1-5的LNP，而具有调配物编号6-16的LNP使用上述交叉流程序制备，但使用PD-10管柱(GE Healthcare Life Sciences)纯化且使用Amicon离心过滤单元(Millipore Sigma)浓缩。作为阴性对照，向相应基因型的小鼠单独投用媒剂(Tris-盐水-蔗糖缓冲液(TSS))。施用具有表25中的调配物编号2-5的LNP的人类化TTR小鼠的背景是50％129S6/SvEvTac 50％C57BL/6NTac；施用具有表25中的调配物编号6-16的LNP的人类化TTR小鼠以及具有鼠类TTR的小鼠(施用LNP700，调配物编号1)的背景是75％C57BL/6NTac 25％129S6/SvEvTac。

[0784] 表25.投药人类化TTR小鼠的LNP调配物。

[0785]

调配物编号	LNP	引导	RNA浓度(mg/ml)	N:P比率	摩尔比(分别脂质A、胆固醇、DSPC及PEG2k-DMG)
1	LNP700	G000282	0.53	4.5	45:44:9:2
2	LNP701	G000481	0.46	4.5	45:44:9:2
3	LNP702	G000489	0.61	4.5	45:44:9:2
4	LNP703	G000494	0.57	4.5	45:44:9:2
5	LNP704	G000499	0.59	4.5	45:44:9:2
6	LNP1148	G000481	0.73	4.5	45:44:9:2
7	LNP1152	G000499	0.45	6.0	50:38:9:3
8	LNP1153	G000482	0.53	6.0	50:38:9:3
9	LNP1155	G000571	0.70	6.0	50:38:9:3
10	LNP1156	G000572	0.58	6.0	50:38:9:3
11	LNP1157	G000480	0.84	6.0	50:38:9:3
12	LNP1159	G000488	0.79	6.0	50:38:9:3
13	LNP1160	G000493	0.71	6.0	50:38:9:3
14	LNP1161	G000500	0.66	6.0	50:38:9:3
15	LNP1162	G000567	0.69	6.0	50:38:9:3
16	LNP1163	G000570	0.66	6.0	50:38:9:3

[0786]

[0787] 具有调配物编号1-5的LNP含有SEQ ID NO:2的Cas9 mRNA且具有调配物编号6-16的LNP含有SEQ ID NO:1的Cas9 mRNA，所有与引导皆呈1:1重量比。LNP分别含有表25中所列举的摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG。具有调配物编号1-5的LNP是以2mg/kg(总RNA含量)投用且具有调配物编号6-16的LNP是以1mg/kg(总RNA含量)投用。使用经设计以使用于NGS分析的所关注区域扩增的引物测定肝编辑结果。调配物编号1-5的肝编辑结果示于图9中且指示人类TTR序列的编辑，其中四个引导中的每一个测试为＞35％编辑(平均值)的值，且G000494及G000499提供接近60％的值。调配物编号6-8、10-13及15-16的肝编辑结果示于图13及表26中，其显示利用所测试每一调配物的人类TTR序列的有效编辑。所有调配物

皆观察到大于38％编辑，其中若干调配物提供大于60％的编辑值。由于PCR扩增子的设计及产生的低测序读数，未显示调配物9及14。

[0788] 在提供调配物编号6-8、10-13及15-16的小鼠中测量血清中人类TTR的含量。参见图14B。图14A是出于比较目的提供的图13的重复。对于所测试每一调配物检测到血清人类TTR的减弱，其与在肝中检测到的编辑量相关(参见图14A对图14B，表26)。

[0789] 表26

[0790]

引导ID	编辑％	血清TTR(％TSS)
TSS(媒剂)	0.06	100
G481	61.28	10.52
G499	65.66	8.39
G482	70.86	4.65
G572	73.52	2.11
G480	77.34	3.48
G488	59.125	27.78
G493	38.55	49.73
G567	47.525	44.24
G570	45.5	41.73
G571	33.88	11.39
G500	44.44	34.28

[0791] 在另一组实验中，向人类化TTR小鼠投用一系列剂量的具有引导G000480、G000488、G000489及G000502的LNP调配物。调配物含有与引导呈1:1的重量比的Cas9 mRNA(SEQ ID NO:1)。LNP含有分别呈50:38:9:3摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG，且N:P比率为6。剂量为1、0.3、0.1或0.03mg/kg(n＝5/组)。使用上述交叉流程序制备LNP且分别使用PD-10管柱及Amicon离心过滤器单元纯化及浓缩。使用经设计以使用于NGS分析的所关注区域扩增的引物测定肝编辑结果，且如上文所述测量血清人类TTR含量。肝编辑的结果示于图26A中且血清人类TTR含量示于图26B-C中。编辑及血清TTR含量的剂量反应是明显的。

[0792] 在另一组实验中，向人类化TTR小鼠投用一系列剂量的具有引导G000481、G000482、G000486及G000499的LNP调配物。调配物含有与引导呈1:1的重量比的Cas9 mRNA(SEQ ID NO:1)。LNP含有分别呈50:38:9:3摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG，且N:P比率为6。剂量为1、0.3或0.1mg/kg(n＝5/组)。使用上述交叉流程序制备LNP且分别使用PD-10管柱及Amicon离心过滤器单元纯化及浓缩。使用经设计以使用于NGS分析的所关注区域扩增的引物测定肝编辑结果，且如上文所述测量血清人类TTR含量。肝编辑的结果示于图27A中且血清人类TTR含量示于图27B-C中。编辑及血清TTR含量的剂量反应是明显的。

[0793] 在另一组实验中，向人类化TTR小鼠投用一系列剂量的具有引导G000480、G000481、G000486、G000499及G000502的LNP调配物。调配物含有与引导呈1:2的重量比的Cas9 mRNA(SEQ ID NO:1)。LNP含有分别呈50:38:9:3摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG，且N:P比率为6。剂量为1、0.3或0.1mg/kg(n＝5/组)。使用上述交叉流程序制备LNP且分别使用PD-10管柱及Amicon离心过滤器单元纯化及浓缩。使用经设计以使用于NGS分析的所关注区域扩增的引物测定肝编辑结果，且如上文所述测量血清人类TTR含量。肝编

辑的结果示于图28A中且血清人类TTR含量示于图28B-C中。编辑及血清TTR含量的剂量反应是明显的。

[0794] 在使用野生型CD-1小鼠的单独实验中，在剂量反应研究中测试包含引导G000502(其在小鼠与食蟹猴之间交叉同源)的LNP调配物。调配物含有与引导呈1:1的重量比的Cas9 mRNA(SEQ ID NO:1)。LNP含有分别呈45:44:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG，且N:P比率为6。剂量为1、0.3、0.1、0.03或0.01mg/kg(n＝5/组)。使用经设计以使用于NGS分析的所关注区域扩增的引物测定肝编辑结果。肝编辑结果示于图15A中且血清小鼠TTR含量示于图15B中。编辑及血清TTR含量的剂量反应是明显的。

[0795] 实例9.LNP以多个剂量递送至小鼠

[0796] 向小鼠(雌性，来自Charles River Laboratory，约6-7周龄)投用LNP调配物LNP705，该LNP调配物是使用如上文所述交叉流及TFF程序制备且含有1:1重量比的G000282(“G282”)及Cas9 mRNA(SEQ ID NO:2)及0.5mg/ml的总RNA浓度。LNP的N:P比率为4.5，且分别含有45:44:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG。每周一次至一周、两周、三周或四周一次(QWx1-4)或每月一次至每两个月或三个月一次(QMx2-3)向各组投药。剂量为0.5mg/kg或1mg/kg(总RNA含量)。对照组在第1天接受0.5、1或2mg/kg的单剂量。每组含有5只小鼠。藉由ELISA分析血清TTR，且在验证时收集各肝、脾及肌肉用于NGS编辑分析。各组示于表27中。X＝处死及验尸。MPK＝mg/kg。

[0797] 表27.研究组

[0798]

组	持续时间/剂量方案	剂量(MPK)	给予的总剂量(MPK)	剂量	剂量	剂量	剂量	NX	剂量	NX
				第1天	第8天	第15天	第22天	第28天	第43天	第49天
1	4周多个剂量/QWx4	0 (TSS对照)	0	X	X	X	X	X		
2	2个月多个剂量/QMx3	1	3	X			X		X	X
3		0.5	1.5	X			X		X	X
4	1个月多个剂量/QMx2	1	2	X			X	X		
5		0.5	1	X			X	X		
6	4周多个剂量/QWx4	1	4	X	X	X	X	X		
7		0.5	2	X	X	X	X	X		
8	3周多	1	3		X	X	X	X		

[0799]

9	个剂量/QWx3	0.5	1.5		X	X	X	X		
10	2周 多	1	2			X	X	X		
11	个剂量/QWx2	0.5	1			X	X	X		
12		1	1				X	X		
13		0.5	0.5				X	X		
14	单剂量/QWx1	2	2				第26天	第32天		

[0800] 表28及图10A-11B显示血清TTR含量结果(KD%＝减弱%)。表29及图12A-C显示肝编辑结果。

[0801] 表28.血清TTR结果。

[0802]

时间 方案	剂量	血清 TTR (μg/mL)	血清 TTR (KD %)
QWx4	TSS	1190.7	-
QMx3	0.5	245.01	79.42
QMx2	0.5	776.73	34.77
QWx4	0.5	347.43	70.82
QWx3	0.5	405.70	65.93
QWx2	0.5	432.25	63.70
QWx1	0.5	804.06	32.47
QMx3	1	91.95	92.28
QMx2	1	176.81	85.15
QWx4	1	119.52	89.96
QWx3	1	167.15	85.96
QWx2	1	130.98	89.00
QWx1	1	573.02	51.88
QWx1	2	219.07	81.60

[0803] 表29.肝编辑结果。

[0804]

时间方案	剂量	肝编辑(%)
QWx4	TSS	0.38
QMx3	0.5	48.18
QMx2	0.5	36.66
QWx4	0.5	56.03
QWx3	0.5	51.35
QWx2	0.5	34.77
QWx1	0.5	24.16
QMx3	1	63.40
QMx2	1	57.37
QWx4	1	62.89
QWx3	1	59.22

[0805]

QWx2	1	60.12
QWx1	1	35.16
QWx1	2	60.57

[0806] 结果显示,随着时间推移,可建立累积剂量及多次施用(包括以每周或每月间隔)的效应,以实现TTR的增加编辑程度及KD%。

[0807] 实例10.RNA运送子:变化mRNA及gRNA比率

[0808] 此研究评估不同比率的gRNA对mRNA的小鼠中的活体内效能。如实例1中所指示用N1-甲基假尿苷三磷酸酯代替尿苷三磷酸酯藉由IVT合成制备具有SEQ ID NO:4的ORF、HSD 5'UTR、人类白蛋白3'UTR、Kozak序列及多-A尾的CleanCap™加帽的Cas9 mRNA。

[0809] 自所述mRNA及如实例1中所述的G282(SEQ ID NO:124)利用呈50:38:9:3摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG且以6的N:P比率制备LNP调配物。调配物的gRNA:Cas9 mRNA重量比示于图19A及19B中。

[0810] 对于活体内表征,将LNP以0.1mg总RNA(mg引导RNA+mg mRNA)/kg施用小鼠(n=5/组)。在投药后7-9天,处死动物,收集血液及肝,且如实例1中所述测量血清TTR及肝编辑。血清TTR及肝编辑结果示于图19A及19B中。向阴性对照小鼠投用TSS媒剂。

[0811] 此外,将上述LNP以0.05mg mRNA/kg的恒定mRNA剂量施用小鼠(n=5/组),同时将gRNA剂量自0.06mg/kg变为0.4mg/kg。在投药后7-9天,处死动物,收集血液及肝,并测量血清TTR及肝编辑。血清TTR及肝编辑结果示于图19C及图19D中。向阴性对照小鼠投用TSS媒剂。

[0812] 实例11.原代人类肝细胞中TTR sgRNA的脱靶分析

[0813] 如实例5中所述在原代人类肝细胞(PHH)中实施靶向TTR的sgRNA的脱靶分析,且具有以下修改。如实例1中所述将PHH以33,000个细胞/孔的密度平铺于胶原涂布的96孔板上。平铺后24小时,用介质洗涤细胞并如实例1所述用Lipofectamine RNAiMAX(ThermoFisher,

目录13778150)进行转染。用含有100ng Cas9 mRNA的脂复合物转染细胞,之后立即添加含有25nM sgRNA及12.5nM供体寡核苷酸的另一脂复合物(0.3μL/孔)。转染后48小时溶解细胞,且提取gDNA并如实例5中进一步阐述进行分析。数据在图20中以图形方式表示。

[0814] 表30显示PHH中检测到的脱靶整合位点数,且与在实例5中使用的HekCas9细胞中检测到的位点数相比较。与HekCas9细胞系相比,每个测试引导在PHH中检测到的位点较少,在单独PHH中未检测到独特位点。

[0815] 表30.经由基于寡核苷酸插入的分析针对PHH中TTR sgRNA检测的脱靶整合位点数

[0816]

引导ID	PHH中的位点数	HekCas9细胞中的位点数(实例5)
G000480	2	11
G000481	0	3
G000482	2	13
G000483	0	5
G000484	0	7
G000485	3	22
G000486	0	12
G000487	0	14
G000488	0	0
G000489	2	19
G000490	0	12
G000491	7	28
G000492	5	97
G000493	1	7
G000494	0	4
G000495	1	13
G000496	0	1
G000497	3	26
G000498	19	82
G000499	1	4
G000500	12	46
G000501	0	4
G000567	0	9
G000568	11	936
G000570	1	19
G000571	1	16

[0817]

G000572	2	15

[0818] 在经由寡核苷酸插入分析鉴别PHH中的潜在脱靶位点之后,例如如实例6中所述,藉由靶向的扩增子测序进一步评估某些潜在位点。除了藉由寡核苷酸插入策略鉴别的潜在脱靶位点之外,在分析中亦包括藉由计算机仿真预测鉴别的额外潜在脱靶位点。

[0819] 为此，如实例4中所述，用包含100ng Cas9 mRNA(SEQ ID NO:1)及14.68nM所关注gRNA(以1:1重量比)的LNP处理PHH。使用上述交叉流程序制备LNP且分别使用PD-10管柱及Amicon离心过滤器单元纯化及浓缩。LNP经调配以具有6.0的N:P比率，且分别含有呈50:38:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG。在LNP处理后，藉由NGS(例如，如实例1及6中所述)分析分离的基因组DNA以确定是否可在潜在脱靶位点检测到插入或缺失，此将指示Cas9介导的裂解事件。表31及32分别显示对于gRNA G000480及G000486进行评估的潜在脱靶位点。

[0820] 如图21A-B及22A-B以及下表33中所示，对于G000480，藉由寡核苷酸插入分析鉴别的潜在脱靶位点中仅两个以低程度检测到插入或缺失，且对于G000486为仅一个以低程度检测到插入或缺失。在任何引导的计算机仿真预测的位点中的任一个皆未检测到插入或缺失。此外，仅在使用LNP的近饱和剂量的该位点检测到插入或缺失，此乃因在G000480及G000486的靶上位点观察到的插入或缺失率分别为约97％及约91％(参见表33)。该位点的基因组坐标亦报告于表31及32中，且各自对应于不编码任何蛋白质的序列。

[0821] 然后实施剂量反应分析以测定其中未检测到脱靶的LNP的最高剂量。如实例4中所述用包含G000480或G000486的LNP处理PHH。相对于gRNA浓度，剂量范围跨越11个点(0.001nM、0.002nM、0.007nM、0.02nM、0.06nM、0.19nM、0.57nM、1.72nM、5.17nM、15.51nM及46.55nM)。如由图21A-B及22A-B中的垂直虚线所表示，对于G000480及G000486不再检测到潜在脱靶位点的最高浓度(相对于gRNA的浓度)分别为0.57nM及15.51nM，其分别导致84.60％及89.50％的靶上插入或缺失率。

[0822] 表31.经由插入检测及针对经由靶向扩增子测序评估的G000480计算机仿真预测鉴别的潜在脱靶位点

引导ID	脱靶(OT)位点ID	所用分析	染色体坐标(hg38)	链
G000480	INS-OT.1	插入检测	chr7:94767406-94767426	+
G000480	INS-OT.2	插入检测	chr2:192658562-192658582	+
G000480	INS-OT.3	插入检测	chr7:4834390-4834410	+
G000480	INS-OT.4	插入检测	chr20:9216118-9216138	-
G000480	INS-OT.5	插入检测	chr10:12547071-12547091	+
G000480	INS-OT.6	插入检测	chr6:168377978-168377998	-
G000480	INS-OT.7	插入检测	chr12:114144669-114144689	-
G000480	INS-OT.8	插入检测	chr10:7376755-7376775	+
G000480	INS-OT.9	插入检测	chr2:52950299-52950319	+
G000480	INS-OT.10	插入检测	chr8:56579165-56579185	-
G000480	INS-OT.11	插入检测	chr1:189992255-189992275	+
G000480	PRED-OT.1	计算机仿真预测	chr10:12547071-12547091	+
G000480	PRE-DOT.2	计算机仿真预测	chrX:119702782-119702802	+
G000480	PRED-OT.3	计算机仿真预测	chr1:116544586-116544606	+
G000480	PRED-OT.4	计算机仿真预测	chr6:88282884-88282904	+
G000480	PRED-OT.6	计算机仿真预测	chr5:121891868-121891888	+
G000480	PRED-OT.7	计算机仿真预测	chr3:52544945-52544965	+
G000480	PRED-OT.8	计算机仿真预测	chr15:36949639-36949659	+
G000480	PRED-OT.9	计算机仿真预测	chr5:33866486-33866506	+
G000480	PRED-OT.10	计算机仿真预测	chr5:159755754-159755774	+
G000480	PRED-OT.11	计算机仿真预测	chr5:31349859-31349879	+
G000480	PRED-OT.12	计算机仿真预测	chr11:79485652-79485672	+
G000480	PRED-OT.13	计算机仿真预测	chr15:29448864-29448884	+
G000480	PRED-OT.14	计算机仿真预测	chr5:171153565-171153585	+
G000480	PRED-OT.15	计算机仿真预测	chr9:84855273-84855293	+

[0823]

[0824]

G000480	PRED-OT.16	计算机仿真预测	chr6:159953060-159953080	+
G000480	PRED-OT.17	计算机仿真预测	chr16:51849024-51849044	+
G000480	PRED-OT.18	计算机仿真预测	chr3:24108809-24108829	+
G000480	PRED-OT.19	计算机仿真预测	chr18:41118310-41118330	+
G000480	PRED-OT.20	计算机仿真预测	chr10:108975241-108975261	+
G000480	PREDO-T.21	计算机仿真预测	chr1:44683633-44683653	+
G000480	PRED-OT.22	计算机仿真预测	chr2:196214849-196214869	+
G000480	PRED-OT.23	计算机仿真预测	chr9:117353544-117353564	+
G000480	PRED-OT.24	计算机仿真预测	chr1:55583322-55583342	+
G000480	PRED-OT.25	计算机仿真预测	chr12:28246827-28246847	+
G000480	PRED-OT.26	计算机仿真预测	chr4:54545361-54545381	+
G000480	PRED-OT.27	计算机仿真预测	chr13:22364836-22364856	+
G000480	PRED-OT.28	计算机仿真预测	chr13:80816049-80816069	+
G000480	PRED-OT.29	计算机仿真预测	chr7:39078622-39078642	+
G000480	PRED-OT.30	计算机仿真预测	chr2:59944386-59944406	+

[0825] “INS-OT.N”是指藉由寡核苷酸插入检测的脱靶位点ID,其中N是上文指定的整数;“PRED-OT.N”是指经由计算机仿真方法预测的脱靶位点ID,其中N是上文指定的整数。

[0826] 表32.经由靶向扩增子测序评估的G000486经由插入检测及计算机仿真预测的鉴别潜在脱靶位点

[0827]

引导ID	脱靶(OT)位点ID	所用分析	染色体坐标(hg38)	链
G000486	INS-OT.1	插入检测	chr14:77332157-77332177	+
G000486	INS-OT.2	插入检测	chr14:54672059-54672079	-
G000486	INS-OT.3	插入检测	chr4:108513169-108513189	-
G000486	INS-OT.4	插入检测	chr5:91397023-91397043	-
G000486	INS-OT.5	插入检测	chr9:116626135-116626155	-
G000486	INS-OT.6	插入检测	chr6:73201226-73201246	+

[0828]

G000486	INS-OT.7	插入检测	chr16:89368352-89368372	-
G000486	INS-OT.8	插入检测	chr7:56308371-56308391	-
G000486	INS-OT.9	插入检测	chr21:43605667-43605687	+
G000486	INS-OT.10	插入检测	chr5:26758030-26758050	+
G000486	INS-OT.11	插入检测	chr17:30656428-30656448	+
G000486	INS-OT.12	插入检测	chr8:130486452-130486472	+
G000486	PRED-OT.1	计算机仿真预测	chr11:44707064-44707084	+
G000486	PRED-OT.2	计算机仿真预测	chr5:50775396-50775416	+
G000486	PRED-OT.3	计算机仿真预测	chr4:141623949-141623969	+
G000486	PRED-OT.4	计算机仿真预测	chr1:223481186-223481206	+
G000486	PRED-OT.5	计算机仿真预测	chr6:39951487-39951507	+
G000486	PRED-OT.6	计算机仿真预测	chrY:5456047-5456067	+
G000486	PRED-OT.8	计算机仿真预测	chr6:129868719-129868739	+
G000486	PRED-OT.9	计算机仿真预测	chrX:80450312-80450332	+
G000486	PRED-OT.10	计算机仿真预测	chr7:27256771-27256791	+
G000486	PRED-OT.11	计算机仿真预测	chr3:181416528-181416548	+
G000486	PRED-OT12	计算机仿真预测	chr7:146425020-146425040	+
G000486	PRED-OT.13	计算机仿真预测	chr3:16980977-16980997	+
G000486	PRED-OT.14	计算机仿真预测	chr7:118161002-118161022	+
G000486	PRED-OT.15	计算机仿真预测	chr6:102220539-102220559	+
G000486	PRED-OT.16	计算机仿真预测	chr12:127278991-127279011	+
G000486	PRED-OT.17	计算机仿真预测	chr2:67686631-67686651	+
G000486	PRED-OT.18	计算机仿真预测	chr1:114467665-114467685	+
G000486	PRED-OT.19	计算机仿真预测	chr3:194514436-194514456	+
G000486	PRED-OT.20	计算机仿真预测	chr14:31767581-31767601	+
G000486	PRED-OT.21	计算机仿真预测	chr16:28706209-28706229	+
G000486	PRED-OT.22	计算机仿真预测	chr8:110526279-110526299	+
G000486	PRED-OT.23	计算机仿真预测	chr19:2899814-2899834	+

[0829]

G000486	PRED-OT.25	计算机仿真预测	chr3:130760261-130760281	+
G000486	PRED-OT.26	计算机仿真预测	chr11:2506046-2506066	+
G000486	PRED-OT.27	计算机仿真预测	chr2:153918318-153918338	+
G000486	PRED-OT.28	计算机仿真预测	chr14:40590226-40590246	+
G000486	PRED-OT.29	计算机仿真预测	chr18:806650-806670	+
G000486	PRED-OT.30	计算机仿真预测	chr2:117707480-117707500	+

[0830] “INS-OT.N”是指藉由寡核苷酸插入检测的脱靶位点ID,其中N是上文指定的整数;“PRED-OT.N”是指经由计算机仿真方法预测的脱靶位点ID,其中N是所指定的整数。

[0831] 表33.经含有100ng mRNA及31.03nM gRNA的LNP处理的PHH中检测到的脱靶位点

[0832]

引导ID	脱靶(OT)位点ID	位点类型	插入或缺失频率(使用具有100 ng Cas9 mRNA及14.68 nM gRNA的LNP)	插入或缺失频率标准偏差
G000480	n/a	靶上	97.33%	1.10%
G000480	INS-OT.2	脱靶	1.43%	0.40%
G000480	INS-OT.4	脱靶	0.97%	0.25%
G000486	n/a	靶上	91.33%	1.97%
G000486	INS-OT.4	脱靶	0.47%	0.06%

[0833] 实例12.至ATTR的人类化小鼠模型的LNP递送

[0834] 此实例中使用表达人类TTR蛋白的V30M病原体突变形式的遗传性ATTR淀粉样变性的公认的人类化转基因小鼠模型。此小鼠模型重述了在ATTR患者中观察的组织中(包括在周围神经系统及胃肠道内)的TTR沉积表型,(参见Santos等人,Neurobiol Aging.2010年2月;31(2):280-9)。

[0835] 向小鼠(约4-5个月龄)投用使用如实例1中所述的交叉流及TFF程序制备的LNP调配物。LNP经调配以具有6.0的N:P比率,且分别含有呈50:38:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG。LNP含有Cas9 mRNA(SEQ ID NO:1)及G000481(“G481”)或非靶向对照引导G000395(“G395”;SEQ ID NO:273),gRNA:mRNA的重量比为1:1。

[0836] 如实例1中所述经由侧尾静脉向小鼠注射1mg/kg(总RNA含量)的单一剂量的LNP,其中n＝10/组。在处理后8周,将小鼠安乐死用于样本收集。如先前由Butler等人,Amyloid.2016年6月;23(2):109-18所述藉由ELISA在血清及脑脊髓液(CSF)中测量人类TTR蛋白含量。如实例1中所述分析肝组织的编辑程度。收集其它组织(胃、结肠、坐骨神经、背根神经节(DRG))且如先前由Gonçalves等人,Amyloid.2014年9月;21(3):175-184所述处理,进行半定量免疫组织化学。使用曼-怀特尼试验法(Mann Whitney test)进行免疫组织化学数据的统计分析,其中p值＜0.0001。

[0837] 如图23A-B中所示,在单剂量的包含G481的LNP后,在人类化小鼠的肝中观察到TTR的稳健编辑(49.4%),且在对照组中未检测到编辑。编辑事件的分析证实,96.8%的事件是

插入,其余是缺失。

[0838] 如图24A-B中所示,TTR蛋白含量在经处理小鼠的血浆中降低但在CSF中不降低,观察到TTR血浆含量减弱大于99%(p<0.001)。

[0839] 经处理动物的血浆中观察到的接近完全减弱的TTR,与分析的组织中TTR蛋白淀粉样沉积的清除率呈相关性。如图25中所示,对照小鼠在组织中出现的淀粉样染色类似在患有ATTR的人类个体中观察到的病理生理学。藉由编辑HuTTR V30M基因座减少循环TTR,导致组织中淀粉样沉积显著减少。在处理后8周,在处理的组织中观察到约85%或更佳的TTR染色减少(图25)。

[0840] 实例13.原代人类肝细胞(PHH)中的TTR mRNA减弱

[0841] 在一个实验中,如实例4中所述,培养PHH且用包含Cas9 mRNA(SEQ ID NO:1)及所关注gRNA(参见图29、表34)的LNP处理。使用上述交叉流程序制备LNP,且分别使用PD-10管柱及Amicon离心过滤器单元纯化及浓缩。LNP经调配以具有6.0的N:P比率,且分别含有呈50:38:9:2摩尔比的脂质A、胆固醇、DSPC及PEG2k-DMG。LNP包含1:2的gRNA:mRNA比率,且以300ng的剂量(相对于所递送mRNA运送子的量)处理细胞。

[0842] 在LNP处理后九十六(96)小时(对于每种条件进行生物学一式三份),根据制造商的方案,使用Dynabeads mRNA DIRECT试剂盒(ThermoFisher Scientific)自PHH细胞纯化mRNA。利用Maxima反转录酶(ThermoFisher Scientific)及聚-dT引物进行反转录(RT)。利用Ampure XP珠粒(Agencourt)纯化所得cDNA。对于定量PCR,利用Taqman Fast Advanced Mastermix及3个Taqman探针组TTR(分析ID:Hs00174914_m1)、GAPDH(分析ID:Hs02786624_g1)及PPIB(分析ID:Hs00168719_m1)扩增2%的纯化的cDNA。根据制造商的说明书(Life Technologies)在QuantStudio 7 Flex实时PCR系统上运行分析。藉由个别地正规化至内源对照(GAPDH及PPIB)计算TTR mRNA的相对表达,且然后取平均值。

[0843] 如图29中所示且在下表34中以数字方式再现,与阴性(未处理)对照相比,测试的每一LNP调配物导致TTR mRNA减弱。藉由每一LNP制备中使用的gRNA ID鉴别图29及表34中的组。TTR mRNA的相对表达绘制于图29中,而TTR mRNA的减弱百分比提供于表34中。

[0844] 表34.

[0845]

引导ID	平均减弱%	标准偏差
G000480	95.19	1.68
G000481	91.39	2.39
G000482	82.31	4.51
G000483	68.78	13.45
G000484	75.22	9.05
G000488	92.77	3.76
G000489	91.85	2.77
G000490	78.34	5.76
G000493	87.53	4.54
G000494	91.15	3.63
G000499	91.38	1.71
G000500	92.90	3.15
G000567	90.89	5.39
G000568	53.44	20.20
G000570	93.38	2.66
G000571	96.17	2.07

[0846]

G000572	55.92	24.53

[0847] 在单独实验中，在用包含G000480、G000486及G000502的LNP处理后，评估TTR mRNA减弱。调配LNP且培养PHH并用LNP处理，各自如此实例中的上述实验中所述，只是以100ng的剂量(相对于所递送mRNA运送子的量)处理细胞。

[0848] 在LNP处理后九十六(96)小时(对于每种条件单次处理)，根据制造商的方案使用Dynabeads mRNA DIRECT试剂盒(ThermoFisher Scientific)自PHH细胞纯化mRNA。根据制造商的说明书用高容量cDNA反转录试剂盒(ThermoFisher Scientific)实施反转录(RT)。对于定量PCR，利用Taqman Fast Advanced Mastermix及3个Taqman探针组TTR(分析ID:Hs00174914_m1)、GAPDH(分析ID:Hs02786624_g1)及PPIB(分析ID:Hs00168719_m1)扩增2%的cDNA。根据制造商的说明书(Life Technologies)在QuantStudio 7 Flex实时PCR系统上运行分析。藉由个别地正规化至内源对照(GAPDH及PPIB)计算TTR mRNA的相对表达，且然后取平均值。

[0849] 如图30中所示且在下表35中以数字方式再现，与阴性(未处理)对照相比，测试的每一LNP调配物导致TTR mRNA减弱。藉由每一LNP制备中使用的gRNA ID鉴别图30及表35中的组。TTR mRNA的相对表达绘制于图30中，而TTR mRNA的减弱百分比提供于表35中。

[0850] 表35.

[0851]

引导ID	平均减弱%	标准偏差
G000480	95.61	0.92
G000486	97.36	0.63

G000502	90.94	2.63

[0852] 序列表

[0853] 以下序列表提供本文揭示的序列清单。应理解，若关于RNA提及DNA序列(包含Ts)，则Ts应经Us(其可根据上下文经修饰或经未修饰)置换，且反之亦然。

[0854]

说明	序列	SEQ ID No.
具有HSD的5’ UTR、	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT	1

[0855]

对应于SEQ ID NO: 204的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物	GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAGAAGT ACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGA ATACAAGGTCCCGAGCAAGAAGTTCAAGG TCCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGATA CACAAGAAGAAAGAACAGAATCTGCTACC TGCAGGAAATCTTCAGCAACGAAATGGCA AAGGTCGACGACAGCTTCTTCCACAGACT GGAAGAAAGCTTCCTGGTCGAAGAAGACA AGAAGCACGAAAGACACCCGATCTTCGGA AACATCGTCGACGAAGTCGCATACCACGAA AAGTACCCGACAATCTACCACCTGAGAAAG AAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACA CATGATCAAGTTCAGAGGACACTTCCTGAT CGAAGGAGACCTGAACCCGGACAACAGCG ACGTCGACAAGCTGTTCATCCAGCTGGTCC AGACATACAACCAGCTGTTCGAAGAAAAC CCGATCAACGCAAGCGGAGTCGACGCAAA GGCAATCCTGAGCGCAAGACTGAGCAAGA GCAGAAGACTGGAAAACCTGATCGCACAG CTGCCGGGAGAAAAGAAGAACGGACTGTT CGGAAACCTGATCGCACTGAGCCTGGGAC TGACACCGAACTTCAAGAGCAACTTCGAC CTGGCAGAAGACGCAAAGCTGCAGCTGAG CAAGGACACATACGACGACGACCTGGACA ACCTGCTGGCACAGATCGGAGACCAGTAC GCAGACCTGTTCCTGGCAGCAAAGAACCT GAGCGACGCAATCCTGCTGAGCGACATCCT GAGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACG ACGAACACCACCAGGACCTGACACTGCTG AAGGCACTGGTCAGACAGCAGCTGCCGGA AAAGTACAAGGAAATCTTCTTCGACCAGA GCAAGAACGGATACGCAGGATACATCGACG GAGGAGCAAGCCAGGAAGAATTCTACAAG TTCATCAAGCCGATCCTGGAAAAGATGGAC GGAACAGAAGAACTGCTGGTCAAGCTGAA CAGAGAAGACCTGCTGAGAAAGCAGAGAA	

[0856]

	CATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGA GAAGACAGGAAGACTTCTACCCGTTCCTG AAGGACAACAGAGAAAAGATCGAAAAGAT CCTGACATTCAGAATCCCGTACTACGTCGG ACCGCTGGCAAGAGGAAACAGCAGATTCG CATGGATGACAAGAAAGAGCGAAGAAACA ATCACACCGTGGAACTTCGAAGAAGTCGTC GACAAGGGAGCAAGCGCACAGAGCTTCAT CGAAAGAATGACAAACTTCGACAAGAACC TGCCGAACGAAAAGGTCCTGCCGAAGCAC AGCCTGCTGTACGAATACTTCACAGTCTAC AACGAACTGACAAAGGTCAAGTACGTCAC AGAAGGAATGAGAAAGCCGGCATTCCTGA GCGGAGAACAGAAGAAGGCAATCGTCGAC CTGCTGTTCAAGACAAACAGAAAGGTCAC AGTCAAGCAGCTGAAGGAAGACTACTTCA AGAAGATCGAATGCTTCGACAGCGTCGAAA TCAGCGGAGTCGAAGACAGATTCAACGCA AGCCTGGGAACATACCACGACCTGCTGAA GATCATCAAGGACAAGGACTTCCTGGACA ACGAAGAAAACGAAGACATCCTGGAAGAC ATCGTCCTGACACTGACACTGTTCGAAGAC AGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCAT GAAGCAGCTGAAGAGAAGAAGATACACAG GATGGGGAAGACTGAGCAGAAAGCTGATC AACGGAATCAGAGACAAGCAGAGCGGAAA GACAATCCTGGACTTCCTGAAGAGCGACG GATTCGCAAACAGAAACTTCATGCAGCTGA TCCACGACGACAGCCTGACATTCAAGGAA GACATCCAGAAGGCACAGGTCAGCGGACA GGGAGACAGCCTGCACGAACACATCGCAA ACCTGGCAGGAAGCCCGGCAATCAAGAAG GGAATCCTGCAGACAGTCAAGGTCGTCGA CGAACTGGTCAAGGTCATGGGAAGACACA AGCCGGAAAACATCGTCATCGAAATGGCA AGAGAAAACCAGACAACACAGAAGGGAC AGAAGAACAGCAGAGAAAGAATGAAGAG AATCGAAGAAGGAATCAAGGAACTGGGAA GCCAGATCCTGAAGGAACACCCGGTCGAA AACACACAGCTGCAGAACGAAAAGCTGTA CCTGTACTACCTGCAGAACGGAAGAGACAT	

[0857]

	GTACGTCGACCAGGAACTGGACATCAACA GACTGAGCGACTACGACGTCGACCACATC GTCCCGCAGAGCTTCCTGAAGGACGACAG CATCGACAACAAGGTCCTGACAAGAAGCG ACAAGAACAGAGGAAAGAGCGACAACGT CCCGAGCGAAGAAGTCGTCAAGAAGATGA AGAACTACTGGAGACAGCTGCTGAACGCA AAGCTGATCACACAGAGAAAGTTCGACAA CCTGACAAAGGCAGAGAGAGGAGGACTGA GCGAACTGGACAAGGCAGGATTCATCAAG AGACAGCTGGTCGAAACAAGACAGATCAC AAAGCACGTCGCACAGATCCTGGACAGCA GAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCA GAAAGGACTTCCAGTTCTACAAGGTCAGA GAAATCAACAACTACCACCACGCACACGA CGCATACCTGAACGCAGTCGTCGGAACAG CACTGATCAAGAAGTACCCGAAGCTGGAA AGCGAATTCGTCTACGGAGACTACAAGGTC TACGACGTCAGAAAGATGATCGCAAAGAG CGAACAGGAAATCGGAAAGGCAACAGCAA AGTACTTCTTCTACAGCAACATCATGAACTT CTTCAAGACAGAAATCACACTGGCAAACG GAGAAATCAGAAAGAGACCGCTGATCGAA ACAAACGGAGAAACAGGAGAAATCGTCTG GGACAAGGGAAGAGACTTCGCAACAGTCA GAAAGGTCCTGAGCATGCCGCAGGTCAAC ATCGTCAAGAAGACAGAAGTCCAGACAGG AGGATTCAGCAAGGAAAGCATCCTGCCGA AGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGG AGGATTCGACAGCCCGACAGTCGCATACAG CGTCCTGGTCGTCGCAAAGGTCGAAAAGG GAAAGAGCAAGAAGCTGAAGAGCGTCAA GGAACTGCTGGGAATCACAATCATGGAAA GAAGCAGCTTCGAAAAGAACCCGATCGAC TTCCTGGAAGCAAAGGGATACAAGGAAGT CAAGAAGGACCTGATCATCAAGCTGCCGA AGTACAGCCTGTTCGAACTGGAAAACGGA AGAAAGAGAATGCTGGCAAGCGCAGGAGA ACTGCAGAAGGGAAACGAACTGGCACTGC CGAGCAAGTACGTCAACTTCCTGTACCTGG	

[0858]

	CAAGCCACTACGAAAAGCTGAAGGGAAGC CCGGAAGACAACGAACAGAAGCAGCTGTT CGTCGAACAGCACAAGCACTACCTGGACG AAATCATCGAACAGATCAGCGAATTCAGCA AGAGAGTCATCCTGGCAGACGCAAACCTG GACAAGGTCCTGAGCGCATACAACAAGCA CAGAGACAAGCCGATCAGAGAACAGGCAG AAAACATCATCCACCTGTTCACACTGACAA ACCTGGGAGCACCGGCAGCATTCAAGTACT TCGACACAACAATCGACAGAAAGAGATAC ACAAGCACAAAGGAAGTCCTGGACGCAAC ACTGATCCACCAGAGCATCACAGGACTGTA CGAAACAAGAATCGACCTGAGCCAGCTGG GAGGAGACGGAGGAGGAAGCCCGAAGAA GAAGAGAAAGGTCTAGCTAGCCATCACATT TAAAAGCATCTCAGCCTACCATGAGAATAA GAGAAAGAAAATGAAGATCAATAGCTTATT CATCTCTTTTTCTTTTTCGTTGGTGTAAAGC CAACACCCTGTCTAAAAAACATAAATTTCT TTAATCATTTTGCCTCTTTTCTCTGTGCTTC AATTAATAAAAAATGGAAAGAACCTCGAG	
包含对应于SEQ ID NO: 205的Cas9 ORF且使用在人类中通常高度表达的密码子的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCATGCCTAAGAAAAAGCGGA AGGTCGACGGGGATAAGAAGTACTCAATCG GGCTGGATATCGGAACTAATTCCGTGGGTT GGGCAGTGATCACGGATGAATACAAAGTGC CGTCCAAGAAGTTCAAGGTCCTGGGGAAC ACCGATAGACACAGCATCAAGAAAAATCTC ATCGGAGCCCTGCTGTTTGACTCCGGCGAA ACCGCAGAAGCGACCCGGCTCAAACGTAC CGCGAGGCGACGCTACACCCGGCGGAAGA ATCGCATCTGCTATCTGCAAGAGATCTTTTC GAACGAAATGGCAAAGGTCGACGACAGCT TCTTCCACCGCCTGGAAGAATCTTTCCTGG TGGAGGAGGACAAGAAGCATGAACGGCAT CCTATCTTTGGAAACATCGTCGACGAAGTG GCGTACCACGAAAAGTACCCGACCATCTAC CATCTGCGGAAGAAGTTGGTTGACTCAACT GACAAGGCCGACCTCAGATTGATCTACTTG GCCCTCGCCCATATGATCAAATTCCGCGGA CACTTCCTGATCGAAGGCGATCTGAACCCT	2

[0859]

	GATAACTCCGACGTGGATAAGCTTTTCATTC AACTGGTGCAGACCTACAACCAACTGTTCG AAGAAAACCCAATCAATGCTAGCGGCGTC GATGCCAAGGCCATCCTGTCCGCCCGGCTG TCGAAGTCGCGGCGCCTCGAAAACCTGATC GCACAGCTGCCGGGAGAGAAAAAGAACG GACTTTTCGGCAACTTGATCGCTCTCTCAC TGGGACTCACTCCCAATTTCAAGTCCAATT TTGACCTGGCCGAGGACGCGAAGCTGCAA CTCTCAAAGGACACCTACGACGACGACTT GGACAATTTGCTGGCACAAATTGGCGATCA GTACGCGGATCTGTTCCTTGCCGCTAAGAA CCTTTCGGACGCAATCTTGCTGTCCGATATC CTGCGCGTGAACACCGAAATAACCAAAGC GCCGCTTAGCGCCTCGATGATTAAGCGGTA CGACGAGCATCACCAGGATCTCACGCTGCT CAAAGCGCTCGTGAGACAGCAACTGCCTG AAAAGTACAAGGAGATCTTCTTCGACCAGT CCAAGAATGGGTACGCAGGGTACATCGATG GAGGCGCTAGCCAGGAAGAGTTCTATAAGT TCATCAAGCCAATCCTGGAAAAGATGGACG GAACCGAAGAACTGCTGGTCAAGCTGAAC AGGGAGGATCTGCTCCGGAAACAGAGAAC CTTTGACAACGGATCCATTCCCCACCAGAT CCATCTGGGTGAGCTGCACGCCATCTTGCG GCGCCAGGAGGACTTTTACCCATTCCTCAA GGACAACCGGGAAAAGATCGAGAAAATTC TGACGTTCCGCATCCCGTATTACGTGGGCC CACTGGCGCGCGGCAATTCGCGCTTCGCGT GGATGACTAGAAAATCAGAGGAAACCATC ACTCCTTGGAATTTCGAGGAAGTTGTGGAT AAGGGAGCTTCGGCACAAAGCTTCATCGA ACGAATGACCAACTTCGACAAGAATCTCCC AAACGAGAAGGTGCTTCCTAAGCACAGCC TCCTTTACGAATACTTCACTGTCTACAACGA ACTGACTAAAGTGAAATACGTTACTGAAGG AATGAGGAAGCCGGCCTTTCTGTCCGGAG AACAGAAGAAAGCAATTGTCGATCTGCTGT TCAAGACCAACCGCAAGGTGACCGTCAAG CAGCTTAAAGAGGACTACTTCAAGAAGATC GAGTGTTTCGACTCAGTGGAAATCAGCGG GGTGGAGGACAGATTCAACGCTTCGCTGG GAACCTATCATGATCTCCTGAAGATCATCAA	

[0860]

	GGACAAGGACTTCCTTGACAACGAGGAGA ACGAGGACATCCTGGAAGATATCGTCCTGA CCTTGACCCTTTTCGAGGATCGCGAGATGA TCGAGGAGAGGCTTAAGACCTACGCTCATC TCTTCGACGATAAGGTCATGAAACAACTCA AGCGCCGCCGGTACACTGGTTGGGGCCGC CTCTCCCGCAAGCTGATCAACGGTATTCGC GATAAACAGAGCGGTAAAACTATCCTGGAT TTCCTCAAATCGGATGGCTTCGCTAATCGTA ACTTCATGCAATTGATCCACGACGACAGCC TGACCTTTAAGGAGGACATCCAAAAAGCA CAAGTGTCCGGACAGGGAGACTCACTCCA TGAACACATCGCGAATCTGGCCGGTTCGCC GGCGATTAAGAAGGGAATTCTGCAAACTGT GAAGGTGGTCGACGAGCTGGTGAAGGTCA TGGGACGGCACAAACCGGAGAATATCGTG ATTGAAATGGCCCGAGAAAACCAGACTAC CCAGAAGGGCCAGAAAAACTCCCGCGAAA GGATGAAGCGGATCGAAGAAGGAATCAAG GAGCTGGGCAGCCAGATCCTGAAAGAGCA CCCGGTGGAAAACACGCAGCTGCAGAACG AGAAGCTCTACCTGTACTATTTGCAAAATG GACGGGACATGTACGTGGACCAAGAGCTG GACATCAATCGGTTGTCTGATTACGACGTG GACCACATCGTTCCACAGTCCTTTCTGAAG GATGACTCGATCGATAACAAGGTGTTGACT CGCAGCGACAAGAACAGAGGGAAGTCAGA TAATGTGCCATCGGAGGAGGTCGTGAAGA AGATGAAGAATTACTGGCGGCAGCTCCTGA ATGCGAAGCTGATTACCCAGAGAAAGTTTG ACAATCTCACTAAAGCCGAGCGCGGCGGA CTCTCAGAGCTGGATAAGGCTGGATTCATC AAACGGCAGCTGGTCGAGACTCGGCAGAT TACCAAGCACGTGGCGCAGATCTTGGACTC CCGCATGAACACTAAATACGACGAGAACGA TAAGCTCATCCGGGAAGTGAAGGTGATTAC CCTGAAAAGCAAACTTGTGTCGGACTTTCG GAAGGACTTTCAGTTTTACAAAGTGAGAG AAATCAACAACTACCATCACGCGCATGACG CATACCTCAACGCTGTGGTCGGTACCGCCC TGATCAAAAAGTACCCTAAACTTGAATCGG AGTTTGTGTACGGAGACTACAAGGTCTACG ACGTGAGGAAGATGATAGCCAAGTCCGAA	

[0861]

	CAGGAAATCGGGAAAGCAACTGCGAAATA CTTCTTTTACTCAAACATCATGAACTTTTTC AAGACTGAAATTACGCTGGCCAATGGAGA AATCAGGAAGAGGCCACTGATCGAAACTA ACGGAGAAACGGGCGAAATCGTGTGGGAC AAGGGCAGGGACTTCGCAACTGTTCGCAA AGTGCTCTCTATGCCGCAAGTCAATATTGTG AAGAAAACCGAAGTGCAAACCGGCGGATT TTCAAAGGAATCGATCCTCCCAAAGAGAAA TAGCGACAAGCTCATTGCACGCAAGAAAG ACTGGGACCCGAAGAAGTACGGAGGATTC GATTCGCCGACTGTCGCATACTCCGTCCTC GTGGTGGCCAAGGTGGAGAAGGGAAAGA GCAAAAAGCTCAAATCCGTCAAAGAGCTG CTGGGGATTACCATCATGGAACGATCCTCG TTCGAGAAGAACCCGATTGATTTCCTCGAG GCGAAGGGTTACAAGGAGGTGAAGAAGGA TCTGATCATCAAACTCCCCAAGTACTCACT GTTCGAACTGGAAAATGGTCGGAAGCGCA TGCTGGCTTCGGCCGGAGAACTCCAAAAA GGAAATGAGCTGGCCTTGCCTAGCAAGTAC GTCAACTTCCTCTATCTTGCTTCGCACTACG AAAAACTCAAAGGGTCACCGGAAGATAAC GAACAGAAGCAGCTTTTCGTGGAGCAGCA CAAGCATTATCTGGATGAAATCATCGAACA AATCTCCGAGTTTTCAAAGCGCGTGATCCT CGCCGACGCCAACCTCGACAAAGTCCTGT CGGCCTACAATAAGCATAGAGATAAGCCGA TCAGAGAACAGGCCGAGAACATTATCCACT TGTTCACCCTGACTAACCTGGGAGCCCCAG CCGCCTTCAAGTACTTCGATACTACTATCGA TCGCAAAAGATACACGTCCACCAAGGAAG TTCTGGACGCGACCCTGATCCACCAAAGCA TCACTGGACTCTACGAAACTAGGATCGATC TGTCGCAGCTGGGTGGCGATTGATAGTCTA GCCATCACATTTAAAAGCATCTCAGCCTAC CATGAGAATAAGAGAAAGAAAATGAAGAT CAATAGCTTATTCATCTCTTTTTCTTTTTCGT TGGTGTAAAGCCAACACCCTGTCTAAAAAA CATAAATTTCTTTAATCATTTTGCCTCTTTTC TCTGTGCTTCAATTAATAAAAAATGGAAAG AACCTCGAG	

[0862]

经修饰sgRNA序列(“N”可为任何天然或非天然核苷酸)	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	3
30/30/39多-A序列	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	4
靶向人类TTR (外显子1)的CR003335 gRNA	CUGCUCCUCCUCUGCCUUGC	5
靶向人类TTR (外显子1)的CR003336 gRNA	CCUCCUCUGCCUUGCUGGAC	6
靶向人类TTR (外显子1)的CR003337 gRNA	CCAGUCCAGCAAGGCAGAGG	7
靶向人类TTR (外显子1)的CR003338 gRNA	AUACCAGUCCAGCAAGGCAG	8
靶向人类TTR (外显子1)的CR003339 gRNA	ACACAAAUACCAGUCCAGCA	9
靶向人类TTR (外显子1)的CR003340 gRNA	UGGACUGGUAUUUGUGUCUG	10
靶向人类TTR (外显子1)的CR003341 gRNA	CUGGUAUUUGUGUCUGAGGC	11
靶向人类TTR (外显子2)的CR003342 gRNA	CUUCUCUACACCCAGGGCAC	12
靶向人类TTR (外显子2)的CR003343 gRNA	CAGAGGACACUUGGAUUCAC	13
靶向人类TTR (外显子2)的CR003344 gRNA	UUUGACCAUCAGAGGACACU	14
靶向人类TTR (外显子2)的CR003345 gRNA	UCUAGAACUUUGACCAUCAG	15

[0863]

靶向人类TTR (外显子2)的CR003346 gRNA	AAAGUUCUAGAUGCUGUCCG	16
靶向人类TTR (外显子2)的CR003347 gRNA	CAUUGAUGGCAGGACUGCCU	17
靶向人类TTR (外显子2)的CR003348 gRNA	AGGCAGUCCUGCCAUCAAUG	18
靶向人类TTR (外显子2)的CR003349 gRNA	UGCACGGCCACAUUGAUGGC	19
靶向人类TTR (外显子2)的CR003350 gRNA	CACAUGCACGGCCACAUUGA	20
靶向人类TTR (外显子2)的CR003351 gRNA	AGCCUUUCUGAACACAUGCA	21
靶向人类TTR (外显子2)的CR003352 gRNA	GAAAGGCUGCUGAUGACACC	22
靶向人类TTR (外显子2)的CR003353 gRNA	AAAGGCUGCUGAUGACACCU	23
靶向人类TTR (外显子2)的CR003354 gRNA	ACCUGGGAGCCAUUUGCCUC	24
靶向人类TTR (外显子2)的CR003355 gRNA	CCCAGAGGCAAAUGGCUCCC	25
靶向人类TTR (外显子2)的CR003356 gRNA	GCAACUUACCCAGAGGCAAA	26
靶向人类TTR (外显子2)的CR003357 gRNA	UUCUUUGGCAACUUACCCAG	27
靶向人类TTR (外显子3)的CR003358 gRNA	AUGCAGCUCUCCAGACUCAC	28
靶向人类TTR (外显子3)的CR003359 gRNA	AGUGAGUCUGGAGAGCUGCA	29

[0864]

靶向人类TTR (外显子3)的CR003360 gRNA	GUGAGUCUGGAGAGCUGCAU	30
靶向人类TTR (外显子3)的CR003361 gRNA	GCUGCAUGGGCUCACAACUG	31
靶向人类TTR (外显子3)的CR003362 gRNA	GCAUGGGCUCACAACUGAGG	32
靶向人类TTR (外显子3)的CR003363 gRNA	ACUGAGGAGGAAUUUGUAGA	33
靶向人类TTR (外显子3)的CR003364 gRNA	CUGAGGAGGAAUUUGUAGAA	34
靶向人类TTR (外显子3)的CR003365 gRNA	UGUAGAAGGGAUAUACAAAG	35
靶向人类TTR (外显子3)的CR003366 gRNA	AAAUAGACACCAAAUCUUAC	36
靶向人类TTR (外显子3)的CR003367 gRNA	AGACACCAAAUCUUACUGGA	37
靶向人类TTR (外显子3)的CR003368 gRNA	AAGUGCCUUCCAGUAAGAUU	38
靶向人类TTR (外显子3)的CR003369 gRNA	CUCUGCAUGCUCAUGGAAUG	39
靶向人类TTR (外显子3)的CR003370 gRNA	CCUCUGCAUGCUCAUGGAAU	40
靶向人类TTR (外显子3)的CR003371 gRNA	ACCUCUGCAUGCUCAUGGAA	41
靶向人类TTR (外显子3)的CR003372 gRNA	UACUCACCUCUGCAUGCUCA	42
靶向人类TTR (外显子4)的CR003373 gRNA	GUAUUCACAGCCAACGACUC	43

[0865]

靶向人类TTR (外显子4)的CR003374 gRNA	GCGGCGGGGGCCGGAGUCGU	44
靶向人类TTR (外显子4)的CR003375 gRNA	AAUGGUGUAGCGGCGGGGGC	45
靶向人类TTR (外显子4)的CR003376 gRNA	CGGCAAUGGUGUAGCGGCGG	46
靶向人类TTR (外显子4)的CR003377 gRNA	GCGGCAAUGGUGUAGCGGCG	47
靶向人类TTR (外显子4)的CR003378 gRNA	GGCGGCAAUGGUGUAGCGGC	48
靶向人类TTR (外显子4)的CR003379 gRNA	GGGCGGCAAUGGUGUAGCGG	49
靶向人类TTR (外显子4)的CR003380 gRNA	GCAGGGCGGCAAUGGUGUAG	50
靶向人类TTR (外显子4)的CR003381 gRNA	GGGGCUCAGCAGGGCGGCAA	51
靶向人类TTR (外显子4)的CR003382 gRNA	GGAGUAGGGGCUCAGCAGGG	52
靶向人类TTR (外显子4)的CR003383 gRNA	AUAGGAGUAGGGGCUCAGCA	53
靶向人类TTR (外显子4)的CR003384 gRNA	AAUAGGAGUAGGGGCUCAGC	54
靶向人类TTR (外显子4)的CR003385 gRNA	CCCCUACUCCUAUUCCACCA	55
靶向人类TTR (外显子4)的CR003386 gRNA	CCGUGGUGGAAUAGGAGUAG	56
靶向人类TTR (外显子4)的CR003387 gRNA	GCCGUGGUGGAAUAGGAGUA	57

[0866]

靶向人类TTR (外显子4)的CR003388 gRNA	GACGACAGCCGUGGUGGAAU	58
靶向人类TTR (外显子4)的CR003389 gRNA	AUUGGUGACGACAGCCGUGG	59
靶向人类TTR (外显子4)的CR003390 gRNA	GGGAUUGGUGACGACAGCCG	60
靶向人类TTR (外显子4)的CR003391 gRNA	GGCUGUCGUCACCAAUCCCA	61
靶向人类TTR (外显子4)的CR003392 gRNA	AGUCCCUCAUUCCUUGGGAU	62
靶向人类TTR (外显子1)的CR005298 gRNA	UCCACUCAUUCUUGGCAGGA	63
靶向人类TTR (外显子4)的CR005299 gRNA	AGCCGUGGUGGAAUAGGAGU	64
靶向人类TTR (外显子1)的CR005300 gRNA	UCACAGAAACACUCACCGUA	65
靶向人类TTR (外显子1)的CR005301 gRNA	GUCACAGAAACACUCACCGU	66
靶向人类TTR (外显子2)的CR005302 gRNA	ACGUGUCUUCUCUACACCCA	67
靶向人类TTR (外显子2)的CR005303 gRNA	UGAAUCCAAGUGUCCUCUGA	68
靶向人类TTR (外显子2)的CR005304 gRNA	GGCCGUGCAUGUGUUCAGAA	69
靶向人类TTR (外显子3)的CR005305 gRNA	UAUAGGAAAACCAGUGAGUC	70
靶向人类TTR (外显子3)的CR005306 gRNA	AAAUCUUACUGGAAGGCACU	71

[0867]

靶向人类TTR (外显子4)的CR005307 gRNA	UGUCUGUCUUCUCUCAUAGG	72
靶向食蟹猴TTR的CR000689 gRNA	ACACAAAUACCAGUCCAGCG	73
靶向食蟹猴TTR的CR005364 gRNA	AAAGGCUGCUGAUGAGACCU	74
靶向食蟹猴TTR的CR005365 gRNA	CAUUGACAGCAGGACUGCCU	75
靶向食蟹猴TTR的CR005366 gRNA	AUACCAGUCCAGCGAGGCAG	76
靶向食蟹猴TTR的CR005367 gRNA	CCAGUCCAGCGAGGCAGAGG	77
靶向食蟹猴TTR的CR005368 gRNA	CCUCCUCUGCCUCGCUGGAC	78
靶向食蟹猴TTR的CR005369 gRNA	AAAGUUCUAGAUGCCGUCCG	79
靶向食蟹猴TTR的CR005370 gRNA	ACUUGUCUUCUCUAUACCCA	80
靶向食蟹猴TTR的CR005371 gRNA	AAGUGACUUCCAGUAAGAUU	81
靶向食蟹猴TTR的CR005372 gRNA	AAAAGGCUGCUGAUGAGACC	82
	未使用	83
	未使用	84
	未使用	85
	未使用	86
靶向人类TTR的G000480 sgRNA修饰的序列	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	87
靶向人类TTR的	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAm	88

[0868]

G000481 sgRNA 修饰的序列	GmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
靶向人类 TTR 的 G000482 sgRNA 修饰的序列	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	89
靶向人类 TTR 的 G000483 sgRNA 修饰的序列	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	90
靶向人类 TTR 的 G000484 sgRNA 修饰的序列	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	91
靶向人类 TTR 的 G000485 sgRNA 修饰的序列	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	92
靶向人类 TTR 的 G000486 sgRNA 修饰的序列	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	93
靶向人类 TTR 的 G000487 sgRNA 修饰的序列	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	94
靶向人类 TTR 的 G000488 sgRNA 修	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUA	95

[0869]

饰的序列	UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	
靶向人类TTR的G000489 sgRNA修饰的序列	mU*mU*mU*GACCAUCAGAGGACACUGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	96
靶向人类TTR的G000490 sgRNA修饰的序列	mA*mA*mA*UAGACACCAAAUCUUACGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	97
靶向人类TTR的G000491 sgRNA修饰的序列	mA*mU*mA*CCAGUCCAGCAAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	98
靶向人类TTR的G000492 sgRNA修饰的序列	mC*mU*mU*CUCUACACCCAGGGCACGUU UUAGAmGmCmUmAmGmAmAmAmUmAmG mCAAGUUAAAAUAAGGCUAGUCCGUUAU CAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	99
靶向人类TTR的G000493 sgRNA修饰的序列	mA*mA*mG*UGCCUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	100
靶向人类TTR的G000494 sgRNA修饰的序列	mG*mU*mG*AGUCUGGAGAGCUGCAUGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	101
靶向人类TTR的G000495 sgRNA修	mC*mA*mG*AGGACACUUGGAUUCACGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUUAAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGmU	102

[0870]

饰的序列	mGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
靶向人类TTR的G000496 sgRNA修饰的序列	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	103
靶向人类TTR的G000497 sgRNA修饰的序列	mC*mU*mG*CUCCUCCUCUGCCUUGCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	104
靶向人类TTR的G000498 sgRNA修饰的序列	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	105
靶向人类TTR的G000499 sgRNA修饰的序列	mU*mG*mA*AUCCAAGUGUCCUCUGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	106
靶向人类TTR的G000500 sgRNA修饰的序列	mC*mC*mA*GUCCAGCAAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	107
靶向人类TTR的G000501 sgRNA修饰的序列	mU*mC*mA*CAGAAACACUCACCGUAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	108
靶向人类TTR的G000567 sgRNA修饰的序列	mG*mA*mA*AGGCUGCUGAUGACACCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGm	109

[0871]

	UmGmCmU*mU*mU*mU	
靶向人类TTR的G000568 sgRNA修饰的序列	mG*mG*mC*UGUCGUCACCAAUCCCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	110
靶向人类TTR的G000570 sgRNA修饰的序列	mC*mA*mU*UGAUGGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	111
靶向人类TTR的G000571 sgRNA修饰的序列	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	112
靶向人类TTR的G000572 sgRNA修饰的序列	mC*mC*mC*CUACUCCUAUUCCACCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	113
靶向食蟹猴TTR的G000502 sgRNA修饰的序列	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	114
靶向食蟹猴TTR的G000503 sgRNA修饰的序列	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	115
靶向食蟹猴TTR的G000504 sgRNA修饰的序列	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	116

[0872]

靶向食蟹猴TTR的G000505 sgRNA修饰的序列	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	117
靶向食蟹猴TTR的G000506 sgRNA修饰的序列	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	118
靶向食蟹猴TTR的G000507 sgRNA修饰的序列	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	119
靶向食蟹猴TTR的G000508 sgRNA修饰的序列	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	120
靶向食蟹猴TTR的G000509 sgRNA修饰的序列	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	121
靶向食蟹猴TTR的G000510 sgRNA修饰的序列	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	122
靶向食蟹猴TTR的G000511 sgRNA修饰的序列	mA*mA*mG*UGACUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	123

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靶向小鼠TTR的G000282 sgRNA修饰的序列	mU*mU*mA*CAGCCACGUCUACAGCAGUU UUAGAmGmCmUmAmGmAmAmAmUmAmG mCAAGUUAAAAUAAGGCUAGUCCGUUAU CAmAmCmUmUmGmAmAmAmAmGmU mGmGmCmAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU	124
	未使用	125 至 200
使用表3中所列示最小尿苷密码子的胸苷类似物的Cas9的DNA编码序列，具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGA CATCGGAACAAACAGCGTCGGATGGGCAG TCATCACAGACGAATACAAGGTCCCGAGC AAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATC GGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAC AGCAAGAAGAAGATACACAAGAAGAAAG AACAGAATCTGCTACCTGCAGGAAATCTT CAGCAACGAAATGGCAAAGGTCGACGAC AGCTTCTTCCACAGACTGGAAGAAAGCTT CCTGGTCGAAGAAGACAAGAAGCACGAA AGACACCCGATCTTCGGAAACATCGTCGA CGAAGTCGCATACCACGAAAAGTACCCGA CAATCTACCACCTGAGAAAGAAGCTGGTC GACAGCACAGACAAGGCAGACCTGAGAC TGATCTACCTGGCACTGGCACACATGATCA AGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCG ACAAGCTGTTCATCCAGCTGGTCCAGACAT ACAACCAGCTGTTCGAAGAAAACCCGATC AACGCAAGCGGAGTCGACGCAAAGGCAA TCCTGAGCGCAAGACTGAGCAAGAGCAG AAGACTGGAAAACCTGATCGCACAGCTGC CGGGAGAAAAGAAGAACGGACTGTTCGG AAACCTGATCGCACTGAGCCTGGGACTGA CACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCAAAGCTGCAGCTGAGCA AGGACACATACGACGACGACCTGGACAAC CTGCTGGCACAGATCGGAGACCAGTACGC AGACCTGTTCCTGGCAGCAAAGAACCTGA GCGACGCAATCCTGCTGAGCGACATCCTG AGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATAC	201

[0874]

	GACGAACACCACCAGGACCTGACACTGCT GAAGGCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCA GAGCAAGAACGGATACGCAGGATACATCG ACGGAGGAGCAAGCCAGGAAGAATTCTAC AAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAG CTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCG CACCAGATCCACCTGGGAGAACTGCACGC AATCCTGAGAAGACAGGAAGACTTCTACC CGTTCCTGAAGGACAACAGAGAAAAGATC GAAAAGATCCTGACATTCAGAATCCCGTA CTACGTCGGACCGCTGGCAAGAGGAAACA GCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGA AGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTT CGACAAGAACCTGCCGAACGAAAAGGTC CTGCCGAAGCACAGCCTGCTGTACGAATA CTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAG CCGGCATTCCTGAGCGGAGAACAGAAGA AGGCAATCGTCGACCTGCTGTTCAAGACA AACAGAAAGGTCACAGTCAAGCAGCTGA AGGAAGACTACTTCAAGAAGATCGAATGC TTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCAAGCCTGGGAACAT ACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACG AAGACATCCTGGAAGACATCGTCCTGACA CTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCACACC TGTTCGACGACAAGGTCATGAAGCAGCTG AAGAGAAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATC AGAGACAAGCAGAGCGGAAAGACAATCC TGGACTTCCTGAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGA CGACAGCCTGACATTCAAGGAAGACATCC AGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGG CAGGAAGCCCGGCAATCAAGAAGGGAAT	

[0875]

	CCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACAAGCCG GAAAACATCGTCATCGAAATGGCAAGAGA AAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAAGAATGAAGAGAATCG AAGAAGGAATCAAGGAACTGGGAAGCCA GATCCTGAAGGAACACCCGGTCGAAAACA CACAGCTGCAGAACGAAAAGCTGTACCTG TACTACCTGCAGAACGGAAGAGACATGTA CGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTC CCGCAGAGCTTCCTGAAGGACGACAGCAT CGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGTCC CGAGCGAAGAAGTCGTCAAGAAGATGAA GAACTACTGGAGACAGCTGCTGAACGCAA AGCTGATCACACAGAGAAAGTTCGACAAC CTGACAAAGGCAGAGAGAGGAGGACTGA GCGAACTGGACAAGGCAGGATTCATCAAG AGACAGCTGGTCGAAACAAGACAGATCA CAAAGCACGTCGCACAGATCCTGGACAGC AGAATGAACACAAAGTACGACGAAAACG ACAAGCTGATCAGAGAAGTCAAGGTCATC ACACTGAAGAGCAAGCTGGTCAGCGACTT CAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACAC GACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAG GTCTACGACGTCAGAAAGATGATCGCAAA GAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATG AACTTCTTCAAGACAGAAATCACACTGGC AAACGGAGAAATCAGAAAGAGACCGCTG ATCGAAACAAACGGAGAAACAGGAGAAA TCGTCTGGGACAAGGGAAGAGACTTCGCA ACAGTCAGAAAGGTCCTGAGCATGCCGCA GGTCAACATCGTCAAGAAGACAGAAGTCC AGACAGGAGGATTCAGCAAGGAAAGCAT CCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGACTGGGACCCGA AGAAGTACGGAGGATTCGACAGCCCGACA GTCGCATACAGCGTCCTGGTCGTCGCAAA	

[0876]

	GGTCGAAAAGGGAAAGAGCAAGAAGCTG AAGAGCGTCAAGGAACTGCTGGGAATCAC AATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGA TACAAGGAAGTCAAGAAGGACCTGATCAT CAAGCTGCCGAAGTACAGCCTGTTCGAAC TGGAAAACGGAAGAAAGAGAATGCTGGC AAGCGCAGGAGAACTGCAGAAGGGAAAC GAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAA AGCTGAAGGGAAGCCCGGAAGACAACGA ACAGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAACAG ATCAGCGAATTCAGCAAGAGAGTCATCCT GGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGC CGATCAGAGAACAGGCAGAAAACATCATC CACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAA CAATCGACAGAAAGAGATACACAAGCACA AAGGAAGTCCTGGACGCAACACTGATCCA CCAGAGCATCACAGGACTGTACGAAACAA GAATCGACCTGAGCCAGCTGGGAGGAGA CGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGTCTAG	
使用在人类中通常高度表达的密码子的Cas9的DNA编码序列	ATGGATAAGAAGTACTCAATCGGGCTGGAT ATCGGAACTAATTCCGTGGGTTGGGCAGT GATCACGGATGAATACAAAGTGCCGTCCA AGAAGTTCAAGGTCCTGGGGAACACCGAT AGACACAGCATCAAGAAAAATCTCATCGG AGCCCTGCTGTTTGACTCCGGCGAAACCG CAGAAGCGACCCGGCTCAAACGTACCGCG AGGCGACGCTACACCCGGCGGAAGAATCG CATCTGCTATCTGCAAGAGATCTTTTCGAA CGAAATGGCAAAGGTCGACGACAGCTTCT TCCACCGCCTGGAAGAATCTTTCCTGGTG GAGGAGGACAAGAAGCATGAACGGCATC CTATCTTTGGAAACATCGTCGACGAAGTG GCGTACCACGAAAAGTACCCGACCATCTA CCATCTGCGGAAGAAGTTGGTTGACTCAA CTGACAAGGCCGACCTCAGATTGATCTACT TGGCCCTCGCCCATATGATCAAATTCCGCG GACACTTCCTGATCGAAGGCGATCTGAAC	202

[0877]

	CCTGATAACTCCGACGTGGATAAGCTTTTC ATTCAACTGGTGCAGACCTACAACCAACT GTTCGAAGAAAACCCAATCAATGCTAGCG GCGTCGATGCCAAGGCCATCCTGTCCGCC CGGCTGTCGAAGTCGCGGCGCCTCGAAAA CCTGATCGCACAGCTGCCGGGAGAGAAAA AGAACGGACTTTTCGGCAACTTGATCGCT CTCTCACTGGGACTCACTCCCAATTTCAAG TCCAATTTTGACCTGGCCGAGGACGCGAA GCTGCAACTCTCAAAGGACACCTACGACG ACGACTTGGACAATTTGCTGGCACAAATT GGCGATCAGTACGCGGATCTGTTCCTTGCC GCTAAGAACCTTTCGGACGCAATCTTGCT GTCCGATATCCTGCGCGTGAACACCGAAAT AACCAAAGCGCCGCTTAGCGCCTCGATGA TTAAGCGGTACGACGAGCATCACCAGGAT CTCACGCTGCTCAAAGCGCTCGTGAGACA GCAACTGCCTGAAAAGTACAAGGAGATCT TCTTCGACCAGTCCAAGAATGGGTACGCA GGGTACATCGATGGAGGCGCTAGCCAGGA AGAGTTCTATAAGTTCATCAAGCCAATCCT GGAAAAGATGGACGGAACCGAAGAACTG CTGGTCAAGCTGAACAGGGAGGATCTGCT CCGGAAACAGAGAACCTTTGACAACGGAT CCATTCCCCACCAGATCCATCTGGGTGAGC TGCACGCCATCTTGCGGCGCCAGGAGGAC TTTTACCCATTCCTCAAGGACAACCGGGA AAAGATCGAGAAAATTCTGACGTTCCGCA TCCCGTATTACGTGGGCCCACTGGCGCGC GGCAATTCGCGCTTCGCGTGGATGACTAG AAAATCAGAGGAAACCATCACTCCTTGGA ATTTCGAGGAAGTTGTGGATAAGGGAGCT TCGGCACAAAGCTTCATCGAACGAATGAC CAACTTCGACAAGAATCTCCCAAACGAGA AGGTGCTTCCTAAGCACAGCCTCCTTTACG AATACTTCACTGTCTACAACGAACTGACTA AAGTGAAATACGTTACTGAAGGAATGAGG AAGCCGGCCTTTCTGTCCGGAGAACAGAA GAAAGCAATTGTCGATCTGCTGTTCAAGA CCAACCGCAAGGTGACCGTCAAGCAGCTT AAAGAGGACTACTTCAAGAAGATCGAGTG TTTCGACTCAGTGGAAATCAGCGGGGTGG AGGACAGATTCAACGCTTCGCTGGGAACC	

[0878]

	TATCATGATCTCCTGAAGATCATCAAGGAC AAGGACTTCCTTGACAACGAGGAGAACG AGGACATCCTGGAAGATATCGTCCTGACCT TGACCCTTTTCGAGGATCGCGAGATGATCG AGGAGAGGCTTAAGACCTACGCTCATCTC TTCGACGATAAGGTCATGAAACAACTCAA GCGCCGCCGGTACACTGGTTGGGGCCGCC TCTCCCGCAAGCTGATCAACGGTATTCGCG ATAAACAGAGCGGTAAAACTATCCTGGATT TCCTCAAATCGGATGGCTTCGCTAATCGTA ACTTCATGCAATTGATCCACGACGACAGC CTGACCTTTAAGGAGGACATCCAAAAAGC ACAAGTGTCCGGACAGGGAGACTCACTCC ATGAACACATCGCGAATCTGGCCGGTTCG CCGGCGATTAAGAAGGGAATTCTGCAAAC TGTGAAGGTGGTCGACGAGCTGGTGAAG GTCATGGGACGGCACAAACCGGAGAATAT CGTGATTGAAATGGCCCGAGAAAACCAGA CTACCCAGAAGGGCCAGAAAAACTCCCGC GAAAGGATGAAGCGGATCGAAGAAGGAA TCAAGGAGCTGGGCAGCCAGATCCTGAAA GAGCACCCGGTGGAAAACACGCAGCTGC AGAACGAGAAGCTCTACCTGTACTATTTGC AAAATGGACGGGACATGTACGTGGACCAA GAGCTGGACATCAATCGGTTGTCTGATTAC GACGTGGACCACATCGTTCCACAGTCCTT TCTGAAGGATGACTCGATCGATAACAAGG TGTTGACTCGCAGCGACAAGAACAGAGG GAAGTCAGATAATGTGCCATCGGAGGAGG TCGTGAAGAAGATGAAGAATTACTGGCGG CAGCTCCTGAATGCGAAGCTGATTACCCA GAGAAAGTTTGACAATCTCACTAAAGCCG AGCGCGGCGGACTCTCAGAGCTGGATAAG GCTGGATTCATCAAACGGCAGCTGGTCGA GACTCGGCAGATTACCAAGCACGTGGCGC AGATCTTGGACTCCCGCATGAACACTAAAT ACGACGAGAACGATAAGCTCATCCGGGAA GTGAAGGTGATTACCCTGAAAAGCAAACT TGTGTCGGACTTTCGGAAGGACTTTCAGT TTTACAAAGTGAGAGAAATCAACAACTAC CATCACGCGCATGACGCATACCTCAACGCT GTGGTCGGTACCGCCCTGATCAAAAAGTA CCCTAAACTTGAATCGGAGTTTGTGTACGG	

[0879]

	AGACTACAAGGTCTACGACGTGAGGAAGA TGATAGCCAAGTCCGAACAGGAAATCGGG AAAGCAACTGCGAAATACTTCTTTTACTCA AACATCATGAACTTTTTCAAGACTGAAATT ACGCTGGCCAATGGAGAAATCAGGAAGA GGCCACTGATCGAAACTAACGGAGAAACG GGCGAAATCGTGTGGGACAAGGGCAGGG ACTTCGCAACTGTTCGCAAAGTGCTCTCTA TGCCGCAAGTCAATATTGTGAAGAAAACC GAAGTGCAAACCGGCGGATTTTCAAAGGA ATCGATCCTCCCAAAGAGAAATAGCGACA AGCTCATTGCACGCAAGAAAGACTGGGAC CCGAAGAAGTACGGAGGATTCGATTCGCC GACTGTCGCATACTCCGTCCTCGTGGTGGC CAAGGTGGAGAAGGGAAAGAGCAAAAAG CTCAAATCCGTCAAAGAGCTGCTGGGGAT TACCATCATGGAACGATCCTCGTTCGAGAA GAACCCGATTGATTTCCTCGAGGCGAAGG GTTACAAGGAGGTGAAGAAGGATCTGATC ATCAAACTCCCCAAGTACTCACTGTTCGA ACTGGAAAATGGTCGGAAGCGCATGCTGG CTTCGGCCGGAGAACTCCAAAAAGGAAAT GAGCTGGCCTTGCCTAGCAAGTACGTCAA CTTCCTCTATCTTGCTTCGCACTACGAAAA ACTCAAAGGGTCACCGGAAGATAACGAAC AGAAGCAGCTTTTCGTGGAGCAGCACAAG CATTATCTGGATGAAATCATCGAACAAATC TCCGAGTTTTCAAAGCGCGTGATCCTCGC CGACGCCAACCTCGACAAAGTCCTGTCGG CCTACAATAAGCATAGAGATAAGCCGATCA GAGAACAGGCCGAGAACATTATCCACTTG TTCACCCTGACTAACCTGGGAGCCCCAGC CGCCTTCAAGTACTTCGATACTACTATCGA TCGCAAAAGATACACGTCCACCAAGGAAG TTCTGGACGCGACCCTGATCCACCAAAGC ATCACTGGACTCTACGAAACTAGGATCGAT CTGTCGCAGCTGGGTGGCGATGGCGGTGG ATCTCCGAAAAAGAAGAGAAAGGTGTAAT GA	
具有一个核定位信号(1xNLS)作为C-末	MDKKYSIGLDIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK	203

[0880]

	端7个氨基酸的Cas9的氨基酸序列	VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGGGSPKKKRK V	

[0881]

使用最小尿苷密码子的 Cas9 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG ACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG	204

[0882]

	CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC	

[0883]

	AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU	

		CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGC CCGAAGAAGAAGAGAAAGGUCUAG	
[0884]	使用在人类中通常高度表达的密码子的Cas9 mRNA ORF，具有起始及终止密码子	AUGGAUAAGAAGUACUCAAUCGGGCUGG AUAUCGGAACUAAUUCCGUGGGUUGGGC AGUGAUCACGGAUGAAUACAAAGUGCCG UCCAAGAAGUUCAAGGUCCUGGGGAACA CCGAUAGACACAGCAUCAAGAAAAAUCU CAUCGGAGCCCUGCUGUUUGACUCCGGC GAAACCGCAGAAGCGACCCGGCUCAAAC GUACCGCGAGGCGACGCUACACCCGGCG GAAGAAUCGCAUCUGCUAUCUGCAAGAG AUCUUUUCGAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACCGCCUGGAAGA AUCUUUCCUGGUGGAGGAGGACAAGAAG CAUGAACGGCAUCCUAUCUUUGGAAACA UCGUCGACGAAGUGGCGUACCACGAAAA GUACCCGACCAUCUACCAUCUGCGGAAG AAGUUGGUUGACUCAACUGACAAGGCCG ACCUCAGAUUGAUCUACUUGGCCCUCGC CCAUAUGAUCAAAUUCCGCGGACACUUC CUGAUCGAAGGCGAUCUGAACCCUGAUA ACUCCGACGUGGAUAAGCUUUUCAUUCA ACUGGUGCAGACCUACAACCAACUGUUC GAAGAAAACCCAAUCAAUGCUAGCGGCG	205

[0885]

	UCGAUGCCAAGGCCAUCCUGUCCGCCCG GCUGUCGAAGUCGCGGCGCCUCGAAAAC CUGAUCGCACAGCUGCCGGGAGAGAAAA AGAACGGACUUUUCGGCAACUUGAUCGC UCUCUCACUGGGACUCACUCCCAAUUUC AAGUCCAAUUUUGACCUGGCCGAGGACG CGAAGCUGCAACUCUCAAAGGACACCUA CGACGACGACUUGGACAAUUUGCUGGCA CAAAUUGGCGAUCAGUACGCGGAUCUGU UCCUUGCCGCUAAGAACCUUUCGGACGC AAUCUUGCUGUCCGAUAUCCUGCGCGUG AACACCGAAAUAACCAAAGCGCCGCUUA GCGCCUCGAUGAUUAAGCGGUACGACGA GCAUCACCAGGAUCUCACGCUGCUCAAA GCGCUCGUGAGACAGCAACUGCCUGAAA AGUACAAGGAGAUCUUCUUCGACCAGUC CAAGAAUGGGUACGCAGGGUACAUCGAU GGAGGCGCUAGCCAGGAAGAGUUCUAUA AGUUCAUCAAGCCAAUCCUGGAAAAGAU GGACGGAACCGAAGAACUGCUGGUCAAG CUGAACAGGGAGGAUCUGCUCCGGAAAC AGAGAACCUUUGACAACGGAUCCAUUCC CCACCAGAUCCAUCUGGGUGAGCUGCAC GCCAUCUUGCGGCGCCAGGAGGACUUUU ACCCAUUCCUCAAGGACAACCGGGAAAA GAUCGAGAAAAUUCUGACGUUCCGCAUC CCGUAUUACGUGGGCCCACUGGCGCGCG GCAAUUCGCGCUUCGCGUGGAUGACUAG AAAAUCAGAGGAAACCAUCACUCCUUGG AAUUUCGAGGAAGUUGUGGAUAAGGGA GCUUCGGCACAAAGCUUCAUCGAACGAA UGACCAACUUCGACAAGAAUCUCCCAAA CGAGAAGGUGCUUCCUAAGCACAGCCUC CUUUACGAAUACUUCACUGUCUACAACG AACUGACUAAAGUGAAAUACGUUACUGA AGGAAUGAGGAAGCCGGCCUUUCUGUCC GGAGAACAGAAGAAAGCAAUUGUCGAUC UGCUGUUCAAGACCAACCGCAAGGUGAC CGUCAAGCAGCUUAAAGAGGACUACUUC AAGAAGAUCGAGUGUUUCGACUCAGUGG AAAUCAGCGGGGUGGAGGACAGAUUCAA CGCUUCGCUGGGAACCUAUCAUGAUCUC CUGAAGAUCAUCAAGGACAAGGACUUCC	

[0886]

	UUGACAACGAGGAGAACGAGGACAUCCU GGAAGAUAUCGUCCUGACCUUGACCCUU UUCGAGGAUCGCGAGAUGAUCGAGGAGA GGCUUAAGACCUACGCUCAUCUCUUCGA CGAUAAGGUCAUGAAACAACUCAAGCGC CGCCGGUACACUGGUUGGGGCCGCCUCU CCCGCAAGCUGAUCAACGGUAUUCGCGA UAAACAGAGCGGUAAAACUAUCCUGGAU UUCCUCAAAUCGGAUGGCUUCGCUAAUC GUAACUUCAUGCAAUUGAUCCACGACGA CAGCCUGACCUUUAAGGAGGACAUCCAA AAAGCACAAGUGUCCGGACAGGGAGACU CACUCCAUGAACACAUCGCGAAUCUGGC CGGUUCGCCGGCGAUUAAGAAGGGAAUU CUGCAAACUGUGAAGGUGGUCGACGAGC UGGUGAAGGUCAUGGGACGGCACAAACC GGAGAAUAUCGUGAUUGAAAUGGCCCGA GAAAACCAGACUACCCAGAAGGGCCAGA AAAACUCCCGCGAAAGGAUGAAGCGGAU CGAAGAAGGAAUCAAGGAGCUGGGCAGC CAGAUCCUGAAAGAGCACCCGGUGGAAA ACACGCAGCUGCAGAACGAGAAGCUCUA CCUGUACUAUUUGCAAAAUGGACGGGAC AUGUACGUGGACCAAGAGCUGGACAUCA AUCGGUUGUCUGAUUACGACGUGGACCA CAUCGUUCCACAGUCCUUUCUGAAGGAU GACUCGAUCGAUAACAAGGUGUUGACUC GCAGCGACAAGAACAGAGGGAAGUCAGA UAAUGUGCCAUCGGAGGAGGUCGUGAAG AAGAUGAAGAAUUACUGGCGGCAGCUCC UGAAUGCGAAGCUGAUUACCCAGAGAAA GUUUGACAAUCUCACUAAAGCCGAGCGC GGCGGACUCUCAGAGCUGGAUAAGGCUG GAUUCAUCAAACGGCAGCUGGUCGAGAC UCGGCAGAUUACCAAGCACGUGGCGCAG AUCUUGGACUCCCGCAUGAACACUAAAU ACGACGAGAACGAUAAGCUCAUCCGGGA AGUGAAGGUGAUUACCCUGAAAAGCAAA CUUGUGUCGGACUUUCGGAAGGACUUUC AGUUUUACAAAGUGAGAGAAAUCAACAA CUACCAUCACGCGCAUGACGCAUACCUC AACGCUGUGGUCGGUACCGCCCUGAUCA AAAAGUACCCUAAACUUGAAUCGGAGUU	

[0887]

	UGUGUACGGAGACUACAAGGUCUACGAC GUGAGGAAGAUGAUAGCCAAGUCCGAAC AGGAAAUCGGGAAAGCAACUGCGAAAUA CUUCUUUUACUCAAACAUCAUGAACUUU UUCAAGACUGAAAUUACGCUGGCCAAUG GAGAAAUCAGGAAGAGGCCACUGAUCGA AACUAACGGAGAAACGGGCGAAAUCGUG UGGGACAAGGGCAGGGACUUCGCAACUG UUCGCAAAGUGCUCUCUAUGCCGCAAGU CAAUAUUGUGAAGAAAACCGAAGUGCAA ACCGGCGGAUUUUCAAAGGAAUCGAUCC UCCCAAAGAGAAAUAGCGACAAGCUCAU UGCACGCAAGAAAGACUGGGACCCGAAG AAGUACGGAGGAUUCGAUUCGCCGACUG UCGCAUACUCCGUCCUCGUGGUGGCCAA GGUGGAGAAGGGAAAGAGCAAAAAGCUC AAAUCCGUCAAAGAGCUGCUGGGGAUUA CCAUCAUGGAACGAUCCUCGUUCGAGAA GAACCCGAUUGAUUUCCUCGAGGCGAAG GGUUACAAGGAGGUGAAGAAGGAUCUG AUCAUCAAACUCCCCAAGUACUCACUGU UCGAACUGGAAAAUGGUCGGAAGCGCAU GCUGGCUUCGGCCGGAGAACUCCAAAAA GGAAAUGAGCUGGCCUUGCCUAGCAAGU ACGUCAACUUCCUCUAUCUUGCUUCGCA CUACGAAAAACUCAAAGGGUCACCGGAA GAUAACGAACAGAAGCAGCUUUUCGUGG AGCAGCACAAGCAUUAUCUGGAUGAAAU CAUCGAACAAAUCUCCGAGUUUUCAAAG CGCGUGAUCCUCGCCGACGCCAACCUCG ACAAAGUCCUGUCGGCCUACAAUAAGCA UAGAGAUAAGCCGAUCAGAGAACAGGCC GAGAACAUUAUCCACUUGUUCACCCUGA CUAACCUGGGAGCCCCAGCCGCCUUCAA GUACUUCGAUACUACUAUCGAUCGCAAA AGAUACACGUCCACCAAGGAAGUUCUGG ACGCGACCCUGAUCCACCAAAGCAUCAC UGGACUCUACGAAACUAGGAUCGAUCUG UCGCAGCUGGGUGGCGAUGGCGGUGGAU CUCCGAAAAAGAAGAGAAAGGUGUAAUG A	

[0888]

Cas9切口酶(D10A)氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT	206

[0889]

	NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGGGSPKKKRK V	
Cas9切口酶(D10A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC	207

[0890]

	GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG	

[0891]

	AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC	

[0892]

	AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGC CCGAAGAAGAAGAGAAAGGUCUAG	
dCas9 (D10A H840A) 氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED	208

[0893]

	ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDAIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGGGSPKKKRK V	
dCas9 (D10A H840A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG	209

[0894]

	ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU	

[0895]

	GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACGCA AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA	

[0896]

	GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG	

[0897]

	GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGC CCGAAGAAGAAGAGAAAGGUCUAG	
使用最小尿苷密码子的Cas9 mRNA编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)	GACAAGAAGUACAGCAUCGGACUGGACA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU	210

[0898]

	UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA	

[0899]

	CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA	

[0900]

	CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAGGAGGAAGCCC GAAGAAGAAGAGAAAGGUC	
使用最小尿苷密码子的Cas9切口酶编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU	211

[0901]

	GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA	

[0902]

	AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG	

[0903]

	CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAGGAGGAAGCCC GAAGAAGAAGAGAAAGGUC	

[0904]

使用最小尿苷密码子的dCas9编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA	212

[0905]

	GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC	

[0906]

	ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA	

		ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAGGAGGAAGCCC GAAGAAGAAGAGAAAGGUC	
[0907]	Cas9的氨基酸序列 (无NLS)	MDKKYSIGLDIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI	213

[0908]

	LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGD	
使用最小尿苷密码子的编码 SEQ ID NO: 213 的 Cas9 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG ACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC	214

[0909]

	GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC	

[0910]

	UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA	

[0911]

	GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
使用最小尿苷密码	GACAAGAAGUACAGCAUCGGACUGGACA UCGGAACAAACAGCGUCGGAUGGGCAGU	215

[0912]

子的编码SEQ ID NO: 213的Cas9编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)	CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA	

[0913]

	AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU	

[0914]

	ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA	

[0915]

	ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGAC	
Cas9切口酶的氨基酸序列(无NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE	216

		HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGD	
[0916]	使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO: 216的Cas9切口酶mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG	217

[0917]

	ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG	

[0918]

	GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG	

[0919]

	UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO:	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU	218

[0920]

216的Cas9切口酶编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)	CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG	

[0921]

	UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA	

[0922]

	GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA	

[0923]

	ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGAC	
dCas9的氨基酸序列 (无NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDAIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK	219

		RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGD	
[0924]	使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO: 219的dCas9 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC	220

[0925]

	AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA	

[0926]

	GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACGCA AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG	

[0927]

	AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO: 219的dCas9编码序列(无起始或终止密码子; 适于纳入融合	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC	221

[0928]

	蛋白编码序列中)	UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA	

[0929]

	GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA	

[0930]

	CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA	

[0931]

	GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAGGAGGAAGC	
具有两个核定位信号(2xNLS)作为C-末端氨基酸的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY	222

		SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGSGSPKKKRK VDGSPKKKRKVDSG	
[0932]	使用最小尿苷密码子的编码 SEQ ID NO: 222 的 Cas9 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG ACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA	223

[0933]

	CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU	

[0934]

	UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU	

[0935]

	CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGC CCGAAGAAGAAGAGAAAGGUCCCGAAGA AGAAGAGAAAGGUC GGAAGCGGAAGCCCGAAGAAGAAGAGAA AGGUCGACGGAAGCCCGAAGAAGAAGAG AAAGGUCGACAGCGGAUAG	
使用最小尿苷密码子的编码SEQ ID NO: 222的Cas9编码序列(无起始或终止	GACAAGAAGUACAGCAUCGGACUGGACA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA	224

[0936]

密码子；适于纳入融合蛋白编码序列中)	ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA	

[0937]

	ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG	

[0938]

	CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA	

		GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAGGAGGAAGCCC GAAGAAGAAGAGAAAGGUCCCGAAGAAG AAGAGAAAGGUC GGAAGCGGAAGCCCGAAGAAGAAGAGAA AGGUCGACGGAAGCCCGAAGAAGAAGAG AAAGGUCGACAGCGGA	
[0939]	具有两个核定位信号作为C-末端氨基酸的Cas9切口酶的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN	225

[0940]

	QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDHIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGSGSPKKKRK VDGSPKKKRKVDSG	
使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO: 25 的 Cas9 切口酶 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC	226

[0941]

	GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC	

[0942]

	UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACCAC AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA	

[0943]

	GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAAGCGGAAGC CCGAAGAAGAAGAGAAAGGUCGACGGAA GCCCGAAGAAGAAGAGAAAGGUCGACAG CGGAUAG	

[0944]

使用最小尿苷密码子的编码SEQ ID NO: 25的Cas9切口酶编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA	227

[0945]

	GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC	

[0946]

	ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA	

		ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAA AGGUCGACGGAAGCCCGAAGAAGAAGAG AAAGGUCGACAGCGGA	
[0947]	具有两个核定位信号作为C-末端氨基酸的dCas9的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSK KFKVLGNTDRHSIKKNLIGALLFDSGETAEA TRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIV DEVAYHEKYPTIYHLRKKLVDSTDKADLRLI YLALAHMIKFRGHFLIEGDLNPDNSDVDKL FIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILR VNTEITKAPLSASMIKRYDEHHQDLTLLKAL VRQQLPEKYKEIFFDQSKNGYAGYIDGGAS QEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLL FKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENED ILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQS	228

[0948]

	GKTILDFLKSDGFANRNFMQLIHDDSLTFKE DIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMAREN QTTQKGQKNSRERMKRIEEGIKELGSQILKE HPVENTQLQNEKLYLYYLQNGRDMYVDQE LDINRLSDYDVDAIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREIN NYHHAHDAYLNAVVGTALIKKYPKLESEFV YGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEI VWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNE QKQLFVEQHKHYLDEIIEQISEFSKRVILADA NLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATL IHQSITGLYETRIDLSQLGGDGSGSPKKKRK VDGSPKKKRKVDSG	
使用最小尿苷密码子的编码 SEQ ID NO: 228 的 dCas9 mRNA ORF，具有起始及终止密码子	AUGGACAAGAAGUACAGCAUCGGACUGG CAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCG AGCAAGAAGUUCAAGGUCCUGGGAAACA CAGACAGACACAGCAUCAAGAAGAACCU GAUCGGAGCACUGCUGUUCGACAGCGGA GAAACAGCAGAAGCAACAAGACUGAAGA GAACAGCAAGAAGAAGAUACACAAGAAG AAAGAACAGAAUCUGCUACCUGCAGGAA AUCUUCAGCAACGAAAUGGCAAAGGUCG ACGACAGCUUCUUCCACAGACUGGAAGA AAGCUUCCUGGUCGAAGAAGACAAGAAG CACGAAAGACACCCGAUCUUCGGAAACA UCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACCUGAGAAAG AAGCUGGUCGACAGCACAGACAAGGCAG ACCUGAGACUGAUCUACCUGGCACUGGC ACACAUGAUCAAGUUCAGAGGACACUUC	229

[0949]

	CUGAUCGAAGGAGACCUGAACCCGGACA ACAGCGACGUCGACAAGCUGUUCAUCCA GCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAG UCGACGCAAAGGCAAUCCUGAGCGCAAG ACUGAGCAAGAGCAGAAGACUGGAAAAC CUGAUCGCACAGCUGCCGGGAGAAAAGA AGAACGGACUGUUCGGAAACCUGAUCGC ACUGAGCCUGGGACUGACACCGAACUUC AAGAGCAACUUCGACCUGGCAGAAGACG CAAAGCUGCAGCUGAGCAAGGACACAUA CGACGACGACCUGGACAACCUGCUGGCA CAGAUCGGAGACCAGUACGCAGACCUGU UCCUGGCAGCAAAGAACCUGAGCGACGC AAUCCUGCUGAGCGACAUCCUGAGAGUC AACACAGAAAUCACAAAGGCACCGCUGA GCGCAAGCAUGAUCAAGAGAUACGACGA ACACCACCAGGACCUGACACUGCUGAAG GCACUGGUCAGACAGCAGCUGCCGGAAA AGUACAAGGAAAUCUUCUUCGACCAGAG CAAGAACGGAUACGCAGGAUACAUCGAC GGAGGAGCAAGCCAGGAAGAAUUCUACA AGUUCAUCAAGCCGAUCCUGGAAAAGAU GGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCC GCACCAGAUCCACCUGGGAGAACUGCAC GCAAUCCUGAGAAGACAGGAAGACUUCU ACCCGUUCCUGAAGGACAACAGAGAAAA GAUCGAAAAGAUCCUGACAUUCAGAAUC CCGUACUACGUCGGACCGCUGGCAAGAG GAAACAGCAGAUUCGCAUGGAUGACAAG AAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAG CAAGCGCACAGAGCUUCAUCGAAAGAAU GACAAACUUCGACAAGAACCUGCCGAAC GAAAAGGUCCUGCCGAAGCACAGCCUGC UGUACGAAUACUUCACAGUCUACAACGA ACUGACAAAGGUCAAGUACGUCACAGAA GGAAUGAGAAAGCCGGCAUUCCUGAGCG GAGAACAGAAGAAGGCAAUCGUCGACCU GCUGUUCAAGACAAACAGAAAGGUCACA GUCAAGCAGCUGAAGGAAGACUACUUCA	

[0950]

	AGAAGAUCGAAUGCUUCGACAGCGUCGA AAUCAGCGGAGUCGAAGACAGAUUCAAC GCAAGCCUGGGAACAUACCACGACCUGC UGAAGAUCAUCAAGGACAAGGACUUCCU GGACAACGAAGAAAACGAAGACAUCCUG GAAGACAUCGUCCUGACACUGACACUGU UCGAAGACAGAGAAAUGAUCGAAGAAAG ACUGAAGACAUACGCACACCUGUUCGAC GACAAGGUCAUGAAGCAGCUGAAGAGAA GAAGAUACACAGGAUGGGGAAGACUGAG CAGAAAGCUGAUCAACGGAAUCAGAGAC AAGCAGAGCGGAAAGACAAUCCUGGACU UCCUGAAGAGCGACGGAUUCGCAAACAG AAACUUCAUGCAGCUGAUCCACGACGAC AGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAG CCUGCACGAACACAUCGCAAACCUGGCA GGAAGCCCGGCAAUCAAGAAGGGAAUCC UGCAGACAGUCAAGGUCGUCGACGAACU GGUCAAGGUCAUGGGAAGACACAAGCCG GAAAACAUCGUCAUCGAAAUGGCAAGAG AAAACCAGACAACACAGAAGGGACAGAA GAACAGCAGAGAAAGAAUGAAGAGAAUC GAAGAAGGAAUCAAGGAACUGGGAAGCC AGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACA UGUACGUCGACCAGGAACUGGACAUCAA CAGACUGAGCGACUACGACGUCGACGCA AUCGUCCCGCAGAGCUUCCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAG AAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGA AGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAG UUCGACAACCUGACAAAGGCAGAGAGAG GAGGACUGAGCGAACUGGACAAGGCAGG AUUCAUCAAGAGACAGCUGGUCGAAACA AGACAGAUCACAAAGCACGUCGCACAGA UCCUGGACAGCAGAAUGAACACAAAGUA CGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGC UGGUCAGCGACUUCAGAAAGGACUUCCA	

[0951]

	GUUCUACAAGGUCAGAGAAAUCAACAAC UACCACCACGCACACGACGCAUACCUGA ACGCAGUCGUCGGAACAGCACUGAUCAA GAAGUACCCGAAGCUGGAAAGCGAAUUC GUCUACGGAGACUACAAGGUCUACGACG UCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUAC UUCUUCUACAGCAACAUCAUGAACUUCU UCAAGACAGAAAUCACACUGGCAAACGG AGAAAUCAGAAAGAGACCGCUGAUCGAA ACAAACGGAGAAACAGGAGAAAUCGUCU GGGACAAGGGAAGAGACUUCGCAACAGU CAGAAAGGUCCUGAGCAUGCCGCAGGUC AACAUCGUCAAGAAGACAGAAGUCCAGA CAGGAGGAUUCAGCAAGGAAAGCAUCCU GCCGAAGAGAAACAGCGACAAGCUGAUC GCAAGAAAGAAGGACUGGGACCCGAAGA AGUACGGAGGAUUCGACAGCCCGACAGU CGCAUACAGCGUCCUGGUCGUCGCAAAG GUCGAAAAGGGAAAGAGCAAGAAGCUGA AGAGCGUCAAGGAACUGCUGGGAAUCAC AAUCAUGGAAAGAAGCAGCUUCGAAAAG AACCCGAUCGACUUCCUGGAAGCAAAGG GAUACAAGGAAGUCAAGAAGGACCUGAU CAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGC UGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUAC GUCAACUUCCUGUACCUGGCAAGCCACU ACGAAAAGCUGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCUGUUCGUCGAA CAGCACAAGCACUACCUGGACGAAAUCA UCGAACAGAUCAGCGAAUUCAGCAAGAG AGUCAUCCUGGCAGACGCAAACCUGGAC AAGGUCCUGAGCGCAUACAACAAGCACA GAGACAAGCCGAUCAGAGAACAGGCAGA AAACAUCAUCCACCUGUUCACACUGACA AACCUGGGAGCACCGGCAGCAUUCAAGU ACUUCGACACAACAAUCGACAGAAAGAG AUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAG GACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGAC	

[0952]

	GGAAGCGGAAGCCCGAAGAAGAAGAGAA AGGUCGACGGAAGCCCGAAGAAGAAGAG AAAGGUCGACAGCGGAUAG	
使用最小尿苷密码子的编码SEQ ID NO: 228的dCas9编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAA UCGGAACAAACAGCGUCGGAUGGGCAGU CAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAG ACAGACACAGCAUCAAGAAGAACCUGAU CGGAGCACUGCUGUUCGACAGCGGAGAA ACAGCAGAAGCAACAAGACUGAAGAGAA CAGCAAGAAGAAGAUACACAAGAAGAAA GAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACG ACAGCUUCUUCCACAGACUGGAAGAAAG CUUCCUGGUCGAAGAAGACAAGAAGCAC GAAAGACACCCGAUCUUCGGAAACAUCG UCGACGAAGUCGCAUACCACGAAAAGUA CCCGACAAUCUACCACCUGAGAAAGAAG CUGGUCGACAGCACAGACAAGGCAGACC UGAGACUGAUCUACCUGGCACUGGCACA CAUGAUCAAGUUCAGAGGACACUUCCUG AUCGAAGGAGACCUGAACCCGGACAACA GCGACGUCGACAAGCUGUUCAUCCAGCU GGUCCAGACAUACAACCAGCUGUUCGAA GAAAACCCGAUCAACGCAAGCGGAGUCG ACGCAAAGGCAAUCCUGAGCGCAAGACU GAGCAAGAGCAGAAGACUGGAAAACCUG AUCGCACAGCUGCCGGGAGAAAAGAAGA ACGGACUGUUCGGAAACCUGAUCGCACU GAGCCUGGGACUGACACCGAACUUCAAG AGCAACUUCGACCUGGCAGAAGACGCAA AGCUGCAGCUGAGCAAGGACACAUACGA CGACGACCUGGACAACCUGCUGGCACAG AUCGGAGACCAGUACGCAGACCUGUUCC UGGCAGCAAAGAACCUGAGCGACGCAAU CCUGCUGAGCGACAUCCUGAGAGUCAAC ACAGAAAUCACAAAGGCACCGCUGAGCG CAAGCAUGAUCAAGAGAUACGACGAACA CCACCAGGACCUGACACUGCUGAAGGCA CUGGUCAGACAGCAGCUGCCGGAAAAGU ACAAGGAAAUCUUCUUCGACCAGAGCAA GAACGGAUACGCAGGAUACAUCGACGGA GGAGCAAGCCAGGAAGAAUUCUACAAGU	230

[0953]

	UCAUCAAGCCGAUCCUGGAAAAGAUGGA CGGAACAGAAGAACUGCUGGUCAAGCUG AACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCA CCAGAUCCACCUGGGAGAACUGCACGCA AUCCUGAGAAGACAGGAAGACUUCUACC CGUUCCUGAAGGACAACAGAGAAAAGAU CGAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGGACCGCUGGCAAGAGGAA ACAGCAGAUUCGCAUGGAUGACAAGAAA GAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAA GCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAA AAGGUCCUGCCGAAGCACAGCCUGCUGU ACGAAUACUUCACAGUCUACAACGAACU GACAAAGGUCAAGUACGUCACAGAAGGA AUGAGAAAGCCGGCAUUCCUGAGCGGAG AACAGAAGAAGGCAAUCGUCGACCUGCU GUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGA AGAUCGAAUGCUUCGACAGCGUCGAAAU CAGCGGAGUCGAAGACAGAUUCAACGCA AGCCUGGGAACAUACCACGACCUGCUGA AGAUCAUCAAGGACAAGGACUUCCUGGA CAACGAAGAAAACGAAGACAUCCUGGAA GACAUCGUCCUGACACUGACACUGUUCG AAGACAGAGAAAUGAUCGAAGAAAGACU GAAGACAUACGCACACCUGUUCGACGAC AAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAG AAAGCUGAUCAACGGAAUCAGAGACAAG CAGAGCGGAAAGACAAUCCUGGACUUCC UGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGC CUGACAUUCAAGGAAGACAUCCAGAAGG CACAGGUCAGCGGACAGGGAGACAGCCU GCACGAACACAUCGCAAACCUGGCAGGA AGCCCGGCAAUCAAGAAGGGAAUCCUGC AGACAGUCAAGGUCGUCGACGAACUGGU CAAGGUCAUGGGAAGACACAAGCCGGAA AACAUCGUCAUCGAAAUGGCAAGAGAAA ACCAGACAACACAGAAGGGACAGAAGAA	

[0954]

	CAGCAGAGAAAGAAUGAAGAGAAUCGAA GAAGGAAUCAAGGAACUGGGAAGCCAGA UCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUG UACUACCUGCAGAACGGAAGAGACAUGU ACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUC GUCCCGCAGAGCUUCCUGAAGGACGACA GCAUCGACAACAAGGUCCUGACAAGAAG CGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGA UGAAGAACUACUGGAGACAGCUGCUGAA CGCAAAGCUGAUCACACAGAGAAAGUUC GACAACCUGACAAAGGCAGAGAGAGGAG GACUGAGCGAACUGGACAAGGCAGGAUU CAUCAAGAGACAGCUGGUCGAAACAAGA CAGAUCACAAAGCACGUCGCACAGAUCC UGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGG UCAGCGACUUCAGAAAGGACUUCCAGUU CUACAAGGUCAGAGAAAUCAACAACUAC CACCACGCACACGACGCAUACCUGAACG CAGUCGUCGGAACAGCACUGAUCAAGAA GUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCA GAAAGAUGAUCGCAAAGAGCGAACAGGA AAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCA AGACAGAAAUCACACUGGCAAACGGAGA AAUCAGAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGG ACAAGGGAAGAGACUUCGCAACAGUCAG AAAGGUCCUGAGCAUGCCGCAGGUCAAC AUCGUCAAGAAGACAGAAGUCCAGACAG GAGGAUUCAGCAAGGAAAGCAUCCUGCC GAAGAGAAACAGCGACAAGCUGAUCGCA AGAAAGAAGGACUGGGACCCGAAGAAGU ACGGAGGAUUCGACAGCCCGACAGUCGC AUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAG AGCGUCAAGGAACUGCUGGGAAUCACAA UCAUGGAAAGAAGCAGCUUCGAAAAGAA	

[0955]

	CCCGAUCGACUUCCUGGAAGCAAAGGGA UACAAGGAAGUCAAGAAGGACCUGAUCA UCAAGCUGCCGAAGUACAGCCUGUUCGA ACUGGAAAACGGAAGAAAGAGAAUGCUG GCAAGCGCAGGAGAACUGCAGAAGGGAA ACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACA ACGAACAGAAGCAGCUGUUCGUCGAACA GCACAAGCACUACCUGGACGAAAUCAUC GAACAGAUCAGCGAAUUCAGCAAGAGAG UCAUCCUGGCAGACGCAAACCUGGACAA GGUCCUGAGCGCAUACAACAAGCACAGA GACAAGCCGAUCAGAGAACAGGCAGAAA ACAUCAUCCACCUGUUCACACUGACAAA CCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAU ACACAAGCACAAAGGAAGUCCUGGACGC AACACUGAUCCACCAGAGCAUCACAGGA CUGUACGAAACAAGAAUCGACCUGAGCC AGCUGGGAGGAGACGGAAGCGGAAGCCC GAAGAAGAAGAGAAAGGUCGACGGAAGC CCGAAGAAGAAGAGAAAGGUCGACAGCG GA	
T7启动子	TAATACGACTCACTATA	231
人类β-珠蛋白5’UTR	ACATTTGCTTCTGACACAACTGTGTTCACT AGCAACCTCAAACAGACACC	232
人类β-珠蛋白3’UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAA AGGTTCCTTTGTTCCCTAAGTCCAACTACT AAACTGGGGGATATTATGAAGGGCCTTGA GCATCTGGATTCTGCCTAATAAAAAACATT TATTTTCATTGC	233
人类α-珠蛋白5’UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGC ACTCTTCTGGTCCCCACAGACTCAGAGAG AACCCACC	234
人类α-珠蛋白3’UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGC CCCTTGGGCCTCCCCCCAGCCCCTCCTCCC CTTCCTGCACCCGTACCCCCGTGGTCTTTG AATAAAGTCTGAGTGGGCGGC	235
非洲爪蟾β-珠蛋白5’UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	236

[0956]

非洲爪蟾β-珠蛋白3’UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTATCTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCT	237
牛生长激素5’UTR	CAGGGTCCTGTGGACAGCTCACCAGCT	238
牛生长激素3’UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA	239
小鼠血红素α，成人链1(Hba-a1)，3’UTR	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAG	240
HSD17B4 5’UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGGCCTTATTC	241
靶向小鼠*TTR*基因的G282单引导RNA	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	242
	未使用	243
具有HSD的5’UTR、对应于SEQ ID NO:204的ORF及ALB的3’UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGGCCTTATTCGGATCCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGA	244

[0957]

	AGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCAC ACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACA GCGACGTCGACAAGCTGTTCATCCAGCTG GTCCAGACATACAACCAGCTGTTCGAAGA AAACCCGATCAACGCAAGCGGAGTCGAC GCAAAGGCAATCCTGAGCGCAAGACTGA GCAAGAGCAGAAGACTGGAAAACCTGAT CGCACAGCTGCCGGGAGAAAAGAAGAAC GGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCA ACTTCGACCTGGCAGAAGACGCAAAGCTG CAGCTGAGCAAGGACACATACGACGACGA CCTGGACAACCTGCTGGCACAGATCGGAG ACCAGTACGCAGACCTGTTCCTGGCAGCA AAGAACCTGAGCGACGCAATCCTGCTGAG CGACATCCTGAGAGTCAACACAGAAATCA CAAAGGCACCGCTGAGCGCAAGCATGATC AAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGC AGCTGCCGGAAAAGTACAAGGAAATCTTC TTCGACCAGAGCAAGAACGGATACGCAGG ATACATCGACGGAGGAGCAAGCCAGGAAG AATTCTACAAGTTCATCAAGCCGATCCTGG AAAAGATGGACGGAACAGAAGAACTGCT GGTCAAGCTGAACAGAGAAGACCTGCTG AGAAAGCAGAGAACATTCGACAACGGAA GCATCCCGCACCAGATCCACCTGGGAGAA CTGCACGCAATCCTGAGAAGACAGGAAG ACTTCTACCCGTTCCTGAAGGACAACAGA GAAAAGATCGAAAAGATCCTGACATTCAG AATCCCGTACTACGTCGGACCGCTGGCAA GAGGAAACAGCAGATTCGCATGGATGACA AGAAAGAGCGAAGAAACAATCACACCGT GGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAA TGACAAACTTCGACAAGAACCTGCCGAAC GAAAAGGTCCTGCCGAAGCACAGCCTGCT GTACGAATACTTCACAGTCTACAACGAACT GACAAAGGTCAAGTACGTCACAGAAGGA ATGAGAAAGCCGGCATTCCTGAGCGGAGA ACAGAAGAAGGCAATCGTCGACCTGCTGT	

[0958]

	TCAAGACAAACAGAAAGGTCACAGTCAA GCAGCTGAAGGAAGACTACTTCAAGAAGA TCGAATGCTTCGACAGCGTCGAAATCAGC GGAGTCGAAGACAGATTCAACGCAAGCCT GGGAACATACCACGACCTGCTGAAGATCA TCAAGGACAAGGACTTCCTGGACAACGA AGAAAACGAAGACATCCTGGAAGACATCG TCCTGACACTGACACTGTTCGAAGACAGA GAAATGATCGAAGAAAGACTGAAGACATA CGCACACCTGTTCGACGACAAGGTCATGA AGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCA ACGGAATCAGAGACAAGCAGAGCGGAAA GACAATCCTGGACTTCCTGAAGAGCGACG GATTCGCAAACAGAAACTTCATGCAGCTG ATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGA CAGGGAGACAGCCTGCACGAACACATCGC AAACCTGGCAGGAAGCCCGGCAATCAAG AAGGGAATCCTGCAGACAGTCAAGGTCGT CGACGAACTGGTCAAGGTCATGGGAAGAC ACAAGCCGGAAAACATCGTCATCGAAATG GCAAGAGAAAACCAGACAACACAGAAGG GACAGAAGAACAGCAGAGAAAGAATGAA GAGAATCGAAGAAGGAATCAAGGAACTG GGAAGCCAGATCCTGAAGGAACACCCGGT CGAAAACACACAGCTGCAGAACGAAAAG CTGTACCTGTACTACCTGCAGAACGGAAG AGACATGTACGTCGACCAGGAACTGGACA TCAACAGACTGAGCGACTACGACGTCGAC CACATCGTCCCGCAGAGCTTCCTGAAGGA CGACAGCATCGACAACAAGGTCCTGACAA GAAGCGACAAGAACAGAGGAAAGAGCGA CAACGTCCCGAGCGAAGAAGTCGTCAAG AAGATGAAGAACTACTGGAGACAGCTGCT GAACGCAAAGCTGATCACACAGAGAAAG TTCGACAACCTGACAAAGGCAGAGAGAG GAGGACTGAGCGAACTGGACAAGGCAGG ATTCATCAAGAGACAGCTGGTCGAAACAA GACAGATCACAAAGCACGTCGCACAGATC CTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTC AAGGTCATCACACTGAAGAGCAAGCTGGT	

[0959]

	CAGCGACTTCAGAAAGGACTTCCAGTTCT ACAAGGTCAGAGAAATCAACAACTACCAC CACGCACACGACGCATACCTGAACGCAGT CGTCGGAACAGCACTGATCAAGAAGTACC CGAAGCTGGAAAGCGAATTCGTCTACGGA GACTACAAGGTCTACGACGTCAGAAAGAT GATCGCAAAGAGCGAACAGGAAATCGGA AAGGCAACAGCAAAGTACTTCTTCTACAG CAACATCATGAACTTCTTCAAGACAGAAA TCACACTGGCAAACGGAGAAATCAGAAA GAGACCGCTGATCGAAACAAACGGAGAA ACAGGAGAAATCGTCTGGGACAAGGGAA GAGACTTCGCAACAGTCAGAAAGGTCCTG AGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGC AAGGAAAGCATCCTGCCGAAGAGAAACA GCGACAAGCTGATCGCAAGAAAGAAGGA CTGGGACCCGAAGAAGTACGGAGGATTCG ACAGCCCGACAGTCGCATACAGCGTCCTG GTCGTCGCAAAGGTCGAAAAGGGAAAGA GCAAGAAGCTGAAGAGCGTCAAGGAACT GCTGGGAATCACAATCATGGAAAGAAGCA GCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGA AGGACCTGATCATCAAGCTGCCGAAGTAC AGCCTGTTCGAACTGGAAAACGGAAGAA AGAGAATGCTGGCAAGCGCAGGAGAACT GCAGAAGGGAAACGAACTGGCACTGCCG AGCAAGTACGTCAACTTCCTGTACCTGGC AAGCCACTACGAAAAGCTGAAGGGAAGC CCGGAAGACAACGAACAGAAGCAGCTGT TCGTCGAACAGCACAAGCACTACCTGGAC GAAATCATCGAACAGATCAGCGAATTCAG CAAGAGAGTCATCCTGGCAGACGCAAACC TGGACAAGGTCCTGAGCGCATACAACAAG CACAGAGACAAGCCGATCAGAGAACAGG CAGAAAACATCATCCACCTGTTCACACTG ACAAACCTGGGAGCACCGGCAGCATTCAA GTACTTCGACACAACAATCGACAGAAAGA GATACACAAGCACAAAGGAAGTCCTGGAC GCAACACTGATCCACCAGAGCATCACAGG ACTGTACGAAACAAGAATCGACCTGAGCC AGCTGGGAGGAGACGGAGGAGGAAGCCC	

[0960]

	GAAGAAGAAGAGAAAGGTCTAGCTAGCC ATCACATTTAAAAGCATCTCAGCCTACCAT GAGAATAAGAGAAAGAAAATGAAGATCAA TAGCTTATTCATCTCTTTTTCTTTTTCGTTG GTGTAAAGCCAACACCCTGTCTAAAAAAC ATAAATTTCTTTAATCATTTTGCCTCTTTTC TCTGTGCTTCAATTAATAAAAAATGGAAAG AACCTCGAG	
具有19.36% U含量的替代Cas9 ORF	ATGGATAAGAAGTACTCGATCGGGCTGGAT ATCGGAACTAATTCCGTGGGTTGGGCAGT GATCACGGATGAATACAAAGTGCCGTCCA AGAAGTTCAAGGTCCTGGGGAACACCGAT AGACACAGCATCAAGAAGAATCTCATCGG AGCCCTGCTGTTTGACTCCGGCGAAACCG CAGAAGCGACCCGGCTCAAACGTACCGCG AGGCGACGCTACACCCGGCGGAAGAATCG CATCTGCTATCTGCAAGAAATCTTTTCGAA CGAAATGGCAAAGGTGGACGACAGCTTCT TCCACCGCCTGGAAGAATCTTTCCTGGTG GAGGAGGACAAGAAGCATGAACGGCATC CTATCTTTGGAAACATCGTGGACGAAGTG GCGTACCACGAAAAGTACCCGACCATCTA CCATCTGCGGAAGAAGTTGGTTGACTCAA CTGACAAGGCCGACCTCAGATTGATCTACT TGGCCCTCGCCCATATGATCAAATTCCGCG GACACTTCCTGATCGAAGGCGATCTGAAC CCTGATAACTCCGACGTGGATAAGCTGTTC ATTCAACTGGTGCAGACCTACAACCAACT GTTCGAAGAAAACCCAATCAATGCCAGCG GCGTCGATGCCAAGGCCATCCTGTCCGCC CGGCTGTCGAAGTCGCGGCGCCTCGAAAA CCTGATCGCACAGCTGCCGGGAGAGAAGA AGAACGGACTTTTCGGCAACTTGATCGCT CTCTCACTGGGACTCACTCCCAATTTCAAG TCCAATTTTGACCTGGCCGAGGACGCGAA GCTGCAACTCTCAAAGGACACCTACGACG ACGACTTGGACAATTTGCTGGCACAAATT GGCGATCAGTACGCGGATCTGTTCCTTGCC GCTAAGAACCTTTCGGACGCAATCTTGCT GTCCGATATCCTGCGCGTGAACACCGAAAT AACCAAAGCGCCGCTTAGCGCCTCGATGA TTAAGCGGTACGACGAGCATCACCAGGAT	245

[0961]

	CTCACGCTGCTCAAAGCGCTCGTGAGACA GCAACTGCCTGAAAAGTACAAGGAGATTT TCTTCGACCAGTCCAAGAATGGGTACGCA GGGTACATCGATGGAGGCGCCAGCCAGGA AGAGTTCTATAAGTTCATCAAGCCAATCCT GGAAAAGATGGACGGAACCGAAGAACTG CTGGTCAAGCTGAACAGGGAGGATCTGCT CCGCAAACAGAGAACCTTTGACAACGGA AGCATTCCACACCAGATCCATCTGGGTGA GCTGCACGCCATCTTGCGGCGCCAGGAGG ACTTTTACCCATTCCTCAAGGACAACCGG GAAAAGATCGAGAAAATTCTGACGTTCCG CATCCCGTATTACGTGGGCCCACTGGCGCG CGGCAATTCGCGCTTCGCGTGGATGACTA GAAAATCAGAGGAAACCATCACTCCTTGG AATTTCGAGGAAGTTGTGGATAAGGGAGC TTCGGCACAATCCTTCATCGAACGAATGAC CAACTTCGACAAGAATCTCCCAAACGAGA AGGTGCTTCCTAAGCACAGCCTCCTTTACG AATACTTCACTGTCTACAACGAACTGACTA AAGTGAAATACGTTACTGAAGGAATGAGG AAGCCGGCCTTTCTGAGCGGAGAACAGA AGAAAGCGATTGTCGATCTGCTGTTCAAG ACCAACCGCAAGGTGACCGTCAAGCAGCT TAAAGAGGACTACTTCAAGAAGATCGAGT GTTTCGACTCAGTGGAAATCAGCGGAGTG GAGGACAGATTCAACGCTTCGCTGGGAAC CTATCATGATCTCCTGAAGATCATCAAGGA CAAGGACTTCCTTGACAACGAGGAGAAC GAGGACATCCTGGAAGATATCGTCCTGAC CTTGACCCTTTTCGAGGATCGCGAGATGAT CGAGGAGAGGCTTAAGACCTACGCTCATC TCTTCGACGATAAGGTCATGAAACAACTC AAGCGCCGCCGGTACACTGGTTGGGGCCG CCTCTCCCGCAAGCTGATCAACGGTATTCG CGATAAACAGAGCGGTAAAACTATCCTGG ATTTCCTCAAATCGGATGGCTTCGCTAATC GTAACTTCATGCAGTTGATCCACGACGAC AGCCTGACCTTTAAGGAGGACATCCAGAA AGCACAAGTGAGCGGACAGGGAGACTCA CTCCATGAACACATCGCGAATCTGGCCGG TTCGCCGGCGATTAAGAAGGGAATCCTGC AAACTGTGAAGGTGGTGGACGAGCTGGT	

[0962]

	GAAGGTCATGGGACGGCACAAACCGGAG AATATCGTGATTGAAATGGCCCGAGAAAA CCAGACTACCCAGAAGGGCCAGAAGAAC TCCCGCGAAAGGATGAAGCGGATCGAAGA AGGAATCAAGGAGCTGGGCAGCCAGATCC TGAAAGAGCACCCGGTGGAAAACACGCA GCTGCAGAACGAGAAGCTCTACCTGTACT ATTTGCAAAATGGACGGGACATGTACGTG GACCAAGAGCTGGACATCAATCGGTTGTC TGATTACGACGTGGACCACATCGTTCCACA GTCCTTTCTGAAGGATGACTCCATCGATAA CAAGGTGTTGACTCGCAGCGACAAGAAC AGAGGGAAGTCAGATAATGTGCCATCGGA GGAGGTCGTGAAGAAGATGAAGAATTACT GGCGGCAGCTCCTGAATGCGAAGCTGATT ACCCAGAGAAAGTTTGACAATCTCACTAA AGCCGAGCGCGGCGGACTCTCAGAGCTG GATAAGGCTGGATTCATCAAACGGCAGCT GGTCGAGACTCGGCAGATTACCAAGCACG TGGCGCAGATCCTGGACTCCCGCATGAAC ACTAAATACGACGAGAACGATAAGCTCAT CCGGGAAGTGAAGGTGATTACCCTGAAAA GCAAACTTGTGTCGGACTTTCGGAAGGAC TTTCAGTTTTACAAAGTGAGAGAAATCAA CAACTACCATCACGCGCATGACGCATACCT CAACGCTGTGGTCGGCACCGCCCTGATCA AGAAGTACCCTAAACTTGAATCGGAGTTT GTGTACGGAGACTACAAGGTCTACGACGT GAGGAAGATGATAGCCAAGTCCGAACAGG AAATCGGGAAAGCAACTGCGAAATACTTC TTTTACTCAAACATCATGAACTTCTTCAAG ACTGAAATTACGCTGGCCAATGGAGAAAT CAGGAAGAGGCCACTGATCGAAACTAACG GAGAAACGGGCGAAATCGTGTGGGACAA GGGCAGGGACTTCGCAACTGTTCGCAAAG TGCTCTCTATGCCGCAAGTCAATATTGTGA AGAAAACCGAAGTGCAAACCGGCGGATTT TCAAAGGAATCGATCCTCCCAAAGAGAAA TAGCGACAAGCTCATTGCACGCAAGAAAG ACTGGGACCCGAAGAAGTACGGAGGATTC GATTCGCCGACTGTCGCATACTCCGTCCTC GTGGTGGCCAAGGTGGAGAAGGGAAAGA GCAAGAAGCTCAAATCCGTCAAAGAGCTG	

[0963]

	CTGGGGATTACCATCATGGAACGATCCTCG TTCGAGAAGAACCCGATTGATTTCCTGGA GGCGAAGGGTTACAAGGAGGTGAAGAAG GATCTGATCATCAAACTGCCCAAGTACTCA CTGTTCGAACTGGAAAATGGTCGGAAGCG CATGCTGGCTTCGGCCGGAGAACTCCAGA AAGGAAATGAGCTGGCCTTGCCTAGCAAG TACGTCAACTTCCTCTATCTTGCTTCGCACT ACGAGAAACTCAAAGGGTCACCGGAAGAT AACGAACAGAAGCAGCTTTTCGTGGAGCA GCACAAGCATTATCTGGATGAAATCATCGA ACAAATCTCCGAGTTTTCAAAGCGCGTGA TCCTCGCCGACGCCAACCTCGACAAAGTC CTGTCGGCCTACAATAAGCATAGAGATAAG CCGATCAGAGAACAGGCCGAGAACATTAT CCACTTGTTCACCCTGACTAACCTGGGAG CTCCAGCCGCCTTCAAGTACTTCGATACTA CTATCGACCGCAAAAGATACACGTCCACC AAGGAAGTTCTGGACGCGACCCTGATCCA CCAAAGCATCACTGGACTCTACGAAACTA GGATCGATCTGTCGCAGCTGGGTGGCGAT GGTGGCGGTGGATCCTACCCATACGACGT GCCTGACTACGCCTCCGGAGGTGGTGGCC CCAAGAAGAAACGGAAGGTGTGATAG	
具有HSD的5’UTR、对应于SEQ ID NO: 245的ORF、Kozak序列及ALB的3’UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCTGCCACCATGGATAAGAAGT ACTCGATCGGGCTGGATATCGGAACTAATT CCGTGGGTTGGGCAGTGATCACGGATGAA TACAAAGTGCCGTCCAAGAAGTTCAAGGT CCTGGGGAACACCGATAGACACAGCATCA AGAAGAATCTCATCGGAGCCCTGCTGTTT GACTCCGGCGAAACCGCAGAAGCGACCC GGCTCAAACGTACCGCGAGGCGACGCTAC ACCCGGCGGAAGAATCGCATCTGCTATCTG CAAGAAATCTTTTCGAACGAAATGGCAAA GGTGGACGACAGCTTCTTCCACCGCCTGG AAGAATCTTTCCTGGTGGAGGAGGACAAG AAGCATGAACGGCATCCTATCTTTGGAAAC ATCGTGGACGAAGTGGCGTACCACGAAAA GTACCCGACCATCTACCATCTGCGGAAGA AGTTGGTTGACTCAACTGACAAGGCCGAC	246

[0964]

	CTCAGATTGATCTACTTGGCCCTCGCCCAT ATGATCAAATTCCGCGGACACTTCCTGATC GAAGGCGATCTGAACCCTGATAACTCCGA CGTGGATAAGCTGTTCATTCAACTGGTGCA GACCTACAACCAACTGTTCGAAGAAAACC CAATCAATGCCAGCGGCGTCGATGCCAAG GCCATCCTGTCCGCCCGGCTGTCGAAGTC GCGGCGCCTCGAAAACCTGATCGCACAGC TGCCGGGAGAGAAGAAGAACGGACTTTT CGGCAACTTGATCGCTCTCTCACTGGGAC TCACTCCCAATTTCAAGTCCAATTTTGACC TGGCCGAGGACGCGAAGCTGCAACTCTCA AAGGACACCTACGACGACGACTTGGACAA TTTGCTGGCACAAATTGGCGATCAGTACGC GGATCTGTTCCTTGCCGCTAAGAACCTTTC GGACGCAATCTTGCTGTCCGATATCCTGCG CGTGAACACCGAAATAACCAAAGCGCCGC TTAGCGCCTCGATGATTAAGCGGTACGACG AGCATCACCAGGATCTCACGCTGCTCAAA GCGCTCGTGAGACAGCAACTGCCTGAAAA GTACAAGGAGATTTTCTTCGACCAGTCCA AGAATGGGTACGCAGGGTACATCGATGGA GGCGCCAGCCAGGAAGAGTTCTATAAGTT CATCAAGCCAATCCTGGAAAAGATGGACG GAACCGAAGAACTGCTGGTCAAGCTGAA CAGGGAGGATCTGCTCCGCAAACAGAGA ACCTTTGACAACGGAAGCATTCCACACCA GATCCATCTGGGTGAGCTGCACGCCATCTT GCGGCGCCAGGAGGACTTTTACCCATTCC TCAAGGACAACCGGGAAAAGATCGAGAA AATTCTGACGTTCCGCATCCCGTATTACGT GGGCCCACTGGCGCGCGGCAATTCGCGCT TCGCGTGGATGACTAGAAAATCAGAGGAA ACCATCACTCCTTGGAATTTCGAGGAAGTT GTGGATAAGGGAGCTTCGGCACAATCCTT CATCGAACGAATGACCAACTTCGACAAGA ATCTCCCAAACGAGAAGGTGCTTCCTAAG CACAGCCTCCTTTACGAATACTTCACTGTC TACAACGAACTGACTAAAGTGAAATACGT TACTGAAGGAATGAGGAAGCCGGCCTTTC TGAGCGGAGAACAGAAGAAAGCGATTGT CGATCTGCTGTTCAAGACCAACCGCAAGG TGACCGTCAAGCAGCTTAAAGAGGACTAC	

[0965]

	TTCAAGAAGATCGAGTGTTTCGACTCAGT GGAAATCAGCGGAGTGGAGGACAGATTCA ACGCTTCGCTGGGAACCTATCATGATCTCC TGAAGATCATCAAGGACAAGGACTTCCTT GACAACGAGGAGAACGAGGACATCCTGG AAGATATCGTCCTGACCTTGACCCTTTTCG AGGATCGCGAGATGATCGAGGAGAGGCTT AAGACCTACGCTCATCTCTTCGACGATAAG GTCATGAAACAACTCAAGCGCCGCCGGTA CACTGGTTGGGGCCGCCTCTCCCGCAAGC TGATCAACGGTATTCGCGATAAACAGAGC GGTAAAACTATCCTGGATTTCCTCAAATCG GATGGCTTCGCTAATCGTAACTTCATGCAG TTGATCCACGACGACAGCCTGACCTTTAA GGAGGACATCCAGAAAGCACAAGTGAGC GGACAGGGAGACTCACTCCATGAACACAT CGCGAATCTGGCCGGTTCGCCGGCGATTA AGAAGGGAATCCTGCAAACTGTGAAGGTG GTGGACGAGCTGGTGAAGGTCATGGGACG GCACAAACCGGAGAATATCGTGATTGAAA TGGCCCGAGAAAACCAGACTACCCAGAA GGGCCAGAAGAACTCCCGCGAAAGGATG AAGCGGATCGAAGAAGGAATCAAGGAGC TGGGCAGCCAGATCCTGAAAGAGCACCCG GTGGAAAACACGCAGCTGCAGAACGAGA AGCTCTACCTGTACTATTTGCAAAATGGAC GGGACATGTACGTGGACCAAGAGCTGGAC ATCAATCGGTTGTCTGATTACGACGTGGAC CACATCGTTCCACAGTCCTTTCTGAAGGAT GACTCCATCGATAACAAGGTGTTGACTCG CAGCGACAAGAACAGAGGGAAGTCAGAT AATGTGCCATCGGAGGAGGTCGTGAAGAA GATGAAGAATTACTGGCGGCAGCTCCTGA ATGCGAAGCTGATTACCCAGAGAAAGTTT GACAATCTCACTAAAGCCGAGCGCGGCGG ACTCTCAGAGCTGGATAAGGCTGGATTCAT CAAACGGCAGCTGGTCGAGACTCGGCAGA TTACCAAGCACGTGGCGCAGATCCTGGAC TCCCGCATGAACACTAAATACGACGAGAA CGATAAGCTCATCCGGGAAGTGAAGGTGA TTACCCTGAAAAGCAAACTTGTGTCGGAC TTTCGGAAGGACTTTCAGTTTTACAAAGT GAGAGAAATCAACAACTACCATCACGCGC	

[0966]

	ATGACGCATACCTCAACGCTGTGGTCGGC ACCGCCCTGATCAAGAAGTACCCTAAACT TGAATCGGAGTTTGTGTACGGAGACTACA AGGTCTACGACGTGAGGAAGATGATAGCC AAGTCCGAACAGGAAATCGGGAAAGCAA CTGCGAAATACTTCTTTTACTCAAACATCA TGAACTTCTTCAAGACTGAAATTACGCTG GCCAATGGAGAAATCAGGAAGAGGCCACT GATCGAAACTAACGGAGAAACGGGCGAA ATCGTGTGGGACAAGGGCAGGGACTTCGC AACTGTTCGCAAAGTGCTCTCTATGCCGCA AGTCAATATTGTGAAGAAAACCGAAGTGC AAACCGGCGGATTTTCAAAGGAATCGATC CTCCCAAAGAGAAATAGCGACAAGCTCAT TGCACGCAAGAAAGACTGGGACCCGAAG AAGTACGGAGGATTCGATTCGCCGACTGT CGCATACTCCGTCCTCGTGGTGGCCAAGG TGGAGAAGGGAAAGAGCAAGAAGCTCAA ATCCGTCAAAGAGCTGCTGGGGATTACCAT CATGGAACGATCCTCGTTCGAGAAGAACC CGATTGATTTCCTGGAGGCGAAGGGTTAC AAGGAGGTGAAGAAGGATCTGATCATCAA ACTGCCCAAGTACTCACTGTTCGAACTGG AAAATGGTCGGAAGCGCATGCTGGCTTCG GCCGGAGAACTCCAGAAAGGAAATGAGC TGGCCTTGCCTAGCAAGTACGTCAACTTCC TCTATCTTGCTTCGCACTACGAGAAACTCA AAGGGTCACCGGAAGATAACGAACAGAA GCAGCTTTTCGTGGAGCAGCACAAGCATT ATCTGGATGAAATCATCGAACAAATCTCCG AGTTTTCAAAGCGCGTGATCCTCGCCGAC GCCAACCTCGACAAAGTCCTGTCGGCCTA CAATAAGCATAGAGATAAGCCGATCAGAG AACAGGCCGAGAACATTATCCACTTGTTC ACCCTGACTAACCTGGGAGCTCCAGCCGC CTTCAAGTACTTCGATACTACTATCGACCG CAAAAGATACACGTCCACCAAGGAAGTTC TGGACGCGACCCTGATCCACCAAAGCATC ACTGGACTCTACGAAACTAGGATCGATCT GTCGCAGCTGGGTGGCGATGGTGGCGGTG GATCCTACCCATACGACGTGCCTGACTACG CCTCCGGAGGTGGTGGCCCCAAGAAGAA ACGGAAGGTGTGATAGCTAGCCATCACATT	

[0967]

	TAAAAGCATCTCAGCCTACCATGAGAATAA GAGAAAGAAAATGAAGATCAATAGCTTAT TCATCTCTTTTTCTTTTTCGTTGGTGTAAAG CCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTT CAATTAATAAAAAATGGAAAGAACCTCGA G	
具有HSD的5'UTR、对应于SEQ ID NO: 245的ORF及ALB的3'UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCTATGGATAAGAAGTACTCGA TCGGGCTGGATATCGGAACTAATTCCGTGG GTTGGGCAGTGATCACGGATGAATACAAA GTGCCGTCCAAGAAGTTCAAGGTCCTGGG GAACACCGATAGACACAGCATCAAGAAGA ATCTCATCGGAGCCCTGCTGTTTGACTCCG GCGAAACCGCAGAAGCGACCCGGCTCAA ACGTACCGCGAGGCGACGCTACACCCGGC GGAAGAATCGCATCTGCTATCTGCAAGAA ATCTTTTCGAACGAAATGGCAAAGGTGGA CGACAGCTTCTTCCACCGCCTGGAAGAAT CTTTCCTGGTGGAGGAGGACAAGAAGCAT GAACGGCATCCTATCTTTGGAAACATCGTG GACGAAGTGGCGTACCACGAAAAGTACCC GACCATCTACCATCTGCGGAAGAAGTTGG TTGACTCAACTGACAAGGCCGACCTCAGA TTGATCTACTTGGCCCTCGCCCATATGATC AAATTCCGCGGACACTTCCTGATCGAAGG CGATCTGAACCCTGATAACTCCGACGTGGA TAAGCTGTTCATTCAACTGGTGCAGACCTA CAACCAACTGTTCGAAGAAAACCCAATCA ATGCCAGCGGCGTCGATGCCAAGGCCATC CTGTCCGCCCGGCTGTCGAAGTCGCGGCG CCTCGAAAACCTGATCGCACAGCTGCCGG GAGAGAAGAAGAACGGACTTTTCGGCAA CTTGATCGCTCTCTCACTGGGACTCACTCC CAATTTCAAGTCCAATTTTGACCTGGCCGA GGACGCGAAGCTGCAACTCTCAAAGGAC ACCTACGACGACGACTTGGACAATTTGCT GGCACAAATTGGCGATCAGTACGCGGATC TGTTCCTTGCCGCTAAGAACCTTTCGGACG CAATCTTGCTGTCCGATATCCTGCGCGTGA ACACCGAAATAACCAAAGCGCCGCTTAGC	247

[0968]

	GCCTCGATGATTAAGCGGTACGACGAGCA TCACCAGGATCTCACGCTGCTCAAAGCGC TCGTGAGACAGCAACTGCCTGAAAAGTAC AAGGAGATTTTCTTCGACCAGTCCAAGAA TGGGTACGCAGGGTACATCGATGGAGGCG CCAGCCAGGAAGAGTTCTATAAGTTCATC AAGCCAATCCTGGAAAAGATGGACGGAAC CGAAGAACTGCTGGTCAAGCTGAACAGG GAGGATCTGCTCCGCAAACAGAGAACCTT TGACAACGGAAGCATTCCACACCAGATCC ATCTGGGTGAGCTGCACGCCATCTTGCGG CGCCAGGAGGACTTTTACCCATTCCTCAA GGACAACCGGGAAAAGATCGAGAAAATT CTGACGTTCCGCATCCCGTATTACGTGGGC CCACTGGCGCGCGGCAATTCGCGCTTCGC GTGGATGACTAGAAAATCAGAGGAAACCA TCACTCCTTGGAATTTCGAGGAAGTTGTG GATAAGGGAGCTTCGGCACAATCCTTCATC GAACGAATGACCAACTTCGACAAGAATCT CCCAAACGAGAAGGTGCTTCCTAAGCACA GCCTCCTTTACGAATACTTCACTGTCTACA ACGAACTGACTAAAGTGAAATACGTTACT GAAGGAATGAGGAAGCCGGCCTTTCTGAG CGGAGAACAGAAGAAAGCGATTGTCGATC TGCTGTTCAAGACCAACCGCAAGGTGACC GTCAAGCAGCTTAAAGAGGACTACTTCAA GAAGATCGAGTGTTTCGACTCAGTGGAAA TCAGCGGAGTGGAGGACAGATTCAACGCT TCGCTGGGAACCTATCATGATCTCCTGAAG ATCATCAAGGACAAGGACTTCCTTGACAA CGAGGAGAACGAGGACATCCTGGAAGATA TCGTCCTGACCTTGACCCTTTTCGAGGATC GCGAGATGATCGAGGAGAGGCTTAAGACC TACGCTCATCTCTTCGACGATAAGGTCATG AAACAACTCAAGCGCCGCCGGTACACTGG TTGGGGCCGCCTCTCCCGCAAGCTGATCA ACGGTATTCGCGATAAACAGAGCGGTAAA ACTATCCTGGATTTCCTCAAATCGGATGGC TTCGCTAATCGTAACTTCATGCAGTTGATC CACGACGACAGCCTGACCTTTAAGGAGGA CATCCAGAAAGCACAAGTGAGCGGACAG GGAGACTCACTCCATGAACACATCGCGAA TCTGGCCGGTTCGCCGGCGATTAAGAAGG	

[0969]

	GAATCCTGCAAACTGTGAAGGTGGTGGAC GAGCTGGTGAAGGTCATGGGACGGCACA AACCGGAGAATATCGTGATTGAAATGGCC CGAGAAAACCAGACTACCCAGAAGGGCC AGAAGAACTCCCGCGAAAGGATGAAGCG GATCGAAGAAGGAATCAAGGAGCTGGGC AGCCAGATCCTGAAAGAGCACCCGGTGGA AAACACGCAGCTGCAGAACGAGAAGCTCT ACCTGTACTATTTGCAAAATGGACGGGACA TGTACGTGGACCAAGAGCTGGACATCAAT CGGTTGTCTGATTACGACGTGGACCACATC GTTCCACAGTCCTTTCTGAAGGATGACTCC ATCGATAACAAGGTGTTGACTCGCAGCGA CAAGAACAGAGGGAAGTCAGATAATGTGC CATCGGAGGAGGTCGTGAAGAAGATGAA GAATTACTGGCGGCAGCTCCTGAATGCGA AGCTGATTACCCAGAGAAAGTTTGACAAT CTCACTAAAGCCGAGCGCGGCGGACTCTC AGAGCTGGATAAGGCTGGATTCATCAAAC GGCAGCTGGTCGAGACTCGGCAGATTACC AAGCACGTGGCGCAGATCCTGGACTCCCG CATGAACACTAAATACGACGAGAACGATA AGCTCATCCGGGAAGTGAAGGTGATTACC CTGAAAAGCAAACTTGTGTCGGACTTTCG GAAGGACTTTCAGTTTTACAAAGTGAGAG AAATCAACAACTACCATCACGCGCATGAC GCATACCTCAACGCTGTGGTCGGCACCGC CCTGATCAAGAAGTACCCTAAACTTGAATC GGAGTTTGTGTACGGAGACTACAAGGTCT ACGACGTGAGGAAGATGATAGCCAAGTCC GAACAGGAAATCGGGAAAGCAACTGCGA AATACTTCTTTTACTCAAACATCATGAACT TCTTCAAGACTGAAATTACGCTGGCCAATG GAGAAATCAGGAAGAGGCCACTGATCGA AACTAACGGAGAAACGGGCGAAATCGTGT GGGACAAGGGCAGGGACTTCGCAACTGTT CGCAAAGTGCTCTCTATGCCGCAAGTCAAT ATTGTGAAGAAAACCGAAGTGCAAACCG GCGGATTTTCAAAGGAATCGATCCTCCCA AAGAGAAATAGCGACAAGCTCATTGCACG CAAGAAAGACTGGGACCCGAAGAAGTAC GGAGGATTCGATTCGCCGACTGTCGCATAC TCCGTCCTCGTGGTGGCCAAGGTGGAGAA	

[0970]

	GGGAAAGAGCAAGAAGCTCAAATCCGTC AAAGAGCTGCTGGGGATTACCATCATGGA ACGATCCTCGTTCGAGAAGAACCCGATTG ATTTCCTGGAGGCGAAGGGTTACAAGGAG GTGAAGAAGGATCTGATCATCAAACTGCC CAAGTACTCACTGTTCGAACTGGAAAATG GTCGGAAGCGCATGCTGGCTTCGGCCGGA GAACTCCAGAAAGGAAATGAGCTGGCCTT GCCTAGCAAGTACGTCAACTTCCTCTATCT TGCTTCGCACTACGAGAAACTCAAAGGGT CACCGGAAGATAACGAACAGAAGCAGCTT TTCGTGGAGCAGCACAAGCATTATCTGGAT GAAATCATCGAACAAATCTCCGAGTTTTCA AAGCGCGTGATCCTCGCCGACGCCAACCT CGACAAAGTCCTGTCGGCCTACAATAAGC ATAGAGATAAGCCGATCAGAGAACAGGCC GAGAACATTATCCACTTGTTCACCCTGACT AACCTGGGAGCTCCAGCCGCCTTCAAGTA CTTCGATACTACTATCGACCGCAAAAGATA CACGTCCACCAAGGAAGTTCTGGACGCGA CCCTGATCCACCAAAGCATCACTGGACTCT ACGAAACTAGGATCGATCTGTCGCAGCTG GGTGGCGATGGTGGCGGTGGATCCTACCC ATACGACGTGCCTGACTACGCCTCCGGAG GTGGTGGCCCCAAGAAGAAACGGAAGGT GTGATAGCTAGCCATCACATTTAAAAGCAT CTCAGCCTACCATGAGAATAAGAGAAAGA AAATGAAGATCAATAGCTTATTCATCTCTTT TTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATT TTGCCTCTTTTCTCTGTGCTTCAATTAATAA AAAATGGAAAGAACCTCGAG	
	未使用	248
包含Kozak序列与Cas9 ORF且使用在人类中通常高度表达的密码子的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGCCTAAGAAAA AGCGGAAGGTCGACGGGGATAAGAAGTA CTCAATCGGGCTGGATATCGGAACTAATTC CGTGGGTTGGGCAGTGATCACGGATGAAT ACAAAGTGCCGTCCAAGAAGTTCAAGGTC CTGGGGAACACCGATAGACACAGCATCAA	249

[0971]

	GAAAAATCTCATCGGAGCCCTGCTGTTTG ACTCCGGCGAAACCGCAGAAGCGACCCG GCTCAAACGTACCGCGAGGCGACGCTACA CCCGGCGGAAGAATCGCATCTGCTATCTGC AAGAGATCTTTTCGAACGAAATGGCAAAG GTCGACGACAGCTTCTTCCACCGCCTGGA AGAATCTTTCCTGGTGGAGGAGGACAAGA AGCATGAACGGCATCCTATCTTTGGAAACA TCGTCGACGAAGTGGCGTACCACGAAAAG TACCCGACCATCTACCATCTGCGGAAGAA GTTGGTTGACTCAACTGACAAGGCCGACC TCAGATTGATCTACTTGGCCCTCGCCCATA TGATCAAATTCCGCGGACACTTCCTGATCG AAGGCGATCTGAACCCTGATAACTCCGAC GTGGATAAGCTTTTCATTCAACTGGTGCAG ACCTACAACCAACTGTTCGAAGAAAACCC AATCAATGCTAGCGGCGTCGATGCCAAGG CCATCCTGTCCGCCCGGCTGTCGAAGTCG CGGCGCCTCGAAAACCTGATCGCACAGCT GCCGGGAGAGAAAAAGAACGGACTTTTC GGCAACTTGATCGCTCTCTCACTGGGACT CACTCCCAATTTCAAGTCCAATTTTGACCT GGCCGAGGACGCGAAGCTGCAACTCTCA AAGGACACCTACGACGACGACTTGGACAA TTTGCTGGCACAAATTGGCGATCAGTACGC GGATCTGTTCCTTGCCGCTAAGAACCTTTC GGACGCAATCTTGCTGTCCGATATCCTGCG CGTGAACACCGAAATAACCAAAGCGCCGC TTAGCGCCTCGATGATTAAGCGGTACGACG AGCATCACCAGGATCTCACGCTGCTCAAA GCGCTCGTGAGACAGCAACTGCCTGAAAA GTACAAGGAGATCTTCTTCGACCAGTCCA AGAATGGGTACGCAGGGTACATCGATGGA GGCGCTAGCCAGGAAGAGTTCTATAAGTT CATCAAGCCAATCCTGGAAAAGATGGACG GAACCGAAGAACTGCTGGTCAAGCTGAA CAGGGAGGATCTGCTCCGGAAACAGAGA ACCTTTGACAACGGATCCATTCCCCACCA GATCCATCTGGGTGAGCTGCACGCCATCTT GCGGCGCCAGGAGGACTTTTACCCATTCC TCAAGGACAACCGGGAAAAGATCGAGAA AATTCTGACGTTCCGCATCCCGTATTACGT GGGCCCACTGGCGCGCGGCAATTCGCGCT	

[0972]

	TCGCGTGGATGACTAGAAAATCAGAGGAA ACCATCACTCCTTGGAATTTCGAGGAAGTT GTGGATAAGGGAGCTTCGGCACAAAGCTT CATCGAACGAATGACCAACTTCGACAAGA ATCTCCCAAACGAGAAGGTGCTTCCTAAG CACAGCCTCCTTTACGAATACTTCACTGTC TACAACGAACTGACTAAAGTGAAATACGT TACTGAAGGAATGAGGAAGCCGGCCTTTC TGTCCGGAGAACAGAAGAAAGCAATTGTC GATCTGCTGTTCAAGACCAACCGCAAGGT GACCGTCAAGCAGCTTAAAGAGGACTACT TCAAGAAGATCGAGTGTTTCGACTCAGTG GAAATCAGCGGGGTGGAGGACAGATTCAA CGCTTCGCTGGGAACCTATCATGATCTCCT GAAGATCATCAAGGACAAGGACTTCCTTG ACAACGAGGAGAACGAGGACATCCTGGA AGATATCGTCCTGACCTTGACCCTTTTCGA GGATCGCGAGATGATCGAGGAGAGGCTTA AGACCTACGCTCATCTCTTCGACGATAAGG TCATGAAACAACTCAAGCGCCGCCGGTAC ACTGGTTGGGGCCGCCTCTCCCGCAAGCT GATCAACGGTATTCGCGATAAACAGAGCG GTAAAACTATCCTGGATTTCCTCAAATCGG ATGGCTTCGCTAATCGTAACTTCATGCAAT TGATCCACGACGACAGCCTGACCTTTAAG GAGGACATCCAAAAAGCACAAGTGTCCG GACAGGGAGACTCACTCCATGAACACATC GCGAATCTGGCCGGTTCGCCGGCGATTAA GAAGGGAATTCTGCAAACTGTGAAGGTGG TCGACGAGCTGGTGAAGGTCATGGGACGG CACAAACCGGAGAATATCGTGATTGAAAT GGCCCGAGAAAACCAGACTACCCAGAAG GGCCAGAAAAACTCCCGCGAAAGGATGA AGCGGATCGAAGAAGGAATCAAGGAGCT GGGCAGCCAGATCCTGAAAGAGCACCCG GTGGAAAACACGCAGCTGCAGAACGAGA AGCTCTACCTGTACTATTTGCAAAATGGAC GGGACATGTACGTGGACCAAGAGCTGGAC ATCAATCGGTTGTCTGATTACGACGTGGAC CACATCGTTCCACAGTCCTTTCTGAAGGAT GACTCGATCGATAACAAGGTGTTGACTCG CAGCGACAAGAACAGAGGGAAGTCAGAT AATGTGCCATCGGAGGAGGTCGTGAAGAA	

[0973]

	GATGAAGAATTACTGGCGGCAGCTCCTGA ATGCGAAGCTGATTACCCAGAGAAAGTTT GACAATCTCACTAAAGCCGAGCGCGGCGG ACTCTCAGAGCTGGATAAGGCTGGATTCAT CAAACGGCAGCTGGTCGAGACTCGGCAGA TTACCAAGCACGTGGCGCAGATCTTGGAC TCCCGCATGAACACTAAATACGACGAGAA CGATAAGCTCATCCGGGAAGTGAAGGTGA TTACCCTGAAAAGCAAACTTGTGTCGGAC TTTCGGAAGGACTTTCAGTTTTACAAAGT GAGAGAAATCAACAACTACCATCACGCGC ATGACGCATACCTCAACGCTGTGGTCGGTA CCGCCCTGATCAAAAAGTACCCTAAACTT GAATCGGAGTTTGTGTACGGAGACTACAA GGTCTACGACGTGAGGAAGATGATAGCCA AGTCCGAACAGGAAATCGGGAAAGCAAC TGCGAAATACTTCTTTTACTCAAACATCAT GAACTTTTTCAAGACTGAAATTACGCTGG CCAATGGAGAAATCAGGAAGAGGCCACTG ATCGAAACTAACGGAGAAACGGGCGAAAT CGTGTGGGACAAGGGCAGGGACTTCGCA ACTGTTCGCAAAGTGCTCTCTATGCCGCAA GTCAATATTGTGAAGAAAACCGAAGTGCA AACCGGCGGATTTTCAAAGGAATCGATCC TCCCAAAGAGAAATAGCGACAAGCTCATT GCACGCAAGAAAGACTGGGACCCGAAGA AGTACGGAGGATTCGATTCGCCGACTGTC GCATACTCCGTCCTCGTGGTGGCCAAGGT GGAGAAGGGAAAGAGCAAAAAGCTCAAA TCCGTCAAAGAGCTGCTGGGGATTACCAT CATGGAACGATCCTCGTTCGAGAAGAACC CGATTGATTTCCTCGAGGCGAAGGGTTAC AAGGAGGTGAAGAAGGATCTGATCATCAA ACTCCCCAAGTACTCACTGTTCGAACTGG AAAATGGTCGGAAGCGCATGCTGGCTTCG GCCGGAGAACTCCAAAAAGGAAATGAGC TGGCCTTGCCTAGCAAGTACGTCAACTTCC TCTATCTTGCTTCGCACTACGAAAAACTCA AAGGGTCACCGGAAGATAACGAACAGAA GCAGCTTTTCGTGGAGCAGCACAAGCATT ATCTGGATGAAATCATCGAACAAATCTCCG AGTTTTCAAAGCGCGTGATCCTCGCCGAC GCCAACCTCGACAAAGTCCTGTCGGCCTA	

[0974]

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移除剪接点的Cas9 ORF；12.75% U含量	ATGGACAAGAAGTACAGCATCGGACTGGA CATCGGAACAAACAGCGTCGGATGGGCAG TCATCACAGACGAATACAAGGTCCCGAGC AAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATC GGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAC AGCAAGAAGAAGATACACAAGAAGAAAG AACAGAATCTGCTACCTGCAGGAAATCTT CAGCAACGAAATGGCAAAGGTCGACGAC AGCTTCTTCCACcggCTGGAAGAAAGCTTC CTGGTCGAAGAAGACAAGAAGCACGAAA GACACCCGATCTTCGGAAACATCGTCGAC GAAGTCGCATACCACGAAAAGTACCCGAC AATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACT GATCTACCTGGCACTGGCACACATGATCAA GTTCAGAGGACACTTCCTGATCGAAGGAG ACCTGAACCCGGACAACAGCGACGTCGA CAAGCTGTTCATCCAGCTGGTCCAGACATA CAACCAGCTGTTCGAAGAAAACCCGATCA ACGCAAGCGGAGTCGACGCAAAGGCAAT CCTGAGCGCAAGACTGAGCAAGAGCAGA AGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGA AACCTGATCGCACTGAGCCTGGGACTGAC ACCGAACTTCAAGAGCAACTTCGACCTGG	250

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	CAGAAGACGCAAAGCTGCAGCTGAGCAA GGACACATACGACGACGACCTGGACAACC TGCTGGCACAGATCGGAGACCAGTACGCA GACCTGTTCCTGGCAGCAAAGAACCTGAG CGACGCAATCCTGCTGAGCGACATCCTGA GAGTCAACACAGAAATCACAAAGGCACC GCTGAGCGCAAGCATGATCAAGAGATACG ACGAACACCACCAGGACCTGACACTGCTG AAGGCACTGGTCAGACAGCAGCTGCCGG AAAAGTACAAGGAAATCTTCTTCGACCAG AGCAAGAACGGATACGCAGGATACATCGA CGGAGGAGCAAGCCAGGAAGAATTCTACA AGTTCATCAAGCCGATCCTGGAAAAGATG GACGGAACAGAAGAACTGCTGGTCAAGC TGAACAGAGAAGACCTGCTGAGAAAGCA GAGAACATTCGACAACGGAAGCATCCCGC ACCAGATCCACCTGGGAGAACTGCACGCA ATCCTGAGAAGACAGGAAGACTTCTACCC GTTCCTGAAGGACAACAGAGAAAAGATC GAAAAGATCCTGACATTCAGAATCCCGTA CTACGTCGGACCGCTGGCAAGAGGAAACA GCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGA AGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTT CGACAAGAACCTGCCGAACGAAAAGGTC CTGCCGAAGCACAGCCTGCTGTACGAATA CTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAG CCGGCATTCCTGAGCGGAGAACAGAAGA AGGCAATCGTCGACCTGCTGTTCAAGACA AACAGAAAGGTCACAGTCAAGCAGCTGA AGGAAGACTACTTCAAGAAGATCGAATGC TTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCAAGCCTGGGAACAT ACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACG AAGACATCCTGGAAGACATCGTCCTGACA CTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCACACC TGTTCGACGACAAGGTCATGAAGCAGCTG AAGAGAAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATC	

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[0977]

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具有HSD的5’UTR、对应于SEQ ID NO: 250的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAGAAGT ACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGA ATACAAGGTCCCGAGCAAGAAGTTCAAGG TCCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGAT	251

[0978]

	ACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACcggCT GGAAGAAAGCTTCCTGGTCGAAGAAGAC AAGAAGCACGAAAGACACCCGATCTTCGG AAACATCGTCGACGAAGTCGCATACCACG AAAAGTACCCGACAATCTACCACCTGAGA AAGAAGCTGGTCGACAGCACAGACAAGG CAGACCTGAGACTGATCTACCTGGCACTG GCACACATGATCAAGTTCAGAGGACACTT CCTGATCGAAGGAGACCTGAACCCGGACA ACAGCGACGTCGACAAGCTGTTCATCCAG CTGGTCCAGACATACAACCAGCTGTTCGA AGAAAACCCGATCAACGCAAGCGGAGTC GACGCAAAGGCAATCCTGAGCGCAAGACT GAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGA ACGGACTGTTCGGAAACCTGATCGCACTG AGCCTGGGACTGACACCGAACTTCAAGAG CAACTTCGACCTGGCAGAAGACGCAAAG CTGCAGCTGAGCAAGGACACATACGACGA CGACCTGGACAACCTGCTGGCACAGATCG GAGACCAGTACGCAGACCTGTTCCTGGCA GCAAAGAACCTGAGCGACGCAATCCTGCT GAGCGACATCCTGAGAGTCAACACAGAAA TCACAAAGGCACCGCTGAGCGCAAGCATG ATCAAGAGATACGACGAACACCACCAGGA CCTGACACTGCTGAAGGCACTGGTCAGAC AGCAGCTGCCGGAAAAGTACAAGGAAATC TTCTTCGACCAGAGCAAGAACGGATACGC AGGATACATCGACGGAGGAGCAAGCCAGG AAGAATTCTACAAGTTCATCAAGCCGATCC TGGAAAAGATGGACGGAACAGAAGAACT GCTGGTCAAGCTGAACAGAGAAGACCTG CTGAGAAAGCAGAGAACATTCGACAACG GAAGCATCCCGCACCAGATCCACCTGGGA GAACTGCACGCAATCCTGAGAAGACAGG AAGACTTCTACCCGTTCCTGAAGGACAAC AGAGAAAAGATCGAAAAGATCCTGACATT CAGAATCCCGTACTACGTCGGACCGCTGG CAAGAGGAAACAGCAGATTCGCATGGATG ACAAGAAAGAGCGAAGAAACAATCACAC CGTGGAACTTCGAAGAAGTCGTCGACAAG	

[0979]

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[0980]

	AAAGTTCGACAACCTGACAAAGGCAGAG AGAGGAGGACTGAGCGAACTGGACAAGG CAGGATTCATCAAGAGACAGCTGGTCGAA ACAAGACAGATCACAAAGCACGTCGCAC AGATCCTGGACAGCAGAATGAACACAAAG TACGACGAAAACGACAAGCTGATCAGAGA AGTCAAGGTCATCACACTGAAGAGCAAGC TGGTCAGCGACTTCAGAAAGGACTTCCAG TTCTACAAGGTCAGAGAAATCAACAACTA CCACCACGCACACGACGCATACCTGAACG CAGTCGTCGGAACAGCACTGATCAAGAAG TACCCGAAGCTGGAAAGCGAATTCGTCTA CGGAGACTACAAGGTCTACGACGTCAGAA AGATGATCGCAAAGAGCGAACAGGAAATC GGAAAGGCAACAGCAAAGTACTTCTTCTA CAGCAACATCATGAACTTCTTCAAGACAG AAATCACACTGGCAAACGGAGAAATCAGA AAGAGACCGCTGATCGAAACAAACGGAG AAACAGGAGAAATCGTCTGGGACAAGGG AAGAGACTTCGCAACAGTCAGAAAGGTC CTGAGCATGCCGCAGGTCAACATCGTCAA GAAGACAGAAGTCCAGACAGGAGGATTC AGCAAGGAAAGCATCCTGCCGAAGAGAA ACAGCGACAAGCTGATCGCAAGAAAGAA GGACTGGGACCCGAAGAAGTACGGAGGA TTCGACAGCCCGACAGTCGCATACAGCGT CCTGGTCGTCGCAAAGGTCGAAAAGGGA AAGAGCAAGAAGCTGAAGAGCGTCAAGG AACTGCTGGGAATCACAATCATGGAAAGA AGCAGCTTCGAAAAGAACCCGATCGACTT CCTGGAAGCAAAGGGATACAAGGAAGTC AAGAAGGACCTGATCATCAAGCTGCCGAA GTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGA ACTGCAGAAGGGAAACGAACTGGCACTG CCGAGCAAGTACGTCAACTTCCTGTACCT GGCAAGCCACTACGAAAAGCTGAAGGGA AGCCCGGAAGACAACGAACAGAAGCAGC TGTTCGTCGAACAGCACAAGCACTACCTG GACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAA ACCTGGACAAGGTCCTGAGCGCATACAAC AAGCACAGAGACAAGCCGATCAGAGAAC	

		AGGCAGAAAACATCATCCACCTGTTCACA CTGACAAACCTGGGAGCACCGGCAGCATT CAAGTACTTCGACACAACAATCGACAGAA AGAGATACACAAGCACAAAGGAAGTCCTG GACGCAACACTGATCCACCAGAGCATCAC AGGACTGTACGAAACAAGAATCGACCTGA GCCAGCTGGGAGGAGACGGAGGAGGAAG CCCGAAGAAGAAGAGAAAGGTCTAGCTA GCCATCACATTTAAAAGCATCTCAGCCTAC CATGAGAATAAGAGAAAGAAAATGAAGAT CAATAGCTTATTCATCTCTTTTTCTTTTTCG TTGGTGTAAAGCCAACACCCTGTCTAAAA AACATAAATTTCTTTAATCATTTTGCCTCTT TTCTCTGTGCTTCAATTAATAAAAAATGGA AAGAACCTCGAG	
[0981]	具有通常频繁用于人类中的最小尿苷密码子的Cas9 ORF；12.75% U含量	ATGGACAAGAAGTACAGCATCGGCCTGGA CATCGGCACCAACAGCGTGGGCTGGGCCG TGATCACCGACGAGTACAAGGTGCCCAGC AAGAAGTTCAAGGTGCTGGGCAACACCG ACAGACACAGCATCAAGAAGAACCTGATC GGCGCCCTGCTGTTCGACAGCGGCGAGAC CGCCGAGGCCACCAGACTGAAGAGAACC GCCAGAAGAAGATACACCAGAAGAAAGA ACAGAATCTGCTACCTGCAGGAGATCTTC AGCAACGAGATGGCCAAGGTGGACGACA GCTTCTTCCACAGACTGGAGGAGAGCTTC CTGGTGGAGGAGGACAAGAAGCACGAGA GACACCCCATCTTCGGCAACATCGTGGAC GAGGTGGCCTACCACGAGAAGTACCCCAC CATCTACCACCTGAGAAAGAAGCTGGTGG ACAGCACCGACAAGGCCGACCTGAGACT GATCTACCTGGCCCTGGCCCACATGATCAA GTTCAGAGGCCACTTCCTGATCGAGGGCG ACCTGAACCCCGACAACAGCGACGTGGA CAAGCTGTTCATCCAGCTGGTGCAGACCT ACAACCAGCTGTTCGAGGAGAACCCCATC AACGCCAGCGGCGTGGACGCCAAGGCCAT CCTGAGCGCCAGACTGAGCAAGAGCAGA AGACTGGAGAACCTGATCGCCCAGCTGCC CGGCGAGAAGAAGAACGGCCTGTTCGGC AACCTGATCGCCCTGAGCCTGGGCCTGAC CCCCAACTTCAAGAGCAACTTCGACCTGG	252

[0982]

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[0983]

	GAGACAAGCAGAGCGGCAAGACCATCCT GGACTTCCTGAAGAGCGACGGCTTCGCCA ACAGAAACTTCATGCAGCTGATCCACGAC GACAGCCTGACCTTCAAGGAGGACATCCA GAAGGCCCAGGTGAGCGGCCAGGGCGAC AGCCTGCACGAGCACATCGCCAACCTGGC CGGCAGCCCCGCCATCAAGAAGGGCATCC TGCAGACCGTGAAGGTGGTGGACGAGCT GGTGAAGGTGATGGGCAGACACAAGCCC GAGAACATCGTGATCGAGATGGCCAGAGA GAACCAGACCACCCAGAAGGGCCAGAAG AACAGCAGAGAGAGAATGAAGAGAATCG AGGAGGGCATCAAGGAGCTGGGCAGCCA GATCCTGAAGGAGCACCCCGTGGAGAACA CCCAGCTGCAGAACGAGAAGCTGTACCTG TACTACCTGCAGAACGGCAGAGACATGTA CGTGGACCAGGAGCTGGACATCAACAGAC TGAGCGACTACGACGTGGACCACATCGTG CCCCAGAGCTTCCTGAAGGACGACAGCAT CGACAACAAGGTGCTGACCAGAAGCGAC AAGAACAGAGGCAAGAGCGACAACGTGC CCAGCGAGGAGGTGGTGAAGAAGATGAA GAACTACTGGAGACAGCTGCTGAACGCCA AGCTGATCACCCAGAGAAAGTTCGACAAC CTGACCAAGGCCGAGAGAGGCGGCCTGA GCGAGCTGGACAAGGCCGGCTTCATCAAG AGACAGCTGGTGGAGACCAGACAGATCA CCAAGCACGTGGCCCAGATCCTGGACAGC AGAATGAACACCAAGTACGACGAGAACG ACAAGCTGATCAGAGAGGTGAAGGTGATC ACCCTGAAGAGCAAGCTGGTGAGCGACTT CAGAAAGGACTTCCAGTTCTACAAGGTGA GAGAGATCAACAACTACCACCACGCCCAC GACGCCTACCTGAACGCCGTGGTGGGCAC CGCCCTGATCAAGAAGTACCCCAAGCTGG AGAGCGAGTTCGTGTACGGCGACTACAAG GTGTACGACGTGAGAAAGATGATCGCCAA GAGCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACAGCAACATCATG AACTTCTTCAAGACCGAGATCACCCTGGC CAACGGCGAGATCAGAAAGAGACCCCTGA TCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCAGAGACTTCGCC	

[0984]

	ACCGTGAGAAAGGTGCTGAGCATGCCCCA GGTGAACATCGTGAAGAAGACCGAGGTG CAGACCGGCGGCTTCAGCAAGGAGAGCAT CCTGCCCAAGAGAAACAGCGACAAGCTG ATCGCCAGAAAGAAGGACTGGGACCCCA AGAAGTACGGCGGCTTCGACAGCCCCACC GTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTG AAGAGCGTGAAGGAGCTGCTGGGCATCAC CATCATGGAGAGAAGCAGCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGC TACAAGGAGGTGAAGAAGGACCTGATCAT CAAGCTGCCCAAGTACAGCCTGTTCGAGC TGGAGAACGGCAGAAAGAGAATGCTGGC CAGCGCCGGCGAGCTGCAGAAGGGCAAC GAGCTGGCCCTGCCCAGCAAGTACGTGAA CTTCCTGTACCTGGCCAGCCACTACGAGA AGCTGAAGGGCAGCCCCGAGGACAACGA GCAGAAGCAGCTGTTCGTGGAGCAGCAC AAGCACTACCTGGACGAGATCATCGAGCA GATCAGCGAGTTCAGCAAGAGAGTGATCC TGGCCGACGCCAACCTGGACAAGGTGCTG AGCGCCTACAACAAGCACAGAGACAAGC CCATCAGAGAGCAGGCCGAGAACATCATC CACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCA CCATCGACAGAAAGAGATACACCAGCACC AAGGAGGTGCTGGACGCCACCCTGATCCA CCAGAGCATCACCGGCCTGTACGAGACCA GAATCGACCTGAGCCAGCTGGGCGGCGAC GGCGGCGGCAGCCCCAAGAAGAAGAGAA AGGTGTGA	
具有HSD的5’UTR、对应于SEQ ID NO:252的ORF、Kozak序列及ALB的3’UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAGAAGT ACAGCATCGGCCTGGACATCGGCACCAAC AGCGTGGGCTGGGCCGTGATCACCGACGA GTACAAGGTGCCCAGCAAGAAGTTCAAGG TGCTGGGCAACACCGACAGACACAGCATC AAGAAGAACCTGATCGGCGCCCTGCTGTT CGACAGCGGCGAGACCGCCGAGGCCACC AGACTGAAGAGAACCGCCAGAAGAAGAT	253

[0985]

	ACACCAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAGATCTTCAGCAACGAGATGGC CAAGGTGGACGACAGCTTCTTCCACAGAC TGGAGGAGAGCTTCCTGGTGGAGGAGGA CAAGAAGCACGAGAGACACCCCATCTTCG GCAACATCGTGGACGAGGTGGCCTACCAC GAGAAGTACCCCACCATCTACCACCTGAG AAAGAAGCTGGTGGACAGCACCGACAAG GCCGACCTGAGACTGATCTACCTGGCCCT GGCCCACATGATCAAGTTCAGAGGCCACT TCCTGATCGAGGGCGACCTGAACCCCGAC AACAGCGACGTGGACAAGCTGTTCATCCA GCTGGTGCAGACCTACAACCAGCTGTTCG AGGAGAACCCCATCAACGCCAGCGGCGTG GACGCCAAGGCCATCCTGAGCGCCAGACT GAGCAAGAGCAGAAGACTGGAGAACCTG ATCGCCCAGCTGCCCGGCGAGAAGAAGA ACGGCCTGTTCGGCAACCTGATCGCCCTG AGCCTGGGCCTGACCCCCAACTTCAAGAG CAACTTCGACCTGGCCGAGGACGCCAAGC TGCAGCTGAGCAAGGACACCTACGACGAC GACCTGGACAACCTGCTGGCCCAGATCGG CGACCAGTACGCCGACCTGTTCCTGGCCG CCAAGAACCTGAGCGACGCCATCCTGCTG AGCGACATCCTGAGAGTGAACACCGAGAT CACCAAGGCCCCCCTGAGCGCCAGCATGA TCAAGAGATACGACGAGCACCACCAGGAC CTGACCCTGCTGAAGGCCCTGGTGAGACA GCAGCTGCCCGAGAAGTACAAGGAGATCT TCTTCGACCAGAGCAAGAACGGCTACGCC GGCTACATCGACGGCGGCGCCAGCCAGGA GGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTG CTGGTGAAGCTGAACAGAGAGGACCTGCT GAGAAAGCAGAGAACCTTCGACAACGGC AGCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGAGAAGACAGGAGG ACTTCTACCCCTTCCTGAAGGACAACAGA GAGAAGATCGAGAAGATCCTGACCTTCAG AATCCCCTACTACGTGGGCCCCCTGGCCA GAGGCAACAGCAGATTCGCCTGGATGACC AGAAAGAGCGAGGAGACCATCACCCCCT GGAACTTCGAGGAGGTGGTGGACAAGGG	

[0986]

	CGCCAGCGCCCAGAGCTTCATCGAGAGAA TGACCAACTTCGACAAGAACCTGCCCAAC GAGAAGGTGCTGCCCAAGCACAGCCTGCT GTACGAGTACTTCACCGTGTACAACGAGC TGACCAAGGTGAAGTACGTGACCGAGGGC ATGAGAAAGCCCGCCTTCCTGAGCGGCGA GCAGAAGAAGGCCATCGTGGACCTGCTGT TCAAGACCAACAGAAAGGTGACCGTGAA GCAGCTGAAGGAGGACTACTTCAAGAAGA TCGAGTGCTTCGACAGCGTGGAGATCAGC GGCGTGGAGGACAGATTCAACGCCAGCCT GGGCACCTACCACGACCTGCTGAAGATCA TCAAGGACAAGGACTTCCTGGACAACGA GGAGAACGAGGACATCCTGGAGGACATCG TGCTGACCCTGACCCTGTTCGAGGACAGA GAGATGATCGAGGAGAGACTGAAGACCTA CGCCCACCTGTTCGACGACAAGGTGATGA AGCAGCTGAAGAGAAGAAGATACACCGG CTGGGGCAGACTGAGCAGAAAGCTGATCA ACGGCATCAGAGACAAGCAGAGCGGCAA GACCATCCTGGACTTCCTGAAGAGCGACG GCTTCGCCAACAGAAACTTCATGCAGCTG ATCCACGACGACAGCCTGACCTTCAAGGA GGACATCCAGAAGGCCCAGGTGAGCGGC CAGGGCGACAGCCTGCACGAGCACATCGC CAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTG GACGAGCTGGTGAAGGTGATGGGCAGAC ACAAGCCCGAGAACATCGTGATCGAGATG GCCAGAGAGAACCAGACCACCCAGAAGG GCCAGAAGAACAGCAGAGAGAGAATGAA GAGAATCGAGGAGGGCATCAAGGAGCTG GGCAGCCAGATCCTGAAGGAGCACCCCGT GGAGAACACCCAGCTGCAGAACGAGAAG CTGTACCTGTACTACCTGCAGAACGGCAG AGACATGTACGTGGACCAGGAGCTGGACA TCAACAGACTGAGCGACTACGACGTGGAC CACATCGTGCCCCAGAGCTTCCTGAAGGA CGACAGCATCGACAACAAGGTGCTGACCA GAAGCGACAAGAACAGAGGCAAGAGCGA CAACGTGCCCAGCGAGGAGGTGGTGAAG AAGATGAAGAACTACTGGAGACAGCTGCT GAACGCCAAGCTGATCACCCAGAGAAAGT	

[0987]

	TCGACAACCTGACCAAGGCCGAGAGAGG CGGCCTGAGCGAGCTGGACAAGGCCGGC TTCATCAAGAGACAGCTGGTGGAGACCAG ACAGATCACCAAGCACGTGGCCCAGATCC TGGACAGCAGAATGAACACCAAGTACGAC GAGAACGACAAGCTGATCAGAGAGGTGA AGGTGATCACCCTGAAGAGCAAGCTGGTG AGCGACTTCAGAAAGGACTTCCAGTTCTA CAAGGTGAGAGAGATCAACAACTACCACC ACGCCCACGACGCCTACCTGAACGCCGTG GTGGGCACCGCCCTGATCAAGAAGTACCC CAAGCTGGAGAGCGAGTTCGTGTACGGCG ACTACAAGGTGTACGACGTGAGAAAGATG ATCGCCAAGAGCGAGCAGGAGATCGGCA AGGCCACCGCCAAGTACTTCTTCTACAGC AACATCATGAACTTCTTCAAGACCGAGAT CACCCTGGCCAACGGCGAGATCAGAAAG AGACCCCTGATCGAGACCAACGGCGAGAC CGGCGAGATCGTGTGGGACAAGGGCAGA GACTTCGCCACCGTGAGAAAGGTGCTGAG CATGCCCCAGGTGAACATCGTGAAGAAGA CCGAGGTGCAGACCGGCGGCTTCAGCAA GGAGAGCATCCTGCCCAAGAGAAACAGC GACAAGCTGATCGCCAGAAAGAAGGACT GGGACCCCAAGAAGTACGGCGGCTTCGAC AGCCCCACCGTGGCCTACAGCGTGCTGGT GGTGGCCAAGGTGGAGAAGGGCAAGAGC AAGAAGCTGAAGAGCGTGAAGGAGCTGC TGGGCATCACCATCATGGAGAGAAGCAGC TTCGAGAAGAACCCCATCGACTTCCTGGA GGCCAAGGGCTACAAGGAGGTGAAGAAG GACCTGATCATCAAGCTGCCCAAGTACAG CCTGTTCGAGCTGGAGAACGGCAGAAAG AGAATGCTGGCCAGCGCCGGCGAGCTGCA GAAGGGCAACGAGCTGGCCCTGCCCAGC AAGTACGTGAACTTCCTGTACCTGGCCAG CCACTACGAGAAGCTGAAGGGCAGCCCCG AGGACAACGAGCAGAAGCAGCTGTTCGT GGAGCAGCACAAGCACTACCTGGACGAGA TCATCGAGCAGATCAGCGAGTTCAGCAAG AGAGTGATCCTGGCCGACGCCAACCTGGA CAAGGTGCTGAGCGCCTACAACAAGCACA GAGACAAGCCCATCAGAGAGCAGGCCGA	

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[0988]	具有通常很少用于人类中的最小尿苷密码子的Cas9 ORF；12.75% U含量	ATGGACAAAAAATACAGCATAGGGCTAGA CATAGGGACGAACAGCGTAGGGTGGGCGG TAATAACGGACGAATACAAAGTACCGAGC AAAAAATTCAAAGTACTAGGGAACACGGA CCGACACAGCATAAAAAAAAACCTAATAG GGGCGCTACTATTCGACAGCGGGGAAACG GCGGAAGCGACGCGACTAAAACGAACGG CGCGACGACGATACACGCGACGAAAAAA CCGAATATGCTACCTACAAGAAATATTCAG CAACGAAATGGCGAAAGTAGACGACAGCT TCTTCCACCGACTAGAAGAAAGCTTCCTA GTAGAAGAAGACAAAAAACACGAACGAC ACCCGATATTCGGGAACATAGTAGACGAA GTAGCGTACCACGAAAAATACCCGACGAT ATACCACCTACGAAAAAAACTAGTAGACA GCACGGACAAAGCGGACCTACGACTAATA TACCTAGCGCTAGCGCACATGATAAAATTC CGAGGGCACTTCCTAATAGAAGGGGACCT AAACCCGGACAACAGCGACGTAGACAAA CTATTCATACAACTAGTACAAACGTACAAC CAACTATTCGAAGAAAACCCGATAAACGC GAGCGGGGTAGACGCGAAAGCGATACTAA GCGCGCGACTAAGCAAAAGCCGACGACTA GAAAACCTAATAGCGCAACTACCGGGGGA AAAAAAAAACGGGCTATTCGGGAACCTAA TAGCGCTAAGCCTAGGGCTAACGCCGAAC TTCAAAAGCAACTTCGACCTAGCGGAAGA	254

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[0991]

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具有HSD的5’UTR、对应于SEQ ID NO: 254的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAAAAAT ACAGCATAGGGCTAGACATAGGGACGAAC AGCGTAGGGTGGGCGGTAATAACGGACGA ATACAAAGTACCGAGCAAAAAATTCAAAG TACTAGGGAACACGGACCGACACAGCATA AAAAAAAACCTAATAGGGGCGCTACTATT CGACAGCGGGGAAACGGCGGAAGCGACG CGACTAAAACGAACGGCGCGACGACGATA CACGCGACGAAAAAACCGAATATGCTACC	255

[0992]

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[0995]	具有AGG作为前三个核苷酸用于CleanCap™、HSD的5’UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及ALB的3’UTR的Cas9转录物	AGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAGAAGT ACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGA ATACAAGGTCCCGAGCAAGAAGTTCAAGG TCCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGAT ACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGAC TGGAAGAAAGCTTCCTGGTCGAAGAAGA CAAGAAGCACGAAAGACACCCGATCTTCG GAAACATCGTCGACGAAGTCGCATACCAC GAAAAGTACCCGACAATCTACCACCTGAG AAAGAAGCTGGTCGACAGCACAGACAAG GCAGACCTGAGACTGATCTACCTGGCACT GGCACACATGATCAAGTTCAGAGGACACT TCCTGATCGAAGGAGACCTGAACCCGGAC AACAGCGACGTCGACAAGCTGTTCATCCA GCTGGTCCAGACATACAACCAGCTGTTCG AAGAAAACCCGATCAACGCAAGCGGAGT CGACGCAAAGGCAATCCTGAGCGCAAGA CTGAGCAAGAGCAGAAGACTGGAAAACC TGATCGCACAGCTGCCGGGAGAAAAGAA GAACGGACTGTTCGGAAACCTGATCGCAC TGAGCCTGGGACTGACACCGAACTTCAAG	256

[0996]

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具有CMV的5’ UTR、对应于SEQ ID NO:	GGGCAGATCGCCTGGAGACGCCATCCACG CTGTTTTGACCTCCATAGAAGACACCGGG ACCGATCCAGCCTCCGCGGCCGGGAACGG	257

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204的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物	TGCATTGGAACGCGGATTCCCCGTGCCAA GAGTGACTCACCGTCCTTGACACGGCCAC CATGGACAAGAAGTACAGCATCGGACTGG ACATCGGAACAAACAGCGTCGGATGGGCA GTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACA GACAGACACAGCATCAAGAAGAACCTGAT CGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAA CAGCAAGAAGAAGATACACAAGAAGAAA GAACAGAATCTGCTACCTGCAGGAAATCT TCAGCAACGAAATGGCAAAGGTCGACGA CAGCTTCTTCCACAGACTGGAAGAAAGCT TCCTGGTCGAAGAAGACAAGAAGCACGA AAGACACCCGATCTTCGGAAACATCGTCG ACGAAGTCGCATACCACGAAAAGTACCCG ACAATCTACCACCTGAGAAAGAAGCTGGT CGACAGCACAGACAAGGCAGACCTGAGA CTGATCTACCTGGCACTGGCACACATGATC AAGTTCAGAGGACACTTCCTGATCGAAGG AGACCTGAACCCGGACAACAGCGACGTC GACAAGCTGTTCATCCAGCTGGTCCAGAC ATACAACCAGCTGTTCGAAGAAAACCCGA TCAACGCAAGCGGAGTCGACGCAAAGGC AATCCTGAGCGCAAGACTGAGCAAGAGC AGAAGACTGGAAAACCTGATCGCACAGCT GCCGGGAGAAAAGAAGAACGGACTGTTC GGAAACCTGATCGCACTGAGCCTGGGACT GACACCGAACTTCAAGAGCAACTTCGACC TGGCAGAAGACGCAAAGCTGCAGCTGAG CAAGGACACATACGACGACGACCTGGACA ACCTGCTGGCACAGATCGGAGACCAGTAC GCAGACCTGTTCCTGGCAGCAAAGAACCT GAGCGACGCAATCCTGCTGAGCGACATCC TGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGAT ACGACGAACACCACCAGGACCTGACACT GCTGAAGGCACTGGTCAGACAGCAGCTGC CGGAAAAGTACAAGGAAATCTTCTTCGAC CAGAGCAAGAACGGATACGCAGGATACAT CGACGGAGGAGCAAGCCAGGAAGAATTCT ACAAGTTCATCAAGCCGATCCTGGAAAAG ATGGACGGAACAGAAGAACTGCTGGTCA	

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具有HBB的5’UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及HBB的3’ UTR的Cas9转录物	GGGACATTTGCTTCTGACACAACTGTGTTC ACTAGCAACCTCAAACAGACACCGGATCT GCCACCATGGACAAGAAGTACAGCATCGG ACTGGACATCGGAACAAACAGCGTCGGAT GGGCAGTCATCACAGACGAATACAAGGTC CCGAGCAAGAAGTTCAAGGTCCTGGGAA ACACAGACAGACACAGCATCAAGAAGAA CCTGATCGGAGCACTGCTGTTCGACAGCG GAGAAACAGCAGAAGCAACAAGACTGAA GAGAACAGCAAGAAGAAGATACACAAGA AGAAAGAACAGAATCTGCTACCTGCAGGA AATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAA AGCTTCCTGGTCGAAGAAGACAAGAAGC ACGAAAGACACCCGATCTTCGGAAACATC GTCGACGAAGTCGCATACCACGAAAAGTA CCCGACAATCTACCACCTGAGAAAGAAGC	258

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具有XBG的5'UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及XBG的3' UTR的Cas9转录物	GGGAAGCTCAGAATAAACGCTCAACTTTG GCCGGATCTGCCACCATGGACAAGAAGTA CAGCATCGGACTGGACATCGGAACAAACA GCGTCGGATGGGCAGTCATCACAGACGAA TACAAGGTCCCGAGCAAGAAGTTCAAGGT CCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGAT ACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGAC TGGAAGAAAGCTTCCTGGTCGAAGAAGA CAAGAAGCACGAAAGACACCCGATCTTCG GAAACATCGTCGACGAAGTCGCATACCAC GAAAAGTACCCGACAATCTACCACCTGAG AAAGAAGCTGGTCGACAGCACAGACAAG GCAGACCTGAGACTGATCTACCTGGCACT GGCACACATGATCAAGTTCAGAGGACACT TCCTGATCGAAGGAGACCTGAACCCGGAC AACAGCGACGTCGACAAGCTGTTCATCCA GCTGGTCCAGACATACAACCAGCTGTTCG AAGAAAACCCGATCAACGCAAGCGGAGT CGACGCAAAGGCAATCCTGAGCGCAAGA CTGAGCAAGAGCAGAAGACTGGAAAACC TGATCGCACAGCTGCCGGGAGAAAAGAA GAACGGACTGTTCGGAAACCTGATCGCAC TGAGCCTGGGACTGACACCGAACTTCAAG AGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGAT CGGAGACCAGTACGCAGACCTGTTCCTGG CAGCAAAGAACCTGAGCGACGCAATCCTG CTGAGCGACATCCTGAGAGTCAACACAGA AATCACAAAGGCACCGCTGAGCGCAAGCA TGATCAAGAGATACGACGAACACCACCAG	259

[1007]

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[1009]

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具有AGG作为前三个核苷酸用于CleanCap™、XBG的5’UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及XBG的3’UTR的Cas9转录物	AGGAAGCTCAGAATAAACGCTCAACTTTG GCCGGATCTGCCACCATGGACAAGAAGTA CAGCATCGGACTGGACATCGGAACAAACA GCGTCGGATGGGCAGTCATCACAGACGAA TACAAGGTCCCGAGCAAGAAGTTCAAGGT CCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGAT ACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGAC TGGAAGAAAGCTTCCTGGTCGAAGAAGA	260

[1010]

	CAAGAAGCACGAAAGACACCCGATCTTCG GAAACATCGTCGACGAAGTCGCATACCAC GAAAAGTACCCGACAATCTACCACCTGAG AAAGAAGCTGGTCGACAGCACAGACAAG GCAGACCTGAGACTGATCTACCTGGCACT GGCACACATGATCAAGTTCAGAGGACACT TCCTGATCGAAGGAGACCTGAACCCGGAC AACAGCGACGTCGACAAGCTGTTCATCCA GCTGGTCCAGACATACAACCAGCTGTTCG AAGAAAACCCGATCAACGCAAGCGGAGT CGACGCAAAGGCAATCCTGAGCGCAAGA CTGAGCAAGAGCAGAAGACTGGAAAACC TGATCGCACAGCTGCCGGGAGAAAAGAA GAACGGACTGTTCGGAAACCTGATCGCAC TGAGCCTGGGACTGACACCGAACTTCAAG AGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGAT CGGAGACCAGTACGCAGACCTGTTCCTGG CAGCAAAGAACCTGAGCGACGCAATCCTG CTGAGCGACATCCTGAGAGTCAACACAGA AATCACAAAGGCACCGCTGAGCGCAAGCA TGATCAAGAGATACGACGAACACCACCAG GACCTGACACTGCTGAAGGCACTGGTCAG ACAGCAGCTGCCGGAAAAGTACAAGGAA ATCTTCTTCGACCAGAGCAAGAACGGATA CGCAGGATACATCGACGGAGGAGCAAGCC AGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAG AACTGCTGGTCAAGCTGAACAGAGAAGA CCTGCTGAGAAAGCAGAGAACATTCGACA ACGGAAGCATCCCGCACCAGATCCACCTG GGAGAACTGCACGCAATCCTGAGAAGAC AGGAAGACTTCTACCCGTTCCTGAAGGAC AACAGAGAAAAGATCGAAAAGATCCTGA CATTCAGAATCCCGTACTACGTCGGACCGC TGGCAAGAGGAAACAGCAGATTCGCATGG ATGACAAGAAAGAGCGAAGAAACAATCA CACCGTGGAACTTCGAAGAAGTCGTCGAC AAGGGAGCAAGCGCACAGAGCTTCATCG AAAGAATGACAAACTTCGACAAGAACCTG CCGAACGAAAAGGTCCTGCCGAAGCACA GCCTGCTGTACGAATACTTCACAGTCTACA	

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	ACGAACTGACAAAGGTCAAGTACGTCACA GAAGGAATGAGAAAGCCGGCATTCCTGAG CGGAGAACAGAAGAAGGCAATCGTCGAC CTGCTGTTCAAGACAAACAGAAAGGTCAC AGTCAAGCAGCTGAAGGAAGACTACTTCA AGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGC AAGCCTGGGAACATACCACGACCTGCTGA AGATCATCAAGGACAAGGACTTCCTGGAC AACGAAGAAAACGAAGACATCCTGGAAG ACATCGTCCTGACACTGACACTGTTCGAA GACAGAGAAATGATCGAAGAAAGACTGA AGACATACGCACACCTGTTCGACGACAAG GTCATGAAGCAGCTGAAGAGAAGAAGATA CACAGGATGGGGAAGACTGAGCAGAAAG CTGATCAACGGAATCAGAGACAAGCAGAG CGGAAAGACAATCCTGGACTTCCTGAAGA GCGACGGATTCGCAAACAGAAACTTCATG CAGCTGATCCACGACGACAGCCTGACATT CAAGGAAGACATCCAGAAGGCACAGGTC AGCGGACAGGGAGACAGCCTGCACGAAC ACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAA GGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATC GAAATGGCAAGAGAAAACCAGACAACAC AGAAGGGACAGAAGAACAGCAGAGAAAG AATGAAGAGAATCGAAGAAGGAATCAAG GAACTGGGAAGCCAGATCCTGAAGGAAC ACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAGA ACGGAAGAGACATGTACGTCGACCAGGAA CTGGACATCAACAGACTGAGCGACTACGA CGTCGACCACATCGTCCCGCAGAGCTTCC TGAAGGACGACAGCATCGACAACAAGGT CCTGACAAGAAGCGACAAGAACAGAGGA AAGAGCGACAACGTCCCGAGCGAAGAAG TCGTCAAGAAGATGAAGAACTACTGGAGA CAGCTGCTGAACGCAAAGCTGATCACACA GAGAAAGTTCGACAACCTGACAAAGGCA GAGAGAGGAGGACTGAGCGAACTGGACA AGGCAGGATTCATCAAGAGACAGCTGGTC GAAACAAGACAGATCACAAAGCACGTCG	

[1012]

	CACAGATCCTGGACAGCAGAATGAACACA AAGTACGACGAAAACGACAAGCTGATCAG AGAAGTCAAGGTCATCACACTGAAGAGCA AGCTGGTCAGCGACTTCAGAAAGGACTTC CAGTTCTACAAGGTCAGAGAAATCAACAA CTACCACCACGCACACGACGCATACCTGA ACGCAGTCGTCGGAACAGCACTGATCAAG AAGTACCCGAAGCTGGAAAGCGAATTCGT CTACGGAGACTACAAGGTCTACGACGTCA GAAAGATGATCGCAAAGAGCGAACAGGA AATCGGAAAGGCAACAGCAAAGTACTTCT TCTACAGCAACATCATGAACTTCTTCAAGA CAGAAATCACACTGGCAAACGGAGAAATC AGAAAGAGACCGCTGATCGAAACAAACG GAGAAACAGGAGAAATCGTCTGGGACAA GGGAAGAGACTTCGCAACAGTCAGAAAG GTCCTGAGCATGCCGCAGGTCAACATCGT CAAGAAGACAGAAGTCCAGACAGGAGGA TTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAG AAGGACTGGGACCCGAAGAAGTACGGAG GATTCGACAGCCCGACAGTCGCATACAGC GTCCTGGTCGTCGCAAAGGTCGAAAAGGG AAAGAGCAAGAAGCTGAAGAGCGTCAAG GAACTGCTGGGAATCACAATCATGGAAAG AAGCAGCTTCGAAAAGAACCCGATCGACT TCCTGGAAGCAAAGGGATACAAGGAAGTC AAGAAGGACCTGATCATCAAGCTGCCGAA GTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGA ACTGCAGAAGGGAAACGAACTGGCACTG CCGAGCAAGTACGTCAACTTCCTGTACCT GGCAAGCCACTACGAAAAGCTGAAGGGA AGCCCGGAAGACAACGAACAGAAGCAGC TGTTCGTCGAACAGCACAAGCACTACCTG GACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAA ACCTGGACAAGGTCCTGAGCGCATACAAC AAGCACAGAGACAAGCCGATCAGAGAAC AGGCAGAAAACATCATCCACCTGTTCACA CTGACAAACCTGGGAGCACCGGCAGCATT CAAGTACTTCGACACAACAATCGACAGAA AGAGATACACAAGCACAAAGGAAGTCCTG	

		GACGCAACACTGATCCACCAGAGCATCAC AGGACTGTACGAAACAAGAATCGACCTGA GCCAGCTGGGAGGAGACGGAGGAGGAAG CCCGAAGAAGAAGAGAAAGGTCTAGCTA GCACCAGCCTCAAGAACACCCGAATGGAG TCTCTAAGCTACATAATACCAACTTACACT TTACAAAATGTTGTCCCCCAAAATGTAGCC ATTCGTATCTGCTCCTAATAAAAAGAAAGT TTCTTCACATTCTCTCGAG	
[1013]	具有AGG作为前三个核苷酸用于CleanCap™、HSD的5’UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及ALB的3’UTR的Cas9转录物	AGGTCCCGCAGTCGGCGTCCAGCGGCTCT GCTTGTTCGTGTGTGTGTCGTTGCAGGCCT TATTCGGATCCGCCACCATGGACAAGAAGT ACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGA ATACAAGGTCCCGAGCAAGAAGTTCAAGG TCCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTT CGACAGCGGAGAAACAGCAGAAGCAACA AGACTGAAGAGAACAGCAAGAAGAAGAT ACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGAC TGGAAGAAAGCTTCCTGGTCGAAGAAGA CAAGAAGCACGAAAGACACCCGATCTTCG GAAACATCGTCGACGAAGTCGCATACCAC GAAAAGTACCCGACAATCTACCACCTGAG AAAGAAGCTGGTCGACAGCACAGACAAG GCAGACCTGAGACTGATCTACCTGGCACT GGCACACATGATCAAGTTCAGAGGACACT TCCTGATCGAAGGAGACCTGAACCCGGAC AACAGCGACGTCGACAAGCTGTTCATCCA GCTGGTCCAGACATACAACCAGCTGTTCG AAGAAAACCCGATCAACGCAAGCGGAGT CGACGCAAAGGCAATCCTGAGCGCAAGA CTGAGCAAGAGCAGAAGACTGGAAAACC TGATCGCACAGCTGCCGGGAGAAAAGAA GAACGGACTGTTCGGAAACCTGATCGCAC TGAGCCTGGGACTGACACCGAACTTCAAG AGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGAT CGGAGACCAGTACGCAGACCTGTTCCTGG	261

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多-A 100序列	AAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAA	263
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使用最小尿苷密码子的编码脑膜炎双球菌Cas9的ORF，具有起始及终止密码子	ATGGCAGCATTCAAGCCGAACTCGATCAA CTACATCCTGGGACTGGACATCGGAATCGC ATCGGTCGGATGGGCAATGGTCGAAATCG ACGAAGAAGAAAACCCGATCAGACTGATC GACCTGGGAGTCAGAGTCTTCGAAAGAGC AGAAGTCCCGAAGACAGGAGACTCGCTG GCAATGGCAAGAAGACTGGCAAGATCGGT CAGAAGACTGACAAGAAGAAGAGCACAC AGACTGCTGAGAACAAGAAGACTGCTGA AGAGAGAAGGAGTCCTGCAGGCAGCAAA CTTCGACGAAAACGGACTGATCAAGTCGC TGCCGAACACACCGTGGCAGCTGAGAGC AGCAGCACTGGACAGAAAGCTGACACCG CTGGAATGGTCGGCAGTCCTGCTGCACCT GATCAAGCACAGAGGATACCTGTCGCAGA GAAAGAACGAAGGAGAAACAGCAGACAA GGAACTGGGAGCACTGCTGAAGGGAGTC GCAGGAAACGCACACGCACTGCAGACAG GAGACTTCAGAACACCGGCAGAACTGGC ACTGAACAAGTTCGAAAAGGAATCGGGA CACATCAGAAACCAGAGATCGGACTACTC GCACACATTCTCGAGAAAGGACCTGCAGG CAGAACTGATCCTGCTGTTCGAAAAGCAG AAGGAATTCGGAAACCCGCACGTCTCGGG AGGACTGAAGGAAGGAATCGAAACACTG CTGATGACACAGAGACCGGCACTGTCGGG AGACGCAGTCCAGAAGATGCTGGGACACT GCACATTCGAACCGGCAGAACCGAAGGC AGCAAAGAACACATACACAGCAGAAAGAT TCATCTGGCTGACAAAGCTGAACAACCTG AGAATCCTGGAACAGGGATCGGAAAGACC GCTGACAGACACAGAAAGAGCAACACTG ATGGACGAACCGTACAGAAAGTCGAAGCT GACATACGCACAGGCAAGAAAGCTGCTGG GACTGGAAGACACAGCATTCTTCAAGGGA CTGAGATACGGAAAGGACAACGCAGAAG CATCGACACTGATGGAAATGAAGGCATAC CACGCAATCTCGAGAGCACTGGAAAAGG AAGGACTGAAGGACAAGAAGTCGCCGCT GAACCTGTCGCCGGAACTGCAGGACGAAA TCGGAACAGCATTCTCGCTGTTCAAGACA	265

[1018]

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[1019]

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使用最小尿苷密码子的编码脑膜炎双球菌Cas9的ORF (无起始或终止密码子; 适于纳入融合蛋白编码序列中)	GCAGCATTCAAGCCGAACTCGATCAACTA CATCCTGGGACTGGACATCGGAATCGCATC GGTCGGATGGGCAATGGTCGAAATCGACG AAGAAGAAAACCCGATCAGACTGATCGAC CTGGGAGTCAGAGTCTTCGAAAGAGCAG AAGTCCCGAAGACAGGAGACTCGCTGGC AATGGCAAGAAGACTGGCAAGATCGGTCA GAAGACTGACAAGAAGAAGAGCACACAG ACTGCTGAGAACAAGAAGACTGCTGAAG AGAGAAGGAGTCCTGCAGGCAGCAAACT TCGACGAAAACGGACTGATCAAGTCGCTG	266

[1020]

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[0004] cggttcgccg gcgattaaga agggaattct gcaaactgtg aaggtggtcg acgagctggt 2340

gaaggtcatg ggacggcaca aaccggagaa tatcgtgatt gaaatggccc gagaaaacca 2400

gactacccag aagggccaga aaaactcccg cgaaaggatg aagcggatcg aagaaggaat 2460

caaggagctg ggcagccaga tcctgaaaga gcacccggtg gaaaacacgc agctgcagaa 2520

cgagaagctc tacctgtact atttgcaaaa tggacgggac atgtacgtgg accaagagct 2580

ggacatcaat cggttgtctg attacgacgt ggaccacatc gttccacagt cctttctgaa 2640

ggatgactcg atcgataaca aggtgttgac tcgcagcgac aagaacagag ggaagtcaga 2700

taatgtgcca tcggaggagg tcgtgaagaa gatgaagaat tactggcggc agctcctgaa 2760

tgcgaagctg attacccaga gaaagtttga caatctcact aaagccgagc gcggcggact 2820

ctcagagctg gataaggctg gattcatcaa acggcagctg gtcgagactc ggcagattac 2880

caagcacgtg gcgcagatct tggactcccg catgaacact aaatacgacg agaacgataa 2940

gctcatccgg gaagtgaagg tgattaccct gaaaagcaaa cttgtgtcgg actttcggaa 3000

ggactttcag ttttacaaag tgagagaaat caacaactac catcacgcgc atgacgcata 3060

cctcaacgct gtggtcggta ccgccctgat caaaaagtac cctaaacttg aatcggagtt 3120

tgtgtacgga gactacaagg tctacgacgt gaggaagatg atagccaagt ccgaacagga 3180

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gaagaagtac ggaggattcg attcgccgac tgtcgcatac tccgtcctcg tggtggccaa 3540

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catggaacga tcctcgttcg agaagaaccc gattgatttc ctcgaggcga agggttacaa 3660

ggaggtgaag aaggatctga tcatcaaact ccccaagtac tcactgttcg aactggaaaa 3720

tggtcggaag cgcatgctgg cttcggccgg agaactccaa aaaggaaatg agctggcctt 3780

gcctagcaag tacgtcaact tcctctatct tgcttcgcac tacgaaaaac tcaaagggtc 3840

accggaagat aacgaacaga agcagctttt cgtggagcag cacaagcatt atctggatga 3900

aatcatcgaa caaatctccg agttttcaaa gcgcgtgatc ctcgccgacg ccaacctcga 3960

caaagtcctg tcggcctaca ataagcatag agataagccg atcagagaac aggccgagaa 4020

cattatccac ttgttcaccc tgactaacct gggagcccca gccgccttca agtacttcga 4080

tactactatc gatcgcaaaa gatacacgtc caccaaggaa gttctggacg cgaccctgat 4140

ccaccaaagc atcactggac tctacgaaac taggatcgat ctgtcgcagc tgggtggcga 4200

ttgatagtct agccatcaca tttaaaagca tctcagccta ccatgagaat aagagaaaga 4260

aaatgaagat caatagctta ttcatctctt tttctttttc gttggtgtaa agccaacacc 4320

ctgtctaaaa aacataaatt tctttaatca ttttgcctct tttctctgtg cttcaattaa 4380

taaaaaatgg aaagaacctc gag 4403

[0005]

<210> 3
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 经修饰sgRNA序列

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
<221> misc_feature
<222> (1)..(20)
<223> n 是 a, c, g, 或 u

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 3
nnnnnnnnnn nnnnnnnnnn guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 4
<211> 105
<212> DNA
<213> 人工序列

<220>
<223> 合成的: 30/30/39 多-A 序列

<400> 4
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa gcgaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

aaaccgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 105

<210> 5
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR (外显子1)的CR003335 gRNA

<400> 5
cugcuccucc ucugccuugc 20

<210> 6
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR (外显子1)的CR003336 gRNA

<400> 6
ccuccucugc cuugcuggac 20

[0006]

<210> 7
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR (外显子1)的CR003337 gRNA

<400> 7
ccaguccagc aaggcagagg 20

<210> 8
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 1)的CR003338 gRNA

<400> 8
auaccagucc agcaaggcag 20

<210> 9
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 1)的CR003339 gRNA

<400> 9
acacaaauac caguccagca 20

<210> 10

<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 1)的CR003340 gRNA

<400> 10
uggacuggua uuugugucug 20

<210> 11
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向人类TTR的 (外显子 1)的CR003341 gRNA

<400> 11
cugguauuug ugucugaggc 20

<210> 12
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003342 gRNA

<400> 12
cuucucuaca cccagggcac 20

[0007]

<210> 13
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003343 gRNA

<400> 13
cagaggacac uuggauucac 20

<210> 14
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向人类TTR的 (外显子 2)的CR003344 gRNA

<400> 14
uuugaccauc agaggacacu 20

<210> 15
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003345 gRNA

<400> 15
ucuagaacuu ugaccaucag 20

<210> 16
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003346 gRNA

<400> 16
aaaguucuag augcuguccg 20

<210> 17
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003347 gRNA

<400> 17
cauugauggc aggacugccu 20

<210> 18
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003348 gRNA

<400> 18
aggcaguccu gccaucaaug 20

<210> 19
<211> 20
<212> RNA
<213> 人工序列

[0008]

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003349 gRNA

<400> 19
ugcacggcca cauugauggc 20

<210> 20
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003350 gRNA

<400> 20
cacaugcacg gccacauuga 20

<210> 21
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向人类TTR的 (外显子 2)的CR003351 gRNA

<400> 21
agccuuucug aacacaugca 20

<210> 22
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向人类TTR的 (外显子 2)的CR003352 gRNA

<400> 22
gaaaggcugc ugaugacacc 20

<210> 23
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003353 gRNA

<400> 23
aaaggcugcu gaugacaccu 20

<210> 24
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向人类TTR的 (外显子 2)的CR003354 gRNA

<400> 24
accugggagc cauuugccuc 20

<210> 25
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003355 gRNA

[0009] <400> 25
cccagaggca aauggcuccc 20

<210> 26
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003356 gRNA

<400> 26
gcaacuuacc cagaggcaaa 20

<210> 27
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 2)的CR003357 gRNA

<400> 27
uucuuuggca acuuacccag 20

<210> 28
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003358 gRNA

<400> 28
augcagcucu ccagacucac 20

<210> 29
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003359 gRNA

<400> 29
agugagucug gagagcugca 20

<210> 30
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003360 gRNA

<400> 30
gugagucugg agagcugcau 20

<210> 31
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003361 gRNA

<400> 31
gcugcauggg cucacaacug 20

[0010]

<210> 32
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003362 gRNA

<400> 32
gcaugggcuc acaacugagg 20

<210> 33
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003363 gRNA

<400> 33
acugaggagg aauuuguaga 20

<210> 34
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003364 gRNA

<400> 34
cugaggagga auuuguagaa 20

<210> 35

<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003365 gRNA

<400> 35
uguagaaggg auauacaaag 20

<210> 36
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003366 gRNA

<400> 36
aaauagacac caaaucuuac 20

<210> 37
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003367 gRNA

<400> 37
agacaccaaa ucuuacugga 20

[0011]

<210> 38
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003368 gRNA

<400> 38
aagugccuuc caguaagauu 20

<210> 39
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003369 gRNA

<400> 39
cucugcaugc ucauggaaug 20

<210> 40
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 3)的CR003370 gRNA

<400> 40
ccucugcaug cucauggaau 20

<210> 41
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 3)的CR003371 gRNA

<400> 41
accucugcau gcucauggaa 20

<210> 42
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 3)的CR003372 gRNA

<400> 42
uacucaccuc ugcaugcuca 20

<210> 43
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003373 gRNA

<400> 43
guauucacag ccaacgacuc 20

<210> 44
<211> 20
<212> RNA
<213> 人工序列

[0012]

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003374 gRNA

<400> 44
gcggcggggg ccggagucgu 20

<210> 45
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003375 gRNA

<400> 45
aaugguguag cggcgggggc 20

<210> 46
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003376 gRNA

<400> 46
cggcaauggu guagcggcgg 20

<210> 47
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003377 gRNA

<400> 47
gcggcaaugg uguagcggcg 20

<210> 48
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003378 gRNA

<400> 48
ggcggcaaug guguagcggc 20

<210> 49
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003379 gRNA

<400> 49
gggcggcaau gguguagcgg 20

<210> 50
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003380 gRNA

[0013]
<400> 50
gcagggcggc aaugguguag 20

<210> 51
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003381 gRNA

<400> 51
ggggcucagc agggcggcaa 20

<210> 52
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003382 gRNA

<400> 52
ggaguagggg cucagcaggg 20

<210> 53
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003383 gRNA

<400> 53
auaggaguag gggcucagca 20

<210> 54
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003384 gRNA

<400> 54
aauaggagua ggggcucagc 20

<210> 55
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003385 gRNA

<400> 55
ccccuacucc uauuccacca 20

<210> 56
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003386 gRNA

<400> 56
ccguggugga auaggaguag 20

[0014]

<210> 57
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003387 gRNA

<400> 57
gccguggugg aauaggagua 20

<210> 58
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003388 gRNA

<400> 58
gacgacagcc gugguggaau 20

<210> 59
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的 (外显子 4)的CR003389 gRNA

<400> 59
auuggugacg acagccgugg 20

<210> 60

<210> 20
<211> RNA
<212> 人工序列
<213>

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003390 gRNA

<400> 60
gggauuggug acgacagccg 20

<210> 61
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003391 gRNA

<400> 61
ggcugucguc accaauccca 20

<210> 62
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR003392 gRNA

<400> 62
agucccucau uccuugggau 20

[0015]

<210> 63
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 1)的CR005298 gRNA

<400> 63
uccacucauu cuuggcagga 20

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

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR005299 gRNA

<400> 64
agccguggug gaauaggagu 20

<210> 65
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 1)的CR005300 gRNA

<400> 65
ucacagaaac acucaccgua 20

<210> 66
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 1)的CR005301 gRNA

<400> 66
gucacagaaa cacucaccgu 20

<210> 67
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 2)的CR005302 gRNA

<400> 67
acgugucuuc ucuacaccca 20

<210> 68
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 2)的CR005303 gRNA

<400> 68
ugaauccaag uguccucuga 20

<210> 69
<211> 20
<212> RNA
<213> 人工序列

[0016]

<220>
<223> 合成的：靶向人类TTR的（外显子 2)的CR005304 gRNA

<400> 69
ggccgugcau guguucagaa 20

<210> 70
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 3)的CR005305 gRNA

<400> 70
uauaggaaaa ccagugaguc 20

<210> 71
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 3)的CR005306 gRNA

<400> 71
aaaucuuacu ggaaggcacu 20

<210> 72
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的：靶向人类TTR的（外显子 4)的CR005307 gRNA

<400> 72
ugucugucuu cucucauagg 20

<210> 73
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR000689 gRNA

<400> 73
acacaaauac caguccagcg 20

<210> 74
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005364 gRNA

<400> 74
aaaggcugcu gaugagaccu 20

<210> 75
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005365 gRNA

[0017] <400> 75
cauugacagc aggacugccu 20

<210> 76
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005366 gRNA

<400> 76
auaccagucc agcgaggcag 20

<210> 77
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005367 gRNA

<400> 77
ccaguccagc gaggcagagg 20

<210> 78
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005368 gRNA

<400> 78
ccuccucugc cucgcuggac 20

<210> 79
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005369 gRNA

<400> 79
aaaguucuag augccguccg 20

<210> 80
<211> 20
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的CR005370 gRNA

<400> 80
acuugucuuc ucuauaccca 20

<210> 81
<211> 20
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<213> 人工序列

<220>
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<400> 81
aagugacuuc caguaagauu 20

[0018]

<210> 82
<211> 20
<212> RNA
<213> 人工序列

<220>
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<400> 82
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<210> 83

<400> 83
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<210> 84

<400> 84
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<210> 85

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<400> 86
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<210> 87
<211> 100
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<213> 人工序列

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<223> PS 键，2'-O-Me 核苷酸

<400> 87
aaaggcugcu gaugacaccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 88
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<212> RNA
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<220>
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[0019]

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<400> 88
ucuagaacuu ugaccaucag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 89
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<213> 人工序列

<220>
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<220>

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<400> 89
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 90
<211> 100
<212> RNA
<213> 人工序列

<220>
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 91
<211> 100
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<213> 人工序列

<220>
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<400> 91
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 92
<211> 100
<212> RNA
<213> 人工序列

<220>
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<220>
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<223> PS 键, 2'-O-Me 核苷酸

<220>
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<220>
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<220>
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<223> PS 键, 2'-O-Me 核苷酸

<400> 92
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 93
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<220>
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<400> 93
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<212> RNA
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<220>
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[0022] <223> 2'-O-Me 核苷酸

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<223> PS 键, 2'-O-Me 核苷酸

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<220>
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 96
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的G000489 sgRNA修饰的序列

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[0023]

<400> 96
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<212> RNA
<213> 人工序列

<220>
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<400> 97

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 98
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<220>
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<400> 98
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[0024]

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<220>
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<400> 99
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<400> 100
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

[0025]

<210> 101
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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[0026]

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<400> 103
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<213> 人工序列

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<223> PS 键, 2'-O-Me 核苷酸

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<400> 105
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 106
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<223> PS 键, 2'-O-Me 核苷酸

<400> 106
ugaauccaag uguccucuga guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 107
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<223> PS 键, 2'-O-Me 核苷酸

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<223> PS 键, 2'-O-Me 核苷酸

<220>
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<220>

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<400> 108
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 109
<211> 100
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<220>
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<223> PS 键, 2'-O-Me 核苷酸

<400> 109
gaaaggcugc ugaugacacc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 110
<211> 100
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<223> PS 键, 2'-O-Me 核苷酸

<400> 110
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 111
<211> 100
<212> RNA
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<220>
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<220>
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<400> 111
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 112
<211> 100
<212> RNA
<213> 人工序列

<220>
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<223> PS 键, 2'-O-Me 核苷酸

<400> 112

gucacagaaa cacucaccgu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 113
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向人类TTR的G000572 sgRNA修饰的序列

<220>
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<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 113
ccccuacucc uauuccacca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

[0031]

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 114
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000502 sgRNA修饰的序列

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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>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 114
acacaaauac caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 115
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000503 sgRNA修饰的序列

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<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
<221> modified_base
<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>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 115
aaaaggcugc ugaugagacc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

[0032]

<210> 116
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000504 sgRNA修饰的序列

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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>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 116
aaaggcugcu gaugagaccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 117
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000505 sgRNA修饰的序列

<220>
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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>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 117
cauugacagc aggacugccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 118
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000506 sgRNA修饰的序列

[0033]

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
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<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 118
auaccagucc agcgaggcag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 119
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000507 sgRNA修饰的序列

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<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>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 119
ccaguccagc gaggcagagg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 120
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000508 sgRNA修饰的序列

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<222> (1)..(3)
[0034] <223> PS 键, 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 120
ccuccucugc cucgcuggac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 121
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000509 sgRNA修饰的序列

<220>
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<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>

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<221> modified_base
<222> (29)..(40)
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<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 121
aaaguucuag augccguccg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 122
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000510 sgRNA修饰的序列

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<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
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<222> (29)..(40)
[0035] <223> 2'-O-Me 核苷酸

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<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 122
acuugucuuc ucuauaccca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 123
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向食蟹猴TTR的G000511 sgRNA修饰的序列

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<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

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<220>
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<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 123
aagugacuuc caguaagauu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 124
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向小鼠TTR的G000282 sgRNA修饰的序列

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

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<221> modified_base
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<220>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 124
uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<210> 198

<400> 198
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<210> 199

<400> 199
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<210> 200

<400> 200
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<210> 201
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表3中所列示最小尿苷密码子的胸苷类似物的Cas9的DNA编码序列，具有起始及终止密码子

<400> 201
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

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

[0042] gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

cacgaacaca tcgcaaacct ggcaggaagc ccggcaatca agaagggaat cctgcagaca 2220

gtcaaggtcg tcgacgaact ggtcaaggtc atgggaagac acaagccgga aaacatcgtc 2280

atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag cagaatgaac 2820

acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcccga agaagaagag aaaggtctag 4140

[0043]

<210> 202
<211> 4143
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用在人类中通常高度表达的密码子的Cas9的DNA编码序列

<400> 202
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atcacggatg aatacaaagt gccgtccaag aagttcaagg tcctggggaa caccgataga 120

cacagcatca agaaaaatct catcggagcc ctgctgtttg actccggcga aaccgcagaa 180

gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa tcgcatctgc 240

tatctgcaag agatcttttc gaacgaaatg gcaaaggtcg acgacagctt cttccaccgc 300

ctggaagaat ctttcctggt ggaggaggac aagaagcatg aacggcatcc tatctttgga 360

aacatcgtcg acgaagtggc gtaccacgaa aagtacccga ccatctacca tctgcggaag 420

aagttggttg actcaactga caaggccgac ctcagattga tctacttggc cctcgcccat 480

atgatcaaat tccgcggaca cttcctgatc gaaggcgatc tgaaccctga taactccgac 540

gtggataagc ttttcattca actggtgcag acctacaacc aactgttcga agaaaaccca 600

atcaatgcta gcggcgtcga tgccaaggcc atcctgtccg cccggctgtc gaagtcgcgg 660

cgcctcgaaa acctgatcgc acagctgccg ggagagaaaa agaacggact tttcggcaac 720

ttgatcgctc tctcactggg actcactccc aatttcaagt ccaattttga cctggccgag 780

gacgcgaagc tgcaactctc aaaggacacc tacgacgacg acttggacaa tttgctggca 840

caaattggcg atcagtacgc ggatctgttc cttgccgcta agaacctttc ggacgcaatc 900

ttgctgtccg atatcctgcg cgtgaacacc gaaataacca aagcgccgct tagcgcctcg 960

atgattaagc ggtacgacga gcatcaccag gatctcacgc tgctcaaagc gctcgtgaga 1020

cagcaactgc ctgaaaagta caaggagatc ttcttcgacc agtccaagaa tgggtacgca 1080

gggtacatcg atggaggcgc tagccaggaa gagttctata agttcatcaa gccaatcctg 1140

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gttgtggata agggagcttc ggcacaaagc ttcatcgaac gaatgaccaa cttcgacaag 1500

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ccgatcagag aacaggccga gaacattatc cacttgttca ccctgactaa cctgggagcc 3960

ccagccgcct tcaagtactt cgatactact atcgatcgca aaagatacac gtccaccaag 4020

gaagttctgg acgcgaccct gatccaccaa agcatcactg gactctacga aactaggatc 4080

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tga 4143

<210> 203
<211> 1379
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有一个核定位信号(1xNLS)作为C-末端7个氨基酸的Cas9的氨基酸序列

<400> 203

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
```

[0046]

```
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
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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
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

[0047] 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

[0048] 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

[0049] 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

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

[0050] Gly Gly Gly Ser Pro Lys Lys Lys Arg Lys Val
1370 1375

<210> 204
<211> 4140
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的Cas9 mRNA ORF，具有起始及终止密码子

<400> 204
auggacaaga aguacagcau cggacuggac aucggaacaa acagcgucgg augggcaguc 60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120

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

[0051]

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cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900
cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960
augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020
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ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140
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gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500
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aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

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gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

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 agacggagga ggaagcccga agaagaagag aaaggucuag 4140

[0052]

<210> 205
<211> 4143
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用在人类中通常高度表达的密码子的Cas9 mRNA ORF，具有起始及终止密码子

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aucacggaug aauacaaagu gccguccaag aaguucaagg uccuggggaa caccgauaga 120

cacagcauca agaaaaaucu caucggagcc cugcuguuug acuccggcga aaccgcagaa 180

gcgacccggc ucaaacguac cgcgaggcga cgcuacaccc ggcggaagaa ucgcaucugc 240

uaucugcaag agaucuuuuc gaacgaaaug gcaaaggucg acgacagcuu cuuccaccgc 300

cuggaagaau cuuuccuggu ggaggaggac aagaagcaug aacggcaucc uaucuuugga 360

aacaucgucg acgaaguggc guaccacgaa aaguacccga ccaucuacca ucugcggaag 420

aaguugguug acucaacuga caaggccgac cucagauuga ucuacuuggc ccucgcccau 480

augaucaaau uccgcggaca cuuccugauc gaaggcgauc ugaacccuga uaacuccgac 540

guggauaagc uuuucauuca acuggugcag accuacaacc aacuguucga agaaaaccca 600

aucaaugcua gcggcgucga ugccaaggcc auccuguccg cccggcuguc gaagucgcgg 660

cgccucgaaa accugaucgc acagcugccg ggagagaaaa agaacggacu uuucggcaac 720

uugaucgcuc ucucacuggg acucacuccc aauuucaagu ccaauuuuga ccuggccgag 780

gacgcgaagc ugcaacucuc aaaggacacc uacgacgacg acuuggacaa uuugcuggca 840

[0053]

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gcaacuguuc gcaaagugcu cucuaugccg caagucaaua uugugaagaa aaccgaagug     3300

caaaccggcg gauuuucaaa ggaaucgauc cucccaaaga gaaauagcga caagcucauu     3360

gcacgcaaga aagacuggga cccgaagaag uacggaggau ucgauucgcc gacugucgca     3420

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gaaguucugg acgcgacccu gauccaccaa agcaucacug gacucuacga aacuaggauc     4080

gaucugucgc agcugggugg cgauggcggu ggaucuccga aaaagaagag aaagguguaa     4140

uga                                                                   4143
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[0054]

<210> 206
<211> 1379
<212> PRT
<213> 人工序列

<220>
<223> 合成的: Cas9切口酶(D10A)氨基酸序列

<400> 206

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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
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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

[0055] 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

[0056] 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

[0057] 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

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

[0058] 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 Pro Lys Lys Lys Arg Lys Val
1370 1375

<210> 207
<211> 4140
<212> RNA
<213> 人工序列

<220>
<223> 合成的: Cas9切口酶(D10A) mRNA ORF

<400> 207

[0059]

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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

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

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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agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

[0060] augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

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aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

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uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

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aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

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 agacggagga ggaagcccga agaagaagag aaaggucuag 4140

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<211> 1379
<212> PRT
<213> 人工序列

<220>
<223> 合成的: dCas9 (D10A H840A)氨基酸序列

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[0061] 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

[0062] 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

[0063] 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

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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
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Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
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[0064]

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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
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Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
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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
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Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
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Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
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Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
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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
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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
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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

[0065]

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

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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1355 1360 1365

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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
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gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600
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[0067]

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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

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uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

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[0069]

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cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

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acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

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cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

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<212> RNA
<213> 人工序列

<220>
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[0072]

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cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480
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[0073]

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<212> PRT
<213> 人工序列

<220>
<223> 合成的：Cas9的氨基酸序列(无NLS)

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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

[0074]

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

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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
    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
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[0075]

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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
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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

[0076] 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

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     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

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140
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[0077]

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

[0078]

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

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<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的编码SEQ ID NO：213的Cas9 mRNA ORF，具有起始及终止密码子

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

[0079]

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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cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

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aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

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cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

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ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> 215
<211> 4101
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的编码SEQ ID NO：213的Cas9编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)

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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

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aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

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cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

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cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

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gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

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cugagccagc ugggaggaga c 4101

<210> 216
<211> 1368
<212> PRT
<213> 人工序列

<220>
<223> 合成的: Cas9切口酶的氨基酸序列(无NLS)

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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
```

[0083]

```
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
```

[0084]

```
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

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

[0085] 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

[0086] 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

[0087] Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

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<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO：216的Cas9切口酶mRNA ORF，具有起始及终止密码子

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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

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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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cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

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uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

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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

[0089] gaccugagcc agcugggagg agacuag 4107

<210> 218
<211> 4101
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO：216的Cas9切口酶编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)

<400> 218
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc 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

[0090]

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 auggggaaga 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

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 cgaccacauc 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 c 4101

[0091]

<210> 219
<211> 1368
<212> PRT
<213> 人工序列

<220>
<223> 合成的: dCas9的氨基酸序列(无NLS)

<400> 219

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala 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

[0092] 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

[0093] 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

[0094] 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

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

[0095] 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> 220
<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO：219的dCas9 mRNA ORF，具有起始及终止密码子

[0096]

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aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120
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
augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020
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
gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320
gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

[0097] gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

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 agacuag 4107

〈210〉 221
〈211〉 4113
〈212〉 RNA
〈213〉 人工序列

〈220〉
〈223〉 合成的: 使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO: 219的dCas9编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)

〈400〉 221
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

[0098] cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

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gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

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gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

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aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

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<210> 222
<211> 1392
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有两个核定位信号(2xNLS)作为C-末端氨基酸的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

[0100] 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

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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
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[0101]

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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
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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

[0102] 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

[0103] 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

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

[0104]

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> 223
<211> 4233
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 使用最小尿苷密码子的编码SEQ ID NO: 222的Cas9 mRNA ORF，具有起始及终止密码子

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[0105]

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gaccugagcc agcugggagg agacggagga ggaagcccga agaagaagag aaaggucccg 4140

aagaagaaga gaaaggucgg aagcggaagc ccgaagaaga agagaaaggu cgacggaagc 4200

ccgaagaaga agagaaaggu cgacagcgga uag 4233

<210> 224
<211> 4227
<212> RNA
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<220>
<223> 合成的: 使用最小尿苷密码子的编码SEQ ID NO: 222的Cas9编码序列(无起始或终止密码子; 适于纳入融合蛋白编码序列中)

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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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aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

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aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

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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

[0108]

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aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860
acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920
cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980
cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040
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cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160
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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 cggaggagga agcccgaaga agaagagaaa ggucccgaag 4140

aagaagagaa aggucggaag cggaagcccg aagaagaaga gaaaggucga cggaagcccg 4200

aagaagaaga gaaaggucga cagcgga 4227

<210> 225
<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-末端氨基酸的Cas9切口酶的氨基酸序列

<400> 225

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala 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

[0109]

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

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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
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[0110]

```
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
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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

[0111] 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

[0112] 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

[0113] 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> 226
<211> 4179
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列示的最小尿苷密码子的编码SEQ ID NO：25的Cas9切口酶mRNA ORF，具有起始及终止密码子

<400> 226

[0114]

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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
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aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560
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gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740
agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

[0115]

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caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980
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ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> 227
<211> 4173
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的编码SEQ ID NO：25的Cas9切口酶编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)

<400> 227

[0116]

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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

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

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cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

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<210> 228
<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
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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

[0118] 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

[0119] 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
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

[0120] 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

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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
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[0121]

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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
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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

[0122] 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

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Lys Lys Lys Arg Lys Val Asp Ser Gly
1385 1390

<210> 229
<211> 4179

<212> RNA
<213> 人工序列

<220>
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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180
gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240
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[0124]

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<210> 230
<211> 4173

〈212〉 RNA
〈213〉 人工序列

〈220〉
〈223〉 合成的：使用最小尿苷密码子的编码SEQ ID NO：228的dCas9编码序列(无起始或终止密码子；适于纳入融合蛋白编码序列中)

〈400〉 230

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acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

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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 auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

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cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

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gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

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<213> 人工序列

<220>
<223> 合成的: T7 启动子

<400> 231
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<210> 232
<211> 50
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(50)
<223> 人类β-珠蛋白 5 UTR

<400> 232
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<210> 233
<211> 132
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(132)
<223> 人类β-珠蛋白 3 UTR

[0127]

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taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gc 132

<210> 234
<211> 66
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(66)
<223> 人类α-珠蛋白 5 UTR

<400> 234
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cccacc 66

<210> 235
<211> 110
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(110)
<223> 人类α-珠蛋白 3 UTR

<400> 235
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ttcctgcacc cgtacccccg tggtctttga ataaagtctg agtgggcggc 110

<210> 236
<211> 29
<212> DNA
<213> 非洲爪蟾

<220>
<221> misc_feature
<222> (1)..(29)
<223> 非洲爪蟾β-珠蛋白 5 UTR

<400> 236
aagctcagaa taaacgctca actttggcc 29

<210> 237
<211> 130
<212> DNA
<213> 非洲爪蟾

<220>
<221> misc_feature
<222> (1)..(130)
<223> 非洲爪蟾β-珠蛋白 3 UTR

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caaaatgttg tcccccaaaa tgtagccatt cgtatctgct cctaataaaa agaaagtttc 120

ttcacattct 130

[0128]

<210> 238
<211> 27
<212> DNA
<213> 普通牛

<220>
<221> misc_feature
<222> (1)..(27)
<223> 牛生长激素 5 UTR

<400> 238
cagggtcctg tggacagctc accagct 27

<210> 239
<211> 102
<212> DNA
<213> 普通牛

<220>
<221> misc_feature
<222> (1)..(102)
<223> 牛生长激素 3 UTR

<400> 239
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tcccactgtc ctttcctaat aaaatgagga aattgcatcg ca 102

<210> 240
<211> 93
<212> DNA
<213> 小鼠

<220>
<221> misc_feature
<222> (1)..(93)
<223> 小鼠血红素α，成人链1 (Hba-a1)，3’UTR

<400> 240
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ctcttggtct ttgaataaag cctgagtagg aag 93

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<211> 61
<212> DNA
<213> 人工序列

<220>
<223> 合成的: HSD17B4 5 UTR

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c 61

<210> 242
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向小鼠TTR基因的G282单引导RNA

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<221> modified_base
<222> (1)..(3)
[0129] <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>
<221> modified_base
<222> (97)..(100)
<223> PS 键, 2’-O-Me 核苷酸

<400> 242
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 243

<400> 243
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<210> 244
<211> 4405
<212> DNA
<213> 人工序列

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<223> 合成的: 具有HSD的5’ UTR、对应于SEQ ID NO: 204的ORF及ALB的3’ UTR的Cas9转录物

<400> 244

[0130]

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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

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gttcggaaac ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga 840

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cctgctggca cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag 960

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gaaaatgaag atcaatagct tattcatctc ttttttcttt tcgttggtgt aaagccaaca 4320

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aataaaaaat ggaaagaacc tcgag 4405

<210> 245

<211> 4188
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有19.36% U含量的替代Cas9 ORF

<400> 245

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cacagcatca agaagaatct catcggagcc ctgctgtttg actccggcga aaccgcagaa 180

gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa tcgcatctgc 240

tatctgcaag aaatcttttc gaacgaaatg gcaaaggtgg acgacagctt cttccaccgc 300

ctggaagaat ctttcctggt ggaggaggac aagaagcatg aacggcatcc tatctttgga 360

aacatcgtgg acgaagtggc gtaccacgaa aagtacccga ccatctacca tctgcggaag 420

aagttggttg actcaactga caaggccgac ctcagattga tctacttggc cctcgcccat 480

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gtggataagc tgttcattca actggtgcag acctacaacc aactgttcga agaaaaccca 600

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ggccaaggtg gagaagggaa agagcaaaaa gctcaaatcc gtcaaagagc tgctggggat 3600

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cttcgatact actatcgatc gcaaaagata cacgtccacc aaggaagttc tggacgcgac 4140

cctgatccac caaagcatca ctggactcta cgaaactagg atcgatctgt cgcagctggg 4200

tggcgattga tagtctagcc atcacattta aaagcatctc agcctaccat gagaataaga 4260

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aattaataaa aaatggaaag aacctcgag 4409

<210> 250
<211> 4140
<212> DNA
<213> 人工序列

[0140] <220>
<223> 合成的：移除剪接点的Cas9 ORF；12.75% U含量

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cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccaccgg 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

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gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

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[0141]

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gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200
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gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

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<210> 251
<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：250的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

[0142]

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cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

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ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

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[0143]

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[0144]

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<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有通常频繁用于人类中的最小尿苷密码子的Cas9 ORF；12.75% U含量

<400> 252
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cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccaccagac tgaagagaac cgccagaaga agatacacca gaagaaagaa cagaatctgc 240

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[0145]

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cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagagagtg 3840

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[0146]

<210> 253
<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：252的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

<400> 253
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cgtgggctgg gccgtgatca ccgacgagta caaggtgccc agcaagaagt tcaaggtgct 180

gggcaacacc gacagacaca gcatcaagaa gaacctgatc ggcgccctgc tgttcgacag 240

cggcgagacc gccgaggcca ccagactgaa gagaaccgcc agaagaagat acaccagaag 300

aaagaacaga atctgctacc tgcaggagat cttcagcaac gagatggcca aggtggacga 360

cagcttcttc cacagactgg aggagagctt cctggtggag gaggacaaga agcacgagag 420

acaccccatc ttcggcaaca tcgtggacga ggtggcctac cacgagaagt accccaccat 480

ctaccacctg agaaagaagc tggtggacag caccgacaag gccgacctga gactgatcta 540

cctggccctg gcccacatga tcaagttcag aggccacttc ctgatcgagg gcgacctgaa 600

ccccgacaac agcgacgtgg acaagctgtt catccagctg gtgcagacct acaaccagct 660

gttcgaggag aaccccatca acgccagcgg cgtggacgcc aaggccatcc tgagcgccag 720

actgagcaag agcagaagac tggagaacct gatcgcccag ctgccccggcg agaagaagaa 780

cggcctgttc ggcaacctga tcgccctgag cctgggcctg acccccaact tcaagagcaa 840

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[0147]

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gtacgagacc agaatcgacc tgagccagct gggcggcgac ggcggcggca gccccaagaa 4200

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<210> 254
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有通常很少用于人类中的最小尿苷密码子的Cas9 ORF；12.75% U含量

<400> 254
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gcgacgcgac taaaacgaac ggcgcgacga cgatacacgc gacgaaaaaa ccgaatatgc 240

tacctacaag aaatattcag caacgaaatg gcgaaagtag acgacagctt cttccaccga 300

ctagaagaaa gcttcctagt agaagaagac aaaaaacacg aacgacaccc gatattcggg 360

aacatagtag acgaagtagc gtaccacgaa aaatacccga cgatatacca cctacgaaaa 420

aaactagtag acagcacgga caaagcggac ctacgactaa tatacctagc gctagcgcac 480

atgataaaat tccgagggca cttcctaata gaaggggacc taaacccgga caacagcgac 540

gtagacaaac tattcataca actagtacaa acgtacaacc aactattcga agaaaacccg 600

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[0149]

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<210> 255
<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: 254的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

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[0151]

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tcaattaata aaaaatggaa agaacctcga g 4411

<210> 256
<211> 4411
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有AGG作为前三个核苷酸用于CleanCapTM、HSD的5’ UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

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gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

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cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

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cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

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<210> 259
<211> 4325
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有XBG的5’ UTR、对应于SEQ ID NO：204的ORF、Kozak序列及XBG的3’ UTR的Cas9转录物

<400> 259

[0159]

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aggtcccgag caagaagttc aaggtcctgg gaaacacaga cagacacagc atcaagaaga 180

acctgatcgg agcactgctg ttcgacagcg gagaaacagc agaagcaaca agactgaaga 240

gaacagcaag aagaagatac acaagaagaa agaacagaat ctgctacctg caggaaatct 300

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[0160]

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tcgag 4325

<210> 260
<211> 4325
<212> DNA
<213> 人工序列

<220>
<223> 合成的: 具有AGG作为前三个核苷酸用于CleanCapTM、XBG的5' UTR、对应于SEQ ID NO: 204的ORF、Kozak序列及XBG的3' UTR的Cas9转录物

<400> 260

[0161]

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<210> 261
<211> 4411
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有AGG作为前三个核苷酸用于CleanCapTM、HSD的5’ UTR、对应于SEQ ID NO：204的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

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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> 262

<400> 262
000

<210> 263
<211> 93
<212> DNA
<213> 人工序列

<220>
<223> 合成的：多-A 100 序列

<400> 263
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

[0165]

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 93

<210> 264
<211> 44
<212> DNA
<213> 人工序列

<220>
<223> 合成的：靶向小鼠TTR基因的G209单引导RNA

<400> 264
aaataagaga gaaaagaaga gtaagaagaa atataagagc cacc 44

<210> 265
<211> 3312
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的编码脑膜炎双球菌Cas9的ORF，具有起始及终止密码子

<400> 265
atggcagcat tcaagccgaa ctcgatcaac tacatcctgg gactggacat cggaatcgca 60

tcggtcggat gggcaatggt cgaaatcgac gaagaagaaa acccgatcag actgatcgac 120

ctgggagtca gagtcttcga aagagcagaa gtcccgaaga caggagactc gctggcaatg 180

gcaagaagac tggcaagatc ggtcagaaga ctgacaagaa gaagagcaca cagactgctg 240

agaacaagaa gactgctgaa gagagaagga gtcctgcagg cagcaaactt cgacgaaaac 300

ggactgatca agtcgctgcc gaacacaccg tggcagctga gagcagcagc actggacaga 360

aagctgacac cgctggaatg gtcggcagtc ctgctgcacc tgatcaagca cagaggatac 420

ctgtcgcaga gaaagaacga aggagaaaca gcagacaagg aactgggagc actgctgaag 480

ggagtcgcag gaaacgcaca cgcactgcag acaggagact tcagaacacc ggcagaactg 540

gcactgaaca agttcgaaaa ggaatcggga cacatcagaa accagagatc ggactactcg 600

cacacattct cgagaaagga cctgcaggca gaactgatcc tgctgttcga aaagcagaag 660

gaattcggaa acccgcacgt ctcgggagga ctgaaggaag gaatcgaaac actgctgatg 720

acacagagac cggcactgtc gggagacgca gtccagaaga tgctgggaca ctgcacattc 780

gaaccggcag aaccgaaggc agcaaagaac acatacacag cagaaagatt catctggctg 840

acaaagctga acaacctgag aatcctggaa cagggatcgg aaagaccgct gacagacaca 900

gaaagagcaa cactgatgga cgaaccgtac agaaagtcga agctgacata cgcacaggca 960

agaaagctgc tgggactgga agacacagca ttcttcaagg gactgagata cggaaaggac 1020

aacgcagaag catcgacact gatggaaatg aaggcatacc acgcaatctc gagagcactg 1080

gaaaaggaag gactgaagga caagaagtcg ccgctgaacc tgtcgccgga actgcaggac 1140

gaaatcggaa cagcattctc gctgttcaag acagacgaag acatcacagg aagactgaag 1200

gacagaatcc agccggaaat cctggaagca ctgctgaagc acatctcgtt cgacaagttc 1260

gtccagatct cgctgaaggc actgagaaga atcgtcccgc tgatggaaca gggaaagaga 1320

tacgacgaag catgcgcaga aatctacgga gaccactacg gaaagaagaa cacagaagaa 1380

aagatctacc tgccgccgat cccggcagac gaaatcagaa acccggtcgt cctgagagca 1440

ctgtcgcagg caagaaaggt catcaacgga gtcgtcagaa gatacggatc gccggcaaga 1500

[0166] atccacatcg aaacagcaag agaagtcgga aagtcgttca aggacagaaa ggaaatcgaa 1560

aagagacagg aagaaaacag aaaggacaga gaaaaggcag cagcaaagtt cagagaatac 1620

ttcccgaact tcgtcggaga accgaagtcg aaggacatcc tgaagctgag actgtacgaa 1680

cagcagcacg gaaagtgcct gtactcggga aaggaaatca acctgggaag actgaacgaa 1740

aagggatacg tcgaaatcga ccacgcactg ccgttctcga gaacatggga cgactcgttc 1800

aacaacaagg tcctggtcct gggatcggaa aaccagaaca agggaaacca gacaccgtac 1860

gaatacttca acggaaagga caactcgaga gaatggcagg aattcaaggc aagagtcgaa 1920

acatcgagat tcccgagatc gaagaagcag agaatcctgc tgcagaagtt cgacgaagac 1980

ggattcaagg aaagaaacct gaacgacaca agatacgtca acagattcct gtgccagttc 2040

gtcgcagaca gaatgagact gacaggaaag ggaaagaaga gagtcttcgc atcgaacgga 2100

cagatcacaa acctgctgag aggattctgg ggactgagaa aggtcagagc agaaaacgac 2160

agacaccacg cactggacgc agtcgtcgtc gcatgctcga cagtcgcaat gcagcagaag 2220

atcacaagat tcgtcagata caaggaaatg aacgcattcg acggaaagac aatcgacaag 2280

gaaacaggag aagtcctgca ccagaagaca cacttcccgc agccgtggga attcttcgca 2340

caggaagtca tgatcagagt cttcggaaag ccggacggaa agccggaatt cgaagaagca 2400

gacacactgg aaaagctgag aacactgctg gcagaaaagc tgtcgtcgag accggaagca 2460

gtccacgaat acgtcacacc gctgttcgtc tcgagagcac cgaacagaaa gatgtcggga 2520

cagggacaca tggaaacagt caagtcggca aagagactgg acgaaggagt ctcggtcctg 2580

agagtcccgc tgacacagct gaagctgaag gacctggaaa agatggtcaa cagagaaaga 2640

gaaccgaagc tgtacgaagc actgaaggca agactggaag cacacaagga cgacccggca 2700

aaggcattcg cagaaccgtt ctacaagtac gacaaggcag gaaacagaac acagcaggtc 2760

aaggcagtca gagtcgaaca ggtccagaag acaggagtct gggtcagaaa ccacaacgga 2820

atcgcagaca acgcaacaat ggtcagagta gacgtcttcg aaaagggaga caagtactac 2880

ctggtcccga tctactcgtg gcaggtcgca aagggaatcc tgccggacag agcagtcgtc 2940

cagggaaagg acgaagaaga ctggcagctg atcgacgact cgttcaactt caagttctcg 3000

ctgcacccga acgacctggt cgaagtcatc acaaagaagg caagaatgtt cggatacttc 3060

gcatcgtgcc acagaggaac aggaaacatc aacatcagaa tccacgacct ggaccacaag 3120

atcggaaaga acggaatcct ggaaggaatc ggagtcaaga cagcactgtc gttccagaag 3180

taccagatcg acgaactggg aaaggaaatc agaccgtgca gactgaagaa gagaccgccg 3240

gtcagatccg gaaagagaac agcagacgga tcggaattcg aatcgccgaa gaagaagaga 3300

aaggtcgaat ga 3312

<210> 266
<211> 3306
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用最小尿苷密码子的编码脑膜炎双球菌Cas9的ORF（无起始或终止密码子；适于纳入融合蛋白编码序列中）

<400> 266

[0167]

gcagcattca agccgaactc gatcaactac atcctgggac tggacatcgg aatcgcatcg 60

gtcggatggg caatggtcga aatcgacgaa gaagaaaacc cgatcagact gatcgacctg 120

ggagtcagag tcttcgaaag agcagaagtc ccgaagacag gagactcgct ggcaatggca 180

agaagactgg caagatcggt cagaagactg acaagaagaa gagcacacag actgctgaga 240

acaagaagac tgctgaagag agaaggagtc ctgcaggcag caaacttcga cgaaaacgga 300

ctgatcaagt cgctgccgaa cacaccgtgg cagctgagag cagcagcact ggacagaaag 360

ctgacaccgc tggaatggtc ggcagtcctg ctgcacctga tcaagcacag aggatacctg 420

tcgcagagaa agaacgaagg agaaacagca gacaaggaac tgggagcact gctgaaggga 480

gtcgcaggaa acgcacacgc actgcagaca ggagacttca gaacaccggc agaactggca 540

ctgaacaagt tcgaaaagga atcgggacac atcagaaacc agagatcgga ctactcgcac 600

acattctcga gaaaggacct gcaggcagaa ctgatcctgc tgttcgaaaa gcagaaggaa 660

ttcggaaacc cgcacgtctc gggaggactg aaggaaggaa tcgaaacact gctgatgaca 720

cagagaccgg cactgtcggg agacgcagtc cagaagatgc tgggacactg cacattcgaa 780

ccggcagaac cgaaggcagc aaagaacaca tacacagcag aaagattcat ctggctgaca 840

aagctgaaca acctgagaat cctggaacag ggatcggaaa gaccgctgac agacacagaa 900

agagcaacac tgatggacga accgtacaga aagtcgaagc tgacatacgc acaggcaaga 960

aagctgctgg gactggaaga cacagcattc ttcaagggac tgagatacgg aaaggacaac 1020

gcagaagcat cgacactgat ggaaatgaag gcataccacg caatctcgag agcactggaa 1080

aaggaaggac tgaaggacaa gaagtcgccg ctgaacctgt cgccggaact gcaggacgaa 1140

atcggaacag cattctcgct gttcaagaca gacgaagaca tcacaggaag actgaaggac 1200

[0168]

agaatccagc cggaaatcct ggaagcactg ctgaagcaca tctcgttcga caagttcgtc 1260
cagatctcgc tgaaggcact gagaagaatc gtcccgctga tggaacaggg aaagagatac 1320
gacgaagcat gcgcagaaat ctacggagac cactacggaa agaagaacac agaagaaaag 1380
atctacctgc cgccgatccc ggcagacgaa atcagaaacc cggtcgtcct gagagcactg 1440
tcgcaggcaa gaaaggtcat caacggagtc gtcagaagat acggatcgcc ggcaagaatc 1500
cacatcgaaa cagcaagaga agtcggaaag tcgttcaagg acagaaagga aatcgaaaag 1560
agacaggaag aaaacagaaa ggacagagaa aaggcagcag caaagttcag agaatacttc 1620
ccgaacttcg tcggagaacc gaagtcgaag gacatcctga agctgagact gtacgaacag 1680
cagcacggaa agtgcctgta ctcgggaaag gaaatcaacc tgggaagact gaacgaaaag 1740
ggatacgtcg aaatcgacca cgcactgccg ttctcgagaa catgggacga ctcgttcaac 1800
aacaaggtcc tggtcctggg atcggaaaac cagaacaagg gaaaccagac accgtacgaa 1860
tacttcaacg gaaaggacaa ctcgagagaa tggcaggaat tcaaggcaag agtcgaaaca 1920
tcgagattcc cgagatcgaa gaagcagaga atcctgctgc agaagttcga cgaagacgga 1980
ttcaaggaaa gaaacctgaa cgacacaaga tacgtcaaca gattcctgtg ccagttcgtc 2040
gcagacagaa tgagactgac aggaaaggga aagaagagag tcttcgcatc gaacggacag 2100
atcacaaacc tgctgagagg attctgggga ctgagaaagg tcagagcaga aaacgacaga 2160
caccacgcac tggacgcagt cgtcgtcgca tgctcgacag tcgcaatgca gcagaagatc 2220
acaagattcg tcagatacaa ggaaatgaac gcattcgacg gaaagacaat cgacaaggaa 2280
acaggagaag tcctgcacca gaagacacac ttcccgcagc cgtgggaatt cttcgcacag 2340
gaagtcatga tcagagtctt cggaaagccg gacggaaagc cggaattcga agaagcagac 2400
acactggaaa agctgagaac actgctggca gaaaagctgt cgtcgagacc ggaagcagtc 2460
cacgaatacg tcacaccgct gttcgtctcg agagcaccga acagaaagat gtcgggacag 2520
ggacacatgg aaacagtcaa gtcggcaaag agactggacg aaggagtctc ggtcctgaga 2580
gtcccgctga cacagctgaa gctgaaggac ctggaaaaga tggtcaacag agaaagagaa 2640
ccgaagctgt acgaagcact gaaggcaaga ctggaagcac acaaggacga cccggcaaag 2700
gcattcgcag aaccgttcta caagtacgac aaggcaggaa acagaacaca gcaggtcaag 2760
gcagtcagag tcgaacaggt ccagaagaca ggagtctggg tcagaaacca caacggaatc 2820
gcagacaacg caacaatggt cagagtagac gtcttcgaaa agggagacaa gtactacctg 2880
gtcccgatct actcgtggca ggtcgcaaag ggaatcctgc cggacagagc agtcgtccag 2940
ggaaaggacg aagaagactg gcagctgatc gacgactcgt tcaacttcaa gttctcgctg 3000
cacccgaacg acctggtcga agtcatcaca aagaaggcaa gaatgttcgg atacttcgca 3060
tcgtgccaca gaggaacagg aaacatcaac atcagaatcc acgacctgga ccacaagatc 3120
ggaaagaacg gaatcctgga aggaatcgga gtcaagacag cactgtcgtt ccagaagtac 3180
cagatcgacg aactgggaaa ggaaatcaga ccgtgcagac tgaagaagag accgccggtc 3240
agatccggaa agagaacagc agacggatcg gaattcgaat cgccgaagaa gaagagaaag 3300
gtcgaa 3306

<210> 267
<211> 3636

<212> DNA
<213> 人工序列

<220>
<223> 合成的：包含SEQ ID NO：265（编码脑膜炎双球菌Cas9)的转录物

<400> 267

[0169]

gggagaccca agctggctag cgtttaaact taagcttgga tccgccacca tggcagcatt 60

caagccgaac tcgatcaact acatcctggg actggacatc ggaatcgcat cggtcggatg 120

ggcaatggtc gaaatcgacg aagaagaaaa cccgatcaga ctgatcgacc tgggagtcag 180

agtcttcgaa agagcagaag tcccgaagac aggagactcg ctggcaatgg caagaagact 240

ggcaagatcg gtcagaagac tgacaagaag aagagcacac agactgctga gaacaagaag 300

actgctgaag agagaaggag tcctgcaggc agcaaacttc gacgaaaacg gactgatcaa 360

gtcgctgccg aacacaccgt ggcagctgag agcagcagca ctggacagaa agctgacacc 420

gctggaatgg tcggcagtcc tgctgcacct gatcaagcac agaggatacc tgtcgcagag 480

aaagaacgaa ggagaaacag cagacaagga actgggagca ctgctgaagg gagtcgcagg 540

aaacgcacac gcactgcaga caggagactt cagaacaccg gcagaactgg cactgaacaa 600

gttcgaaaag gaatcgggac acatcagaaa ccagagatcg gactactcgc acacattctc 660

gagaaaggac ctgcaggcag aactgatcct gctgttcgaa aagcagaagg aattcggaaa 720

cccgcacgtc tcgggaggac tgaaggaagg aatcgaaaca ctgctgatga cacagagacc 780

ggcactgtcg ggagacgcag tccagaagat gctgggacac tgcacattcg aaccggcaga 840

accgaaggca gcaaagaaca catacacagc agaaagattc atctggctga caaagctgaa 900

caacctgaga atcctggaac agggatcgga aagaccgctg acagacacag aaagagcaac 960

actgatggac gaaccgtaca gaaagtcgaa gctgacatac gcacaggcaa gaaagctgct 1020

gggactggaa gacacagcat tcttcaaggg actgagatac ggaaaggaca acgcagaagc 1080

atcgacactg atggaaatga aggcatacca cgcaatctcg agagcactgg aaaaggaagg 1140

actgaaggac aagaagtcgc cgctgaacct gtcgccggaa ctgcaggacg aaatcggaac 1200

agcattctcg ctgttcaaga cagacgaaga catcacagga agactgaagg acagaatcca 1260

gccggaaatc ctggaagcac tgctgaagca catctcgttc gacaagttcg tccagatctc 1320

gctgaaggca ctgagaagaa tcgtcccgct gatggaacag ggaaagagat acgacgaagc 1380

atgcgcagaa atctacggag accactacgg aaagaagaac acagaagaaa agatctacct 1440

gccgccgatc ccggcagacg aaatcagaaa cccggtcgtc ctgagagcac tgtcgcaggc 1500

aagaaaggtc atcaacggag tcgtcagaag atacggatcg ccggcaagaa tccacatcga 1560

aacagcaaga gaagtcggaa agtcgttcaa ggacagaaag gaaatcgaaa agagacagga 1620

agaaaacaga aaggacagag aaaaggcagc agcaaagttc agagaatact tcccgaactt 1680

cgtcggagaa ccgaagtcga aggacatcct gaagctgaga ctgtacgaac agcagcacgg 1740

aaagtgcctg tactcgggaa aggaaatcaa cctgggaaga ctgaacgaaa agggatacgt 1800

cgaaatcgac cacgcactgc cgttctcgag aacatgggac gactcgttca acaacaaggt 1860

cctggtcctg ggatcggaaa accagaacaa gggaaaccag acaccgtacg aatacttcaa 1920

cggaaaggac aactcgagag aatggcagga attcaaggca agagtcgaaa catcgagatt 1980

cccgagatcg aagaagcaga gaatcctgct gcagaagttc gacgaagacg gattcaagga 2040

[0170]

aagaaacctg aacgacacaa gatacgtcaa cagattcctg tgccagttcg tcgcagacag 2100

aatgagactg acaggaaagg gaaagaagag agtcttcgca tcgaacggac agatcacaaa 2160

cctgctgaga ggattctggg gactgagaaa ggtcagagca gaaaacgaca gacaccacgc 2220

actggacgca gtcgtcgtcg catgctcgac agtcgcaatg cagcagaaga tcacaagatt 2280

cgtcagatac aaggaaatga acgcattcga cggaaagaca atcgacaagg aaacaggaga 2340

agtcctgcac cagaagacac acttcccgca gccgtgggaa ttcttcgcac aggaagtcat 2400

gatcagagtc ttcggaaagc cggacggaaa gccggaattc gaagaagcag acacactgga 2460

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cgtcacaccg ctgttcgtct cgagagcacc gaacagaaag atgtcgggac agggacacat 2580

ggaaacagtc aagtcggcaa agagactgga cgaaggagtc tcggtcctga gagtcccgct 2640

gacacagctg aagctgaagg acctggaaaa gatggtcaac agagaaagag aaccgaagct 2700

gtacgaagca ctgaaggcaa gactggaagc acacaaggac gacccggcaa aggcattcgc 2760

agaaccgttc tacaagtacg acaaggcagg aaacagaaca cagcaggtca aggcagtcag 2820

agtcgaacag gtccagaaga caggagtctg ggtcagaaac cacaacggaa tcgcagacaa 2880

cgcaacaatg gtcagagtag acgtcttcga aaagggagac aagtactacc tggtcccgat 2940

ctactcgtgg caggtcgcaa agggaatcct gccggacaga gcagtcgtcc agggaaagga 3000

cgaagaagac tggcagctga tcgacgactc gttcaacttc aagttctcgc tgcacccgaa 3060

cgacctggtc gaagtcatca caaagaaggc aagaatgttc ggatacttcg catcgtgcca 3120

cagaggaaca ggaaacatca acatcagaat ccacgacctg gaccacaaga tcggaaagaa 3180

cggaatcctg gaaggaatcg gagtcaagac agcactgtcg ttccagaagt accagatcga 3240

cgaactggga aaggaaatca gaccgtgcag actgaagaag agaccgccgg tcagatccgg 3300

aaagagaaca gcagacggat cggaattcga atcgccgaag aagaagagaa aggtcgaatg 3360

atagctagct cgagtctaga gggcccgttt aaacccgctg atcagcctcg actgtgcctt 3420

ctagttgcca gccatctgtt gtttgcccct cccccgtgcc ttccttgacc ctggaaggtg 3480

ccactcccac tgtcctttcc taataaaatg aggaaattgc atcgcattgt ctgagtaggt 3540

gtcattctat tctgggggggt ggggtggggc aggacagcaa gggggaggat tgggaagaca 3600

atagcaggca tgctggggat gcggtgggct ctatgg 3636

<210> 268
<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> 268

Met Ala Ala Phe Lys Pro Asn Ser Ile Asn Tyr Ile Leu Gly Leu Asp
1 5 10 15

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

[0171] 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

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

[0172] 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

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

[0173]

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

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

[0174] Gly Lys Arg Thr Ala Asp Gly Ser Glu Phe Glu Ser Pro Lys Lys
1085 1090 1095

Lys Arg Lys Val Glu
1100

<210> 269
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的:靶向大鼠TTR基因的G390单引导RNA

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 经修饰的碱基
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 269

gccgagucug gagagcugca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 270
<211> 74
<212> RNA
<213> 人工序列

<220>
<223> 合成的: trRNA

<400> 270
aacagcauag caaguuaaaa uaaggcuagu ccguuaucaa cuugaaaaag uggcaccgag 60

ucggugcuuu uuuu 74

<210> 271

<400> 271
000

<210> 272
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向大鼠TTR基因的G534单引导RNA

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

[0175]

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 经修饰的碱基
<222> (97)..(100)
<223> PS 键, 2'-O-Me 核苷酸

<400> 272
acgcaaauau caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 273
<211> 95
<212> RNA
<213> 人工序列

<220>
<223> 合成的: G000395 5' 截短的无活性sgRNA修饰的序列

<220>
<221> modified_base
<222> (1)..(3)
<223> PS 键, 2'-O-Me 核苷酸

<220>
<221> modified_base

<222> (24)..(35)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (64)..(91)
<223> 2'-O-Me 核苷酸

<220>
<221> modified_base
<222> (92)..(95)
<223> PS 键, 2'-O-Me 核苷酸

<400> 273
gcaauggugu agcggguuuu agagcuagaa auagcaaguu aaaauaaggc uaguccguua 60

ucaacuugaa aaaguggcac cgagucggug cuuuu 95

<210> 274
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的: SV40 NLS

<400> 274

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 275
<211> 7
<212> PRT
<213> 人工序列

[0176]

<220>
<223> 合成的: 替代 SV40 NLS

<400> 275

Pro Lys Lys Lys Arg Arg Val
1 5

<210> 276
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 核浆素 NLS

<400> 276

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> 277
<211> 10
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 示例性 Kozak 序列

<400> 277
gccrccaugg 10

<210> 278
<211> 13

<212> RNA
<213> 人工序列

[0177] <220>
<223> 合成的：示例性 Kozak 序列

<400> 278
gccgccrcca ugg 13

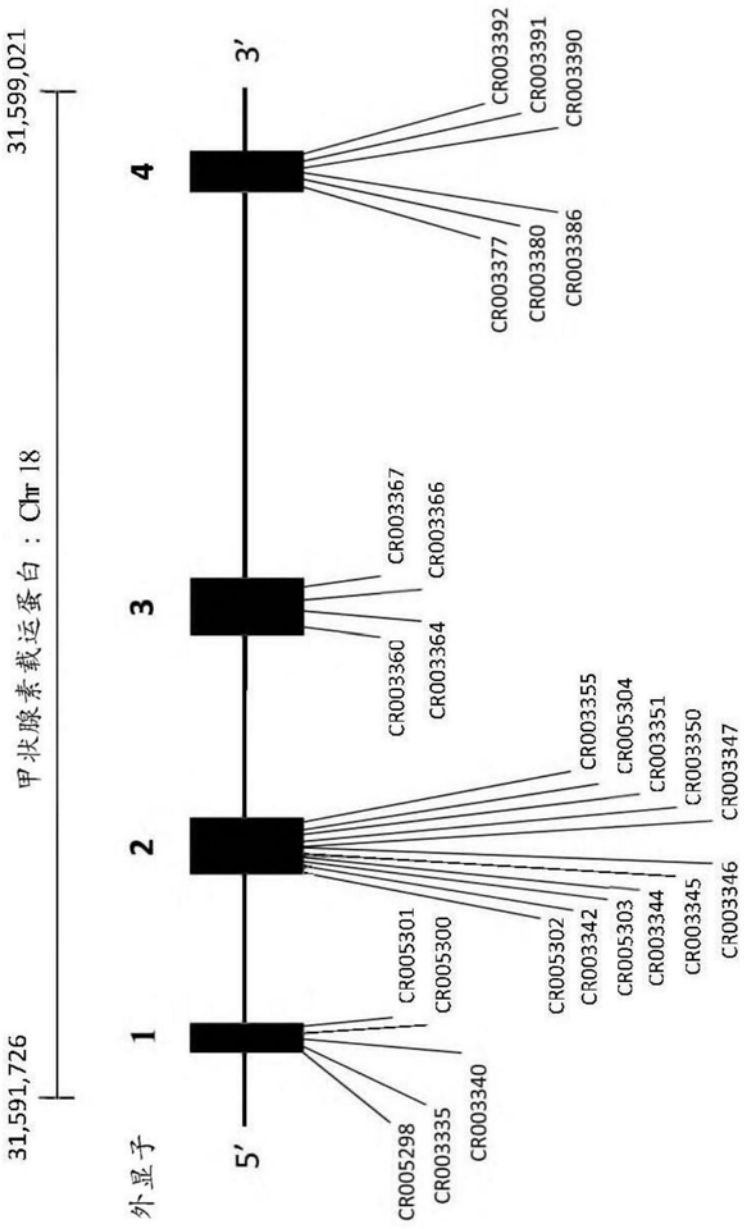

31,599,021
甲状腺素-载运蛋白：Chr 18
31,591,726
外显子
5′
3′
1
2
3
4
CR005298
CR003335
CR003340
CR005301
CR005300
CR005302
CR003342
CR005303
CR003344
CR003345
CR003346
CR003347
CR003350
CR003351
CR005304
CR003355
CR003360
CR003364
CR003367
CR003366
CR003377
CR003380
CR003386
CR003392
CR003391
CR003390

图1

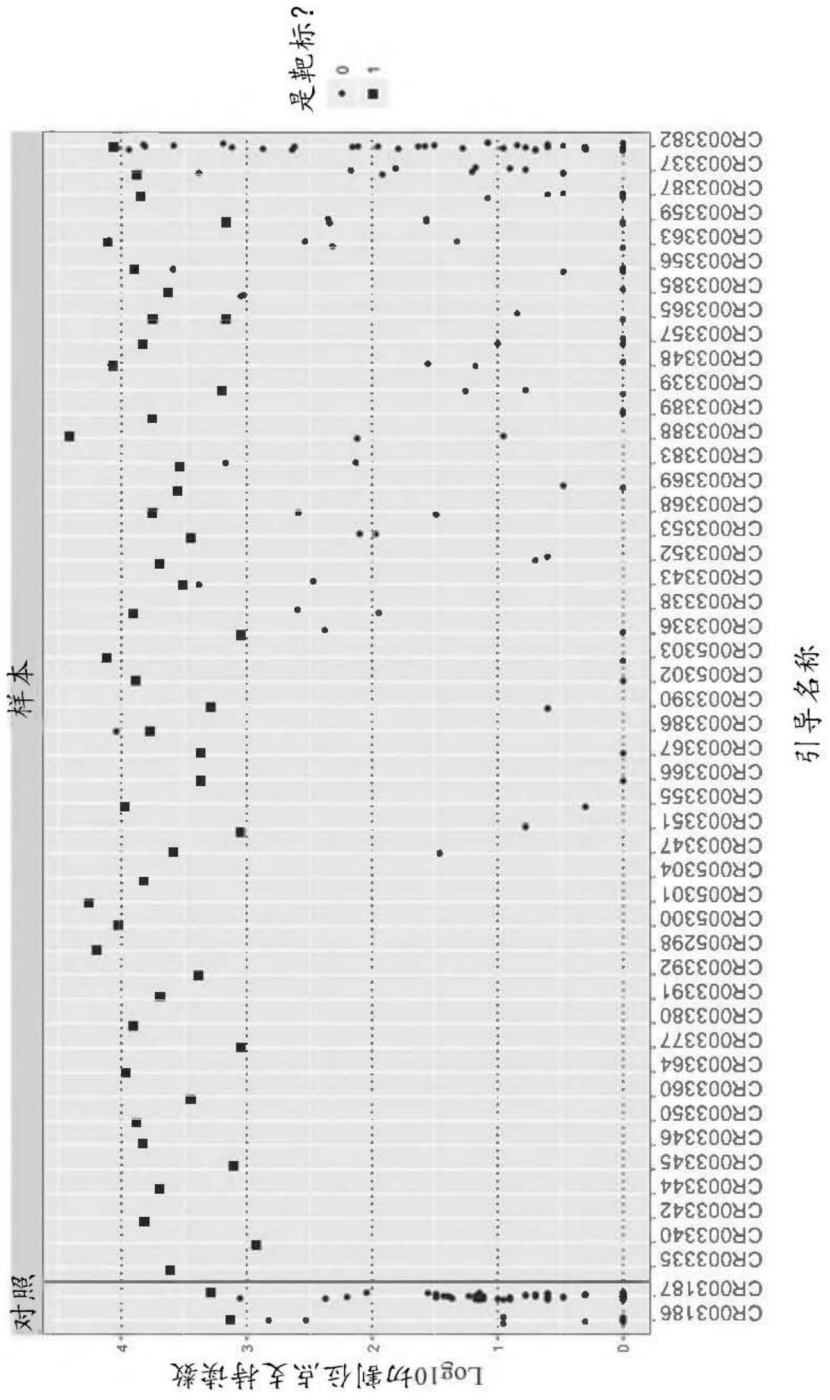

是靶标?
0
1
样本
对照
引导名称
Log10的割位点支持读数
0
1
2
3
4
CR003382
CR003337
CR003387
CR003359
CR003363
CR003356
CR003385
CR003365
CR003357
CR003348
CR003339
CR003389
CR003388
CR003383
CR003369
CR003368
CR003353
CR003352
CR003343
CR003338
CR003336
CR005303
CR005302
CR003390
CR003386
CR003367
CR003366
CR003355
CR003351
CR003347
CR005304
CR005301
CR005300
CR005298
CR003392
CR003391
CR003380
CR003377
CR003364
CR003360
CR003350
CR003346
CR003345
CR003344
CR003342
CR003340
CR003335
CR003187
CR003186

图2

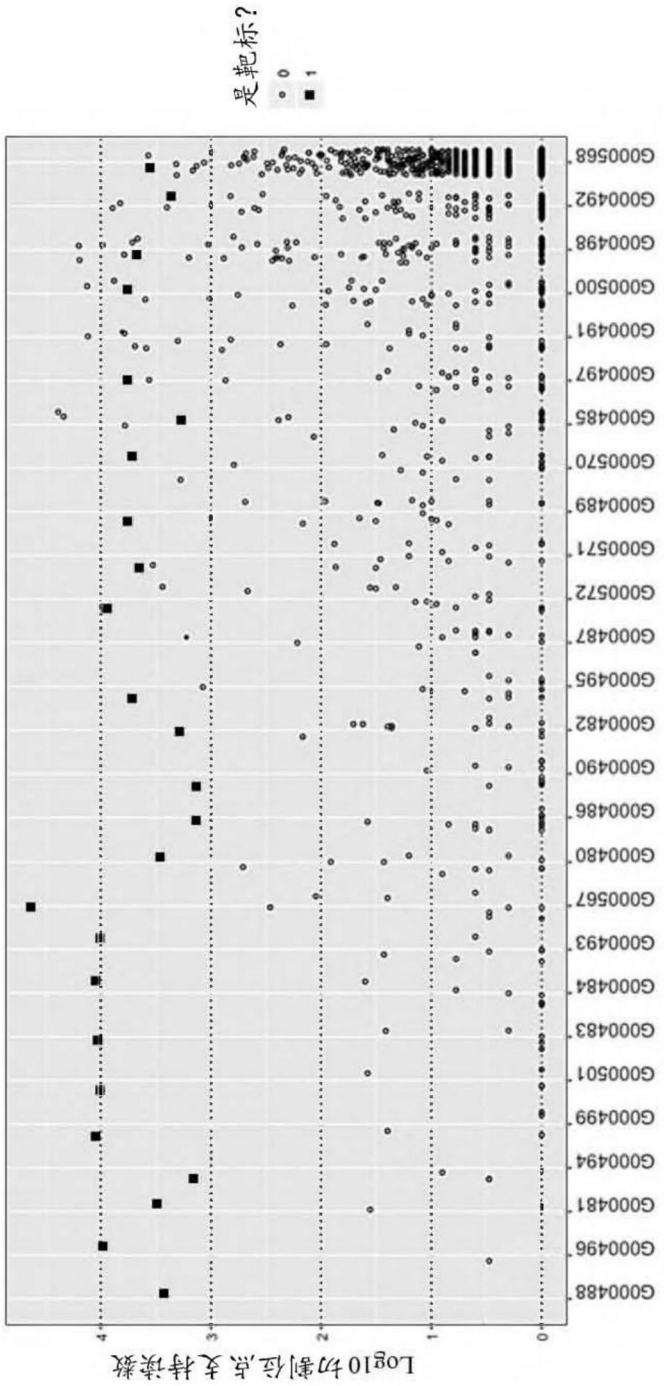

是靶标?
0
1
G000568
G000492
G000498
G000500
G000491
G000497
G000485
G000570
G000489
G000571
G000572
G000487
G000495
G000482
G000490
G000486
G000480
G000567
G000493
G000484
G000483
G000501
G000499
G000494
G000481
G000496
G000488
4
3
2
1
0
Log10 切割位点支持读数

图3

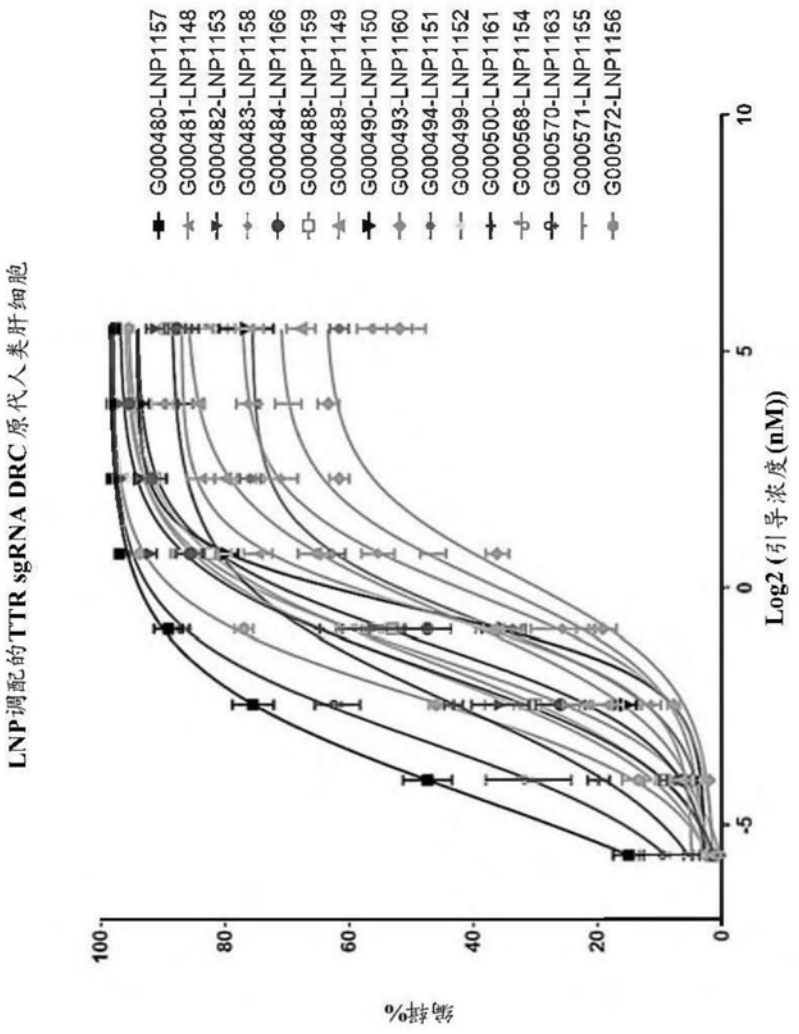

LNP调配的TTR sgRNA DRC 原代人类肝细胞
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
100
80
60
40
20
0
-5
0
5
10
Log2 (引导浓度(nM))
编辑%

图4

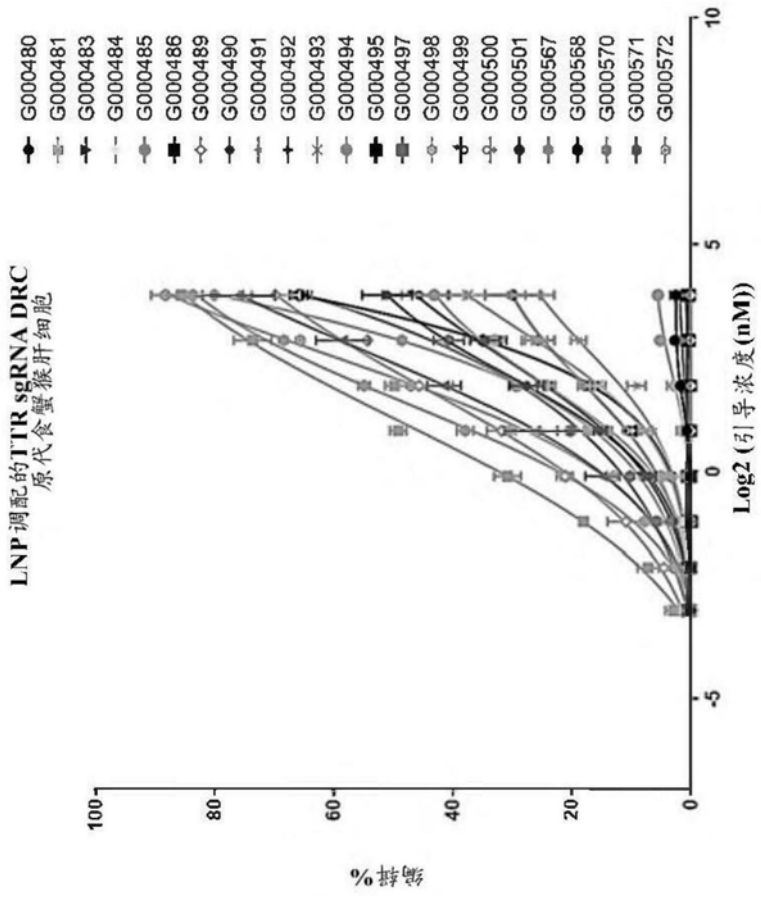

LNP调配的TTR sgRNA DRC
原代食蟹猴肝细胞
G000480
G000481
G000483
G000484
G000485
G000486
G000489
G000490
G000491
G000492
G000493
G000494
G000495
G000497
G000498
G000499
G000500
G000501
G000567
G000568
G000570
G000571
G000572
Log2 (引导浓度(nM))
10
5
0
-5
编辑%
100
80
60
40
20
0

图5

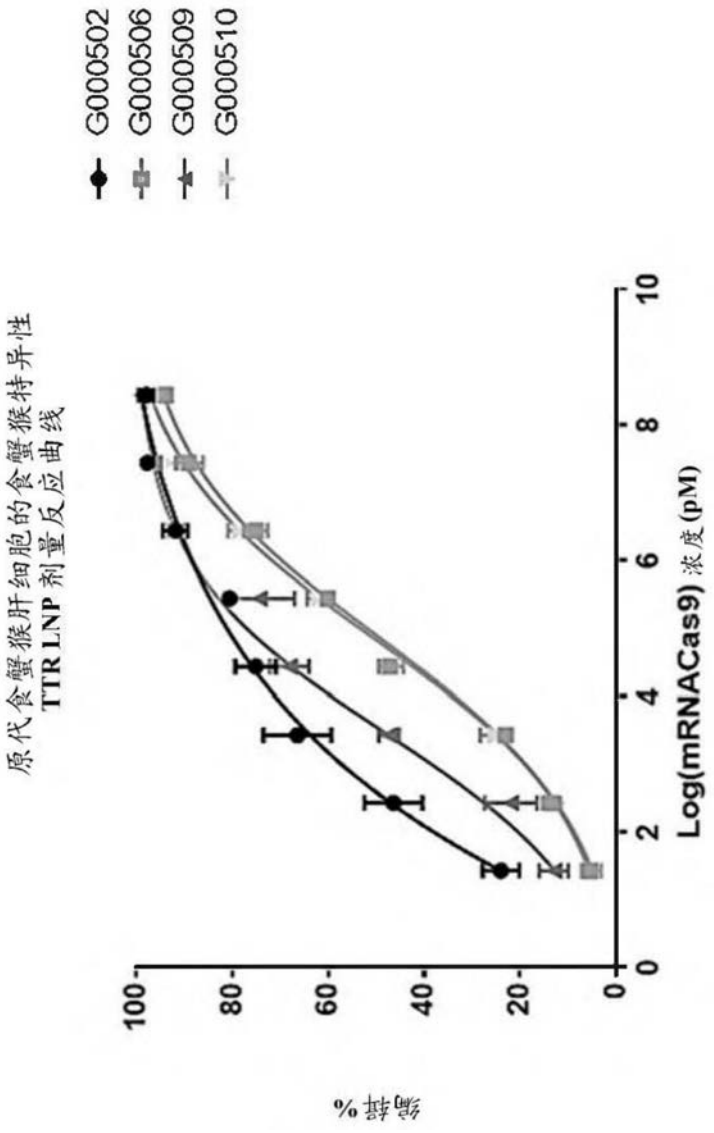

原代食蟹猴肝细胞的食蟹猴特异性
TTR LNP 剂量反应曲线
G000502
G000506
G000509
G000510
编辑%
100
80
60
40
20
0
0
2
4
6
8
10
Log(mRNACas9) 浓度(pM)

图6

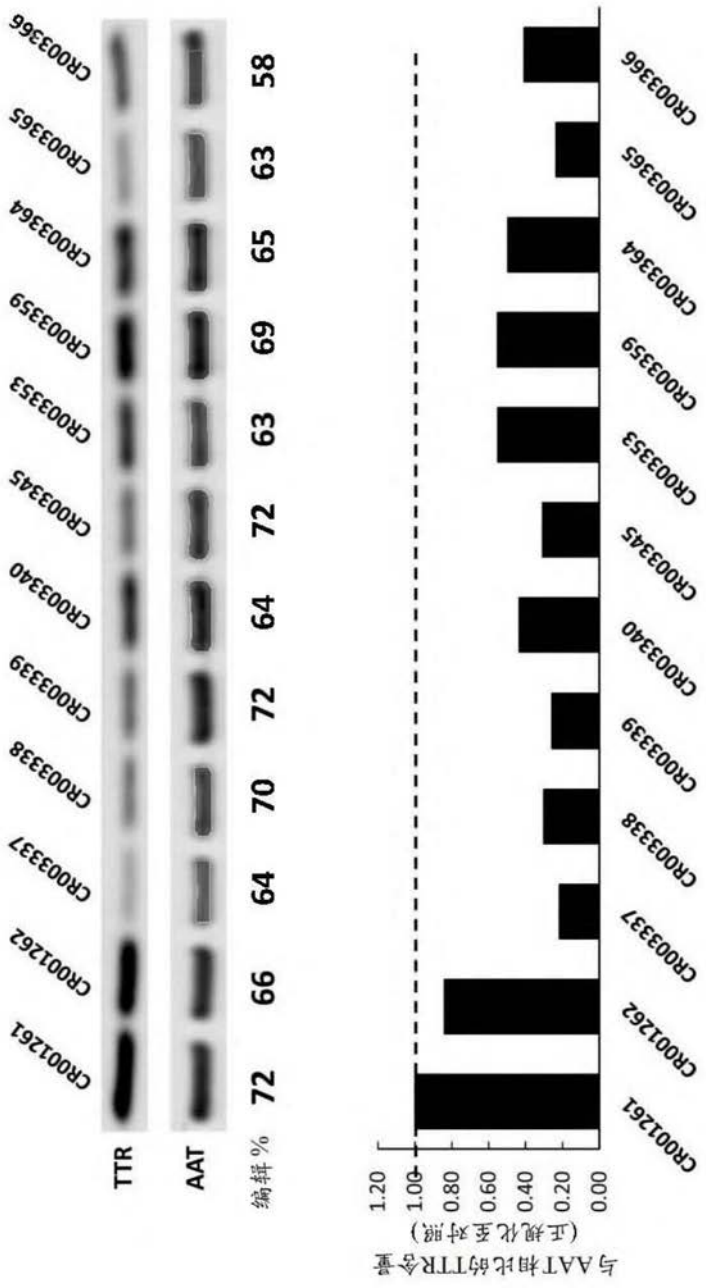

CR001261
CR001262
CR003337
CR003338
CR003339
CR003340
CR003345
CR003353
CR003359
CR003364
CR003365
CR003366
TTR
AAT
72
66
64
70
72
64
72
63
69
65
63
58
编辑 %
1.20
1.00
0.80
0.60
0.40
0.20
0.00
(正规化至对照)
与AAT相比的TTR含量

图7

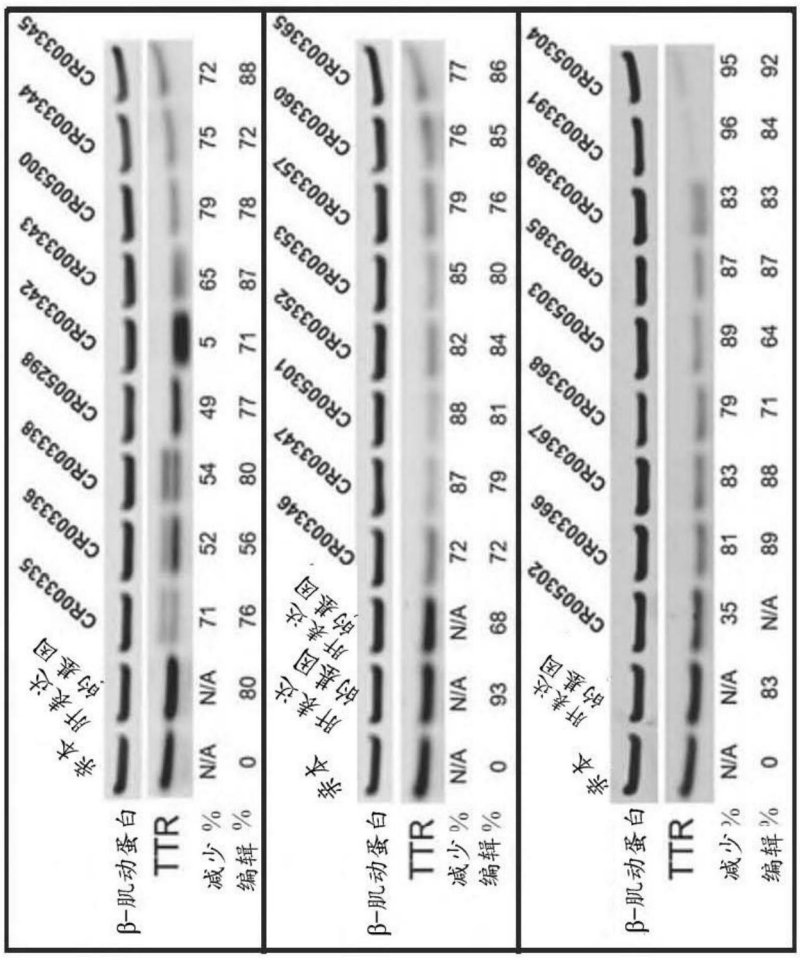

β-肌动蛋白
TTR
减少 %
编辑 %
未处理的疾病对照
CR003335
CR003336
CR003338
CR005298
CR003342
CR003343
CR005300
CR003344
CR003345
N/A
0
N/A
80
71
76
52
56
54
80
49
77
5
71
65
87
79
78
72
72
88
β-肌动蛋白
TTR
减少 %
编辑 %
未处理的疾病对照
CR003346
CR003347
CR005301
CR003352
CR003353
CR003357
CR003360
CR003365
N/A
0
N/A
93
N/A
68
72
72
87
79
88
81
82
84
85
80
79
76
76
85
77
86
β-肌动蛋白
TTR
减少 %
编辑 %
未处理的疾病对照
CR005302
CR003366
CR003367
CR003368
CR005303
CR003385
CR003389
CR003391
CR005304
N/A
0
N/A
83
35
89
81
83
79
88
71
64
89
87
87
83
83
96
84
95
92

图8

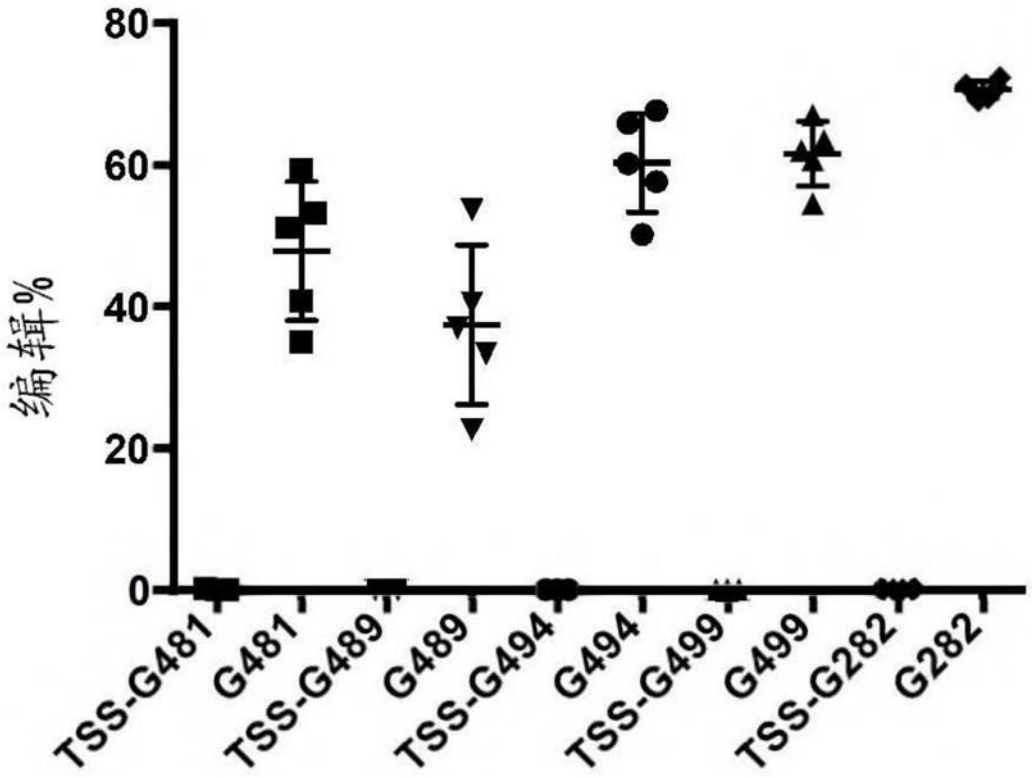

80
60
40
20
0
编辑%
TSS-G481
G481
TSS-G489
G489
TSS-G494
G494
TSS-G499
G499
TSS-G282
G282

图9

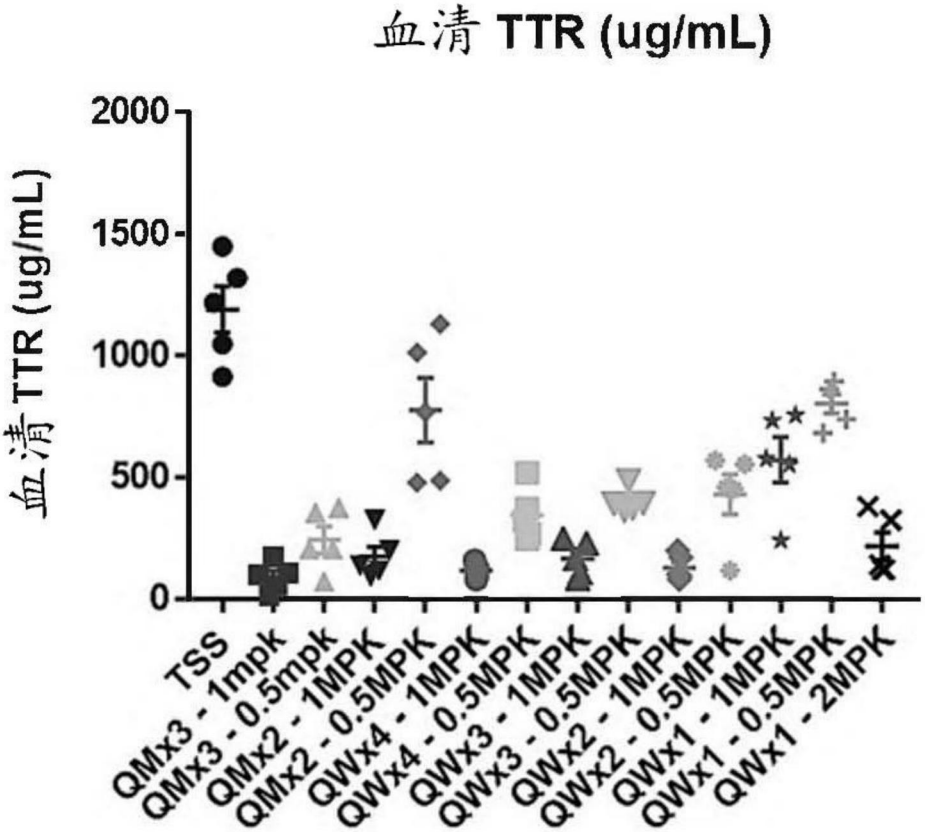

血清 TTR (ug/mL)
血清 TTR (ug/mL)
2000
1500
1000
500
0
TSS
QMx3 - 1mpk
QMx3 - 0.5mpk
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

图10A

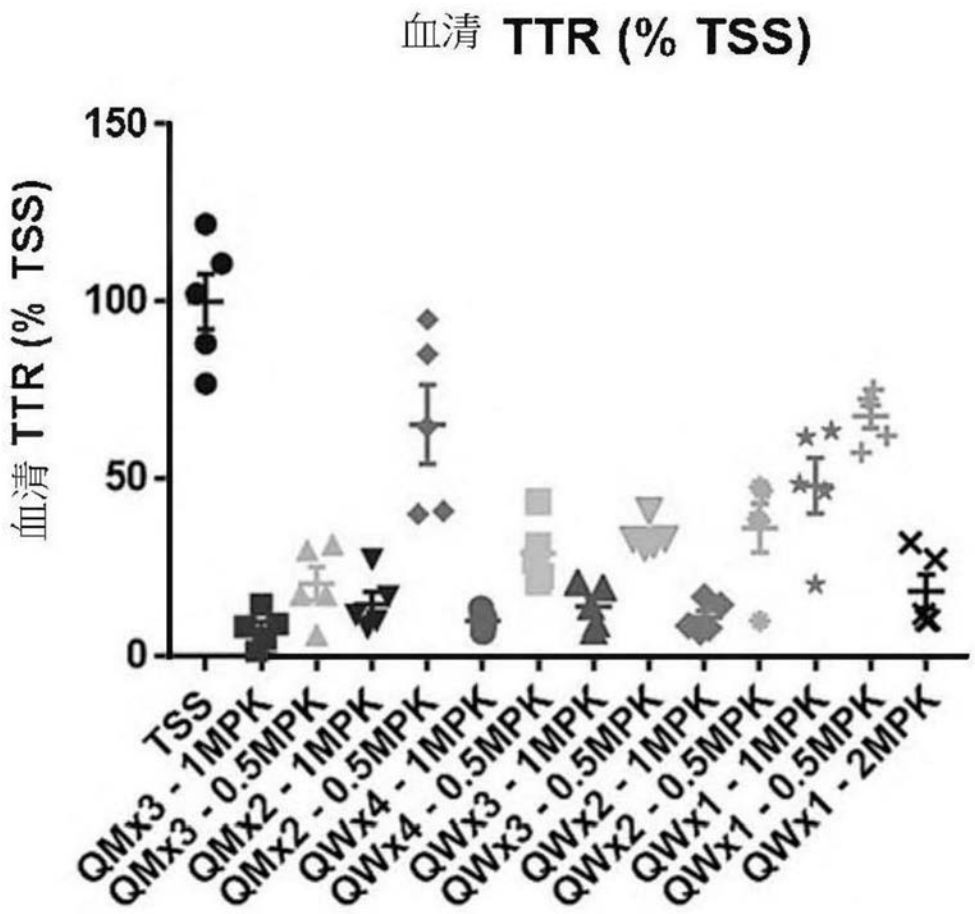

血清 TTR (% TSS)
血清 TTR (% TSS)
150
100
50
0
TSS
QMx3 - 1MPK
QMx3 - 0.5MPK
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

图10B

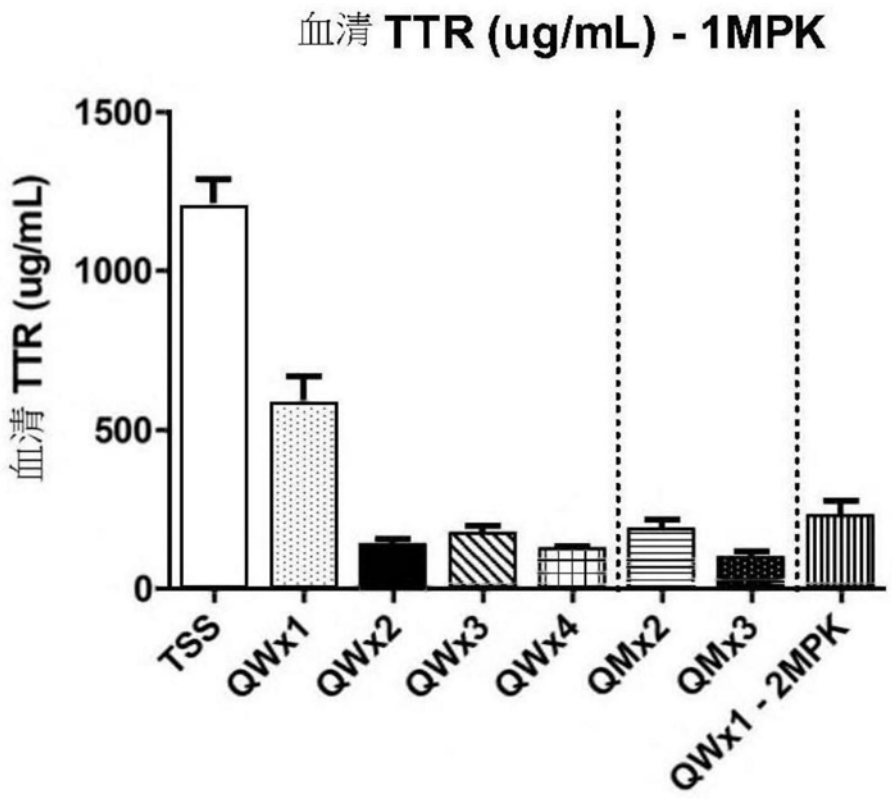

血清 TTR (ug/mL) - 1MPK
血清 TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

图11A

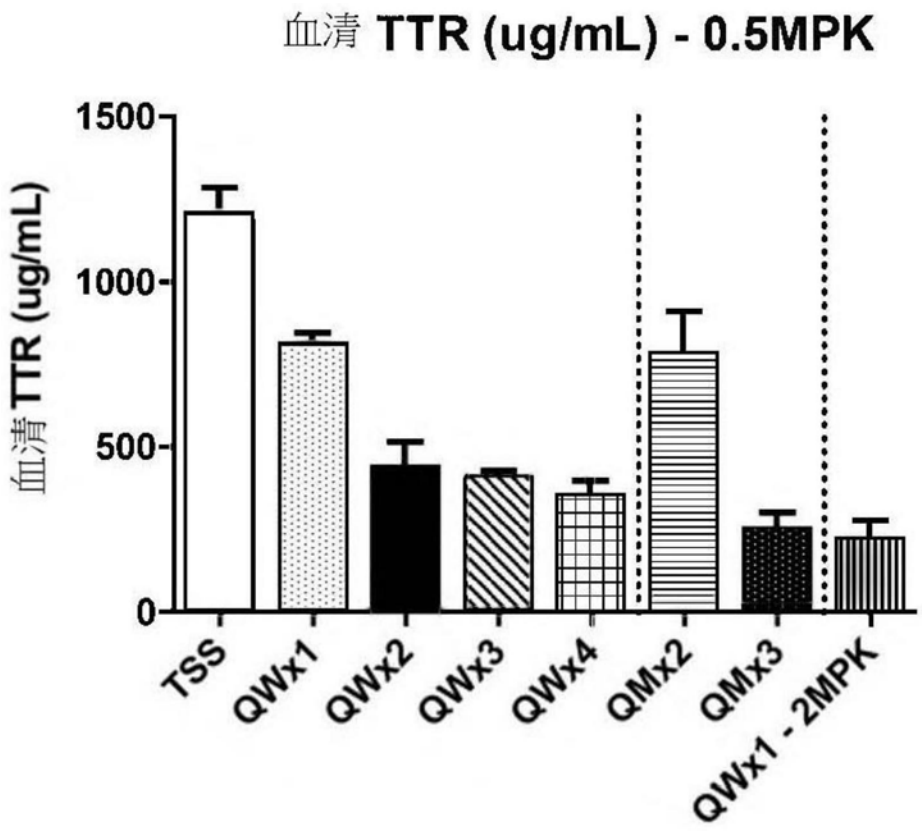

血清 TTR (ug/mL) - 0.5MPK
血清 TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

图11B

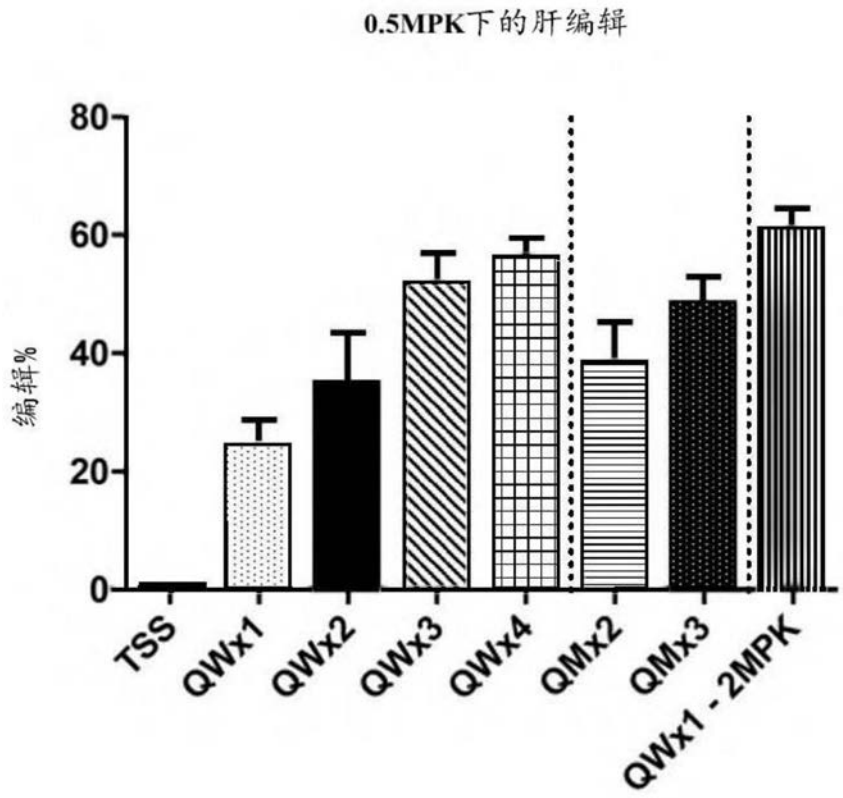

0.5MPK下的肝编辑
编辑%
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

图12A

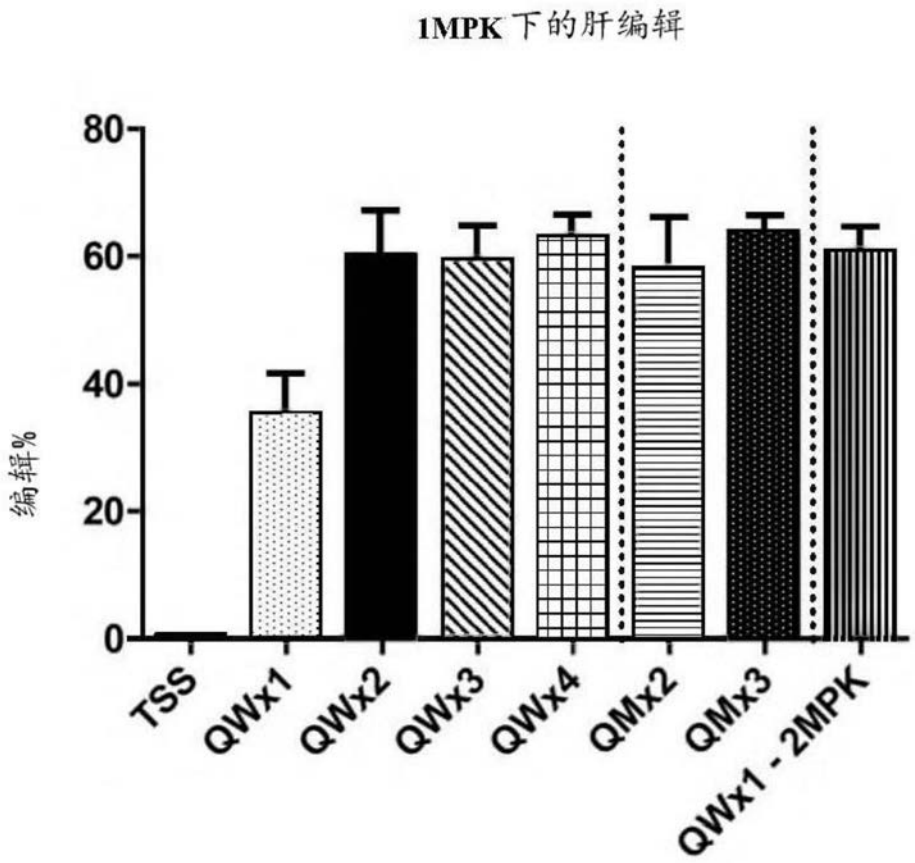

1MPK下的肝编辑
编辑%
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

图12B

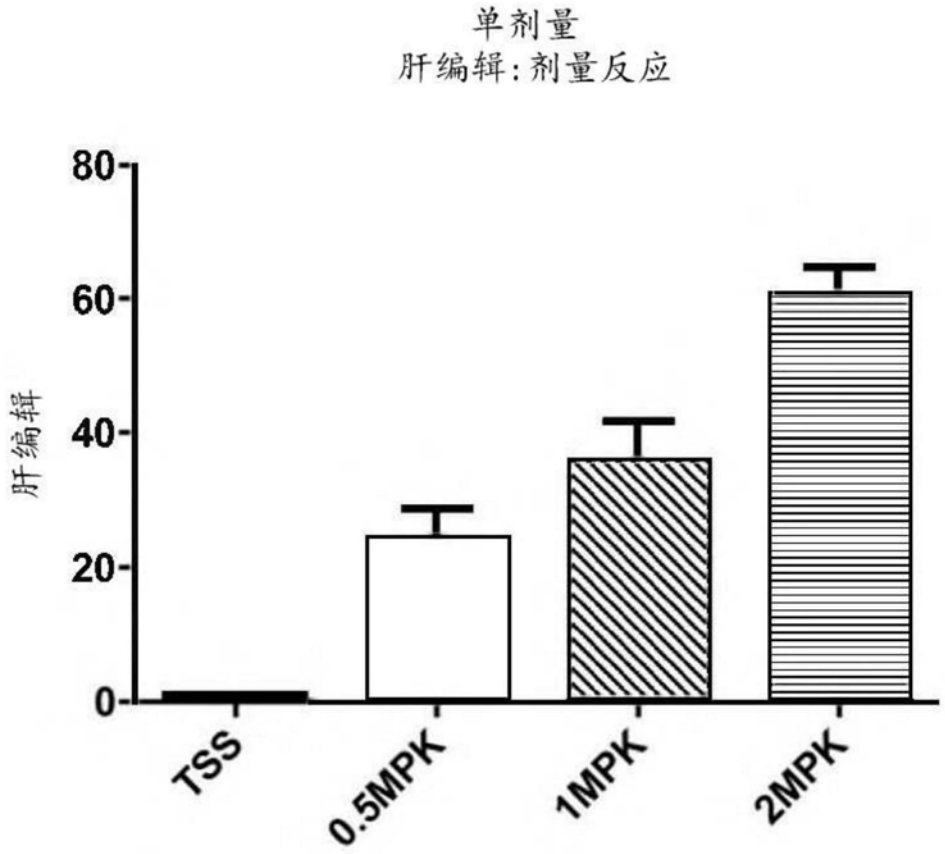

单剂量
肝编辑:剂量反应
肝编辑
80
60
40
20
0
TSS
0.5MPK
1MPK
2MPK

图12C

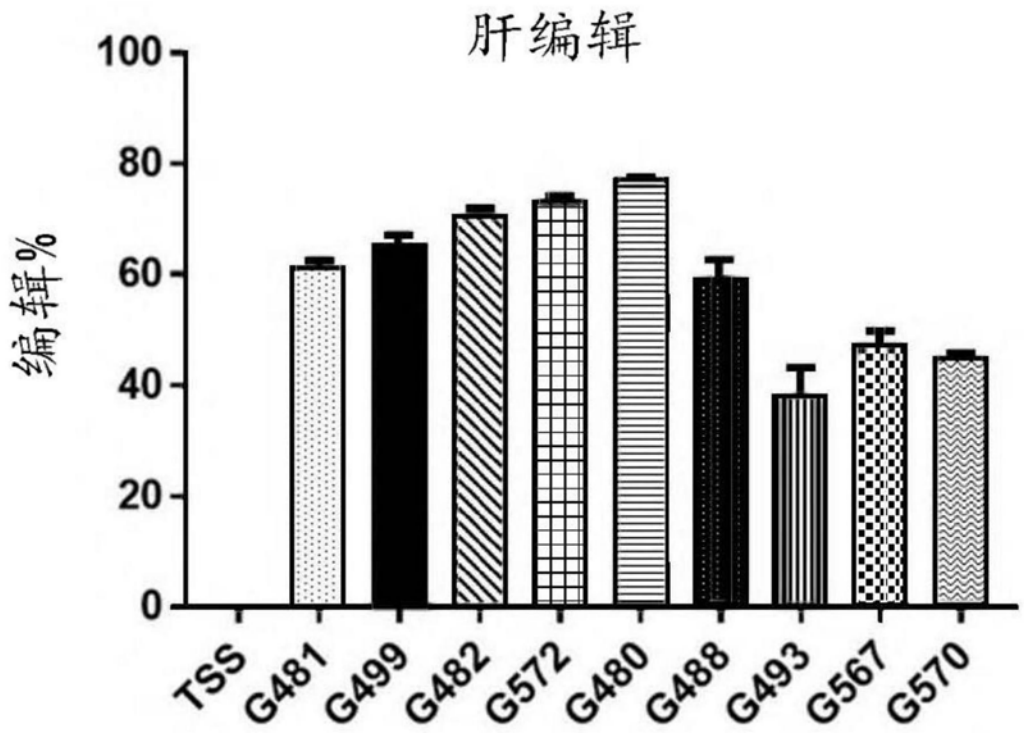

肝编辑
编辑%
100
80
60
40
20
0
TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570

图13

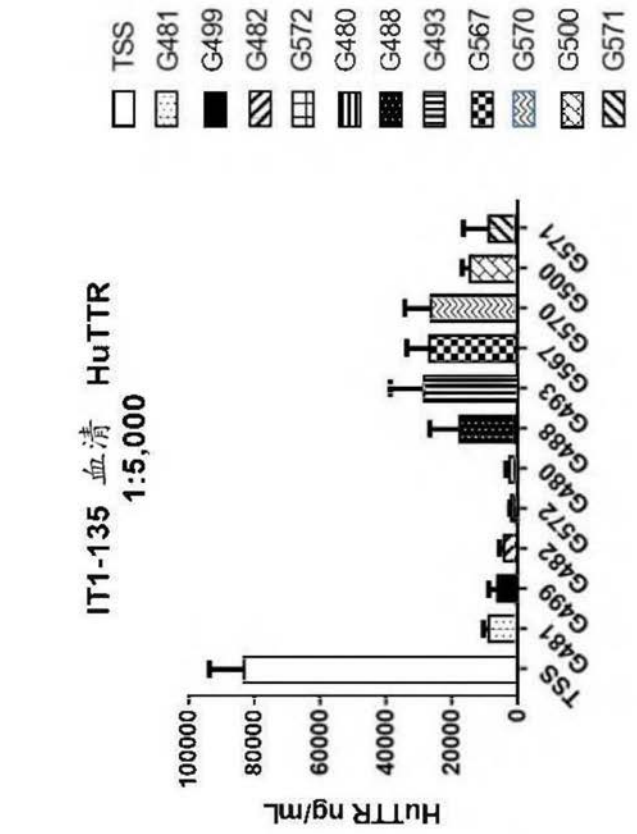

TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570
G500
G571
IT1-135 血清 HuTTR
1:5,000
HuTTR ng/mL
100000
80000
60000
40000
20000
0

图14B

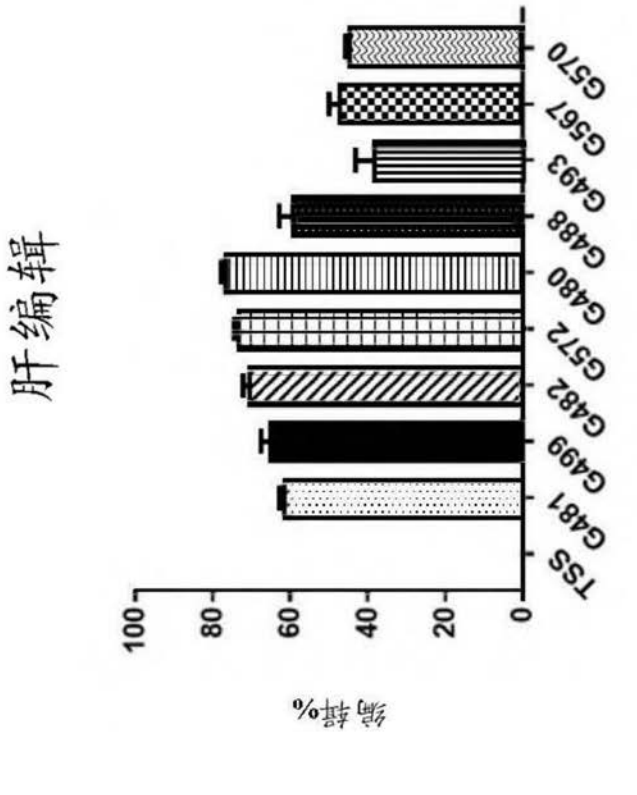

肝编辑
编辑%
100
80
60
40
20
0
TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570

图14A

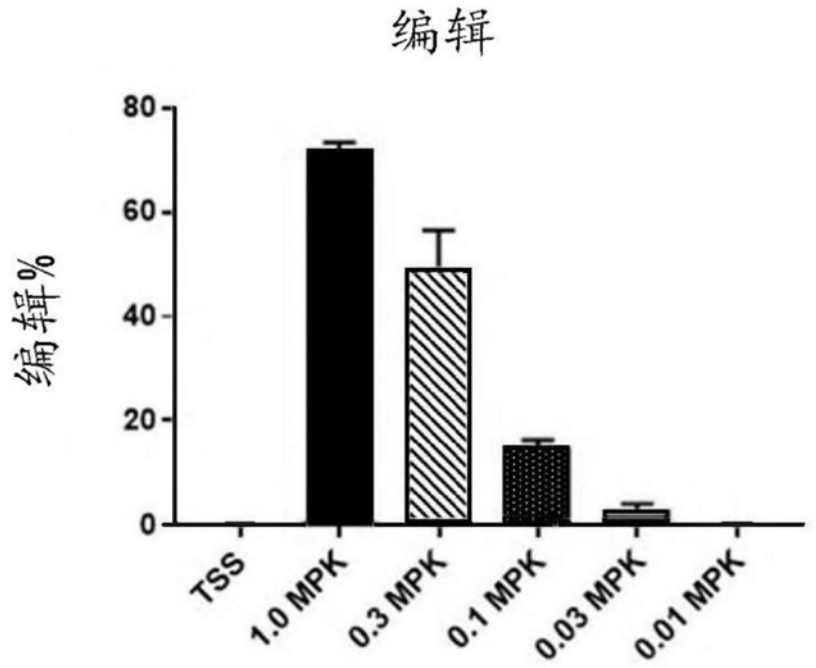

编辑
编辑%
80
60
40
20
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

图15A

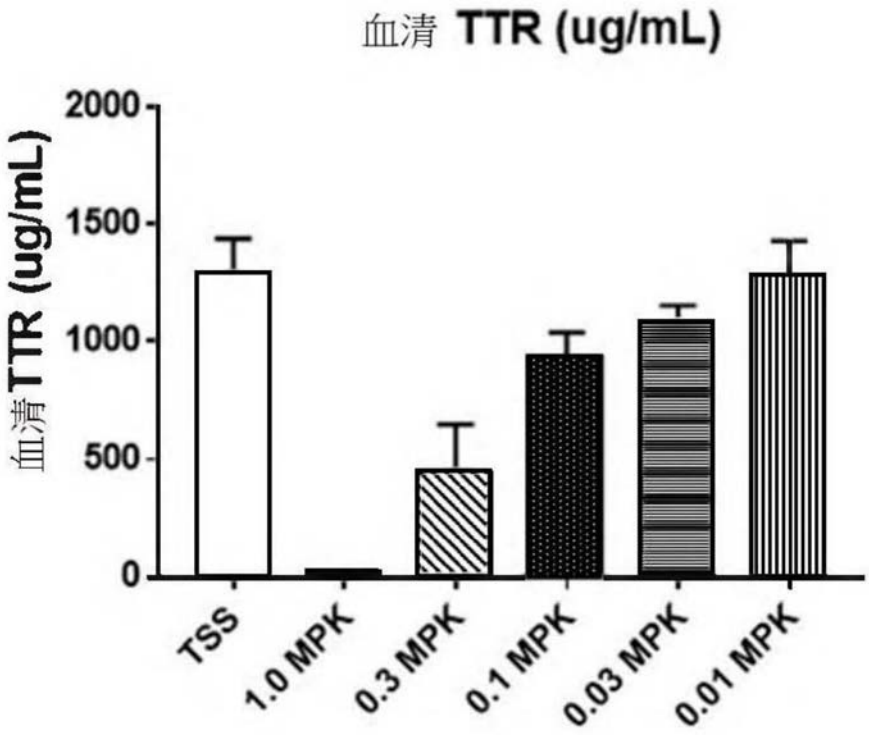

血清 TTR (ug/mL)
血清TTR (ug/mL)
2000
1500
1000
500
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

图15B

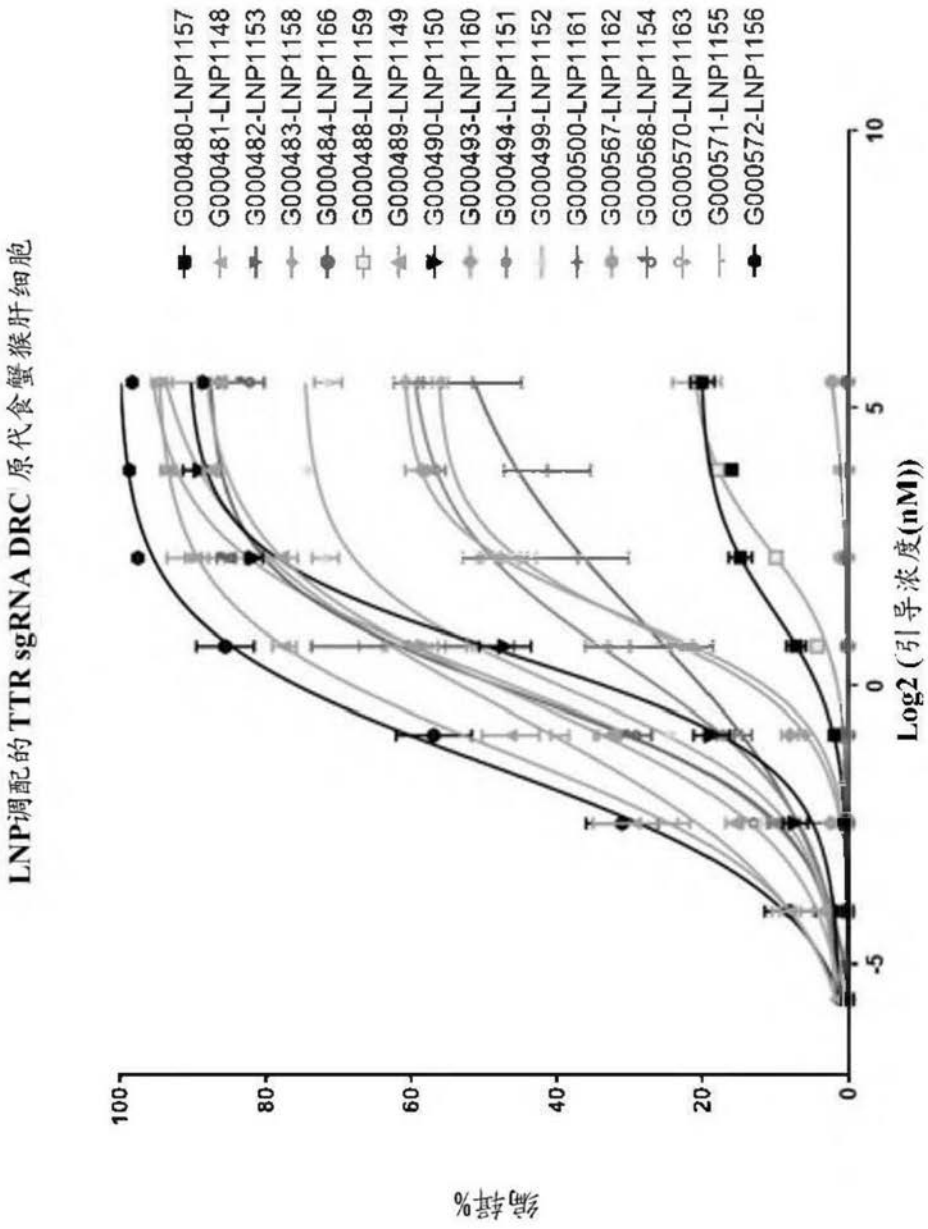

LNP调配的TTR sgRNA DRC 原代食蟹猴肝细胞
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000567-LNP1162
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
100
80
60
40
20
0
-5
0
5
10
Log2 (引导浓度(nM))
%

图16

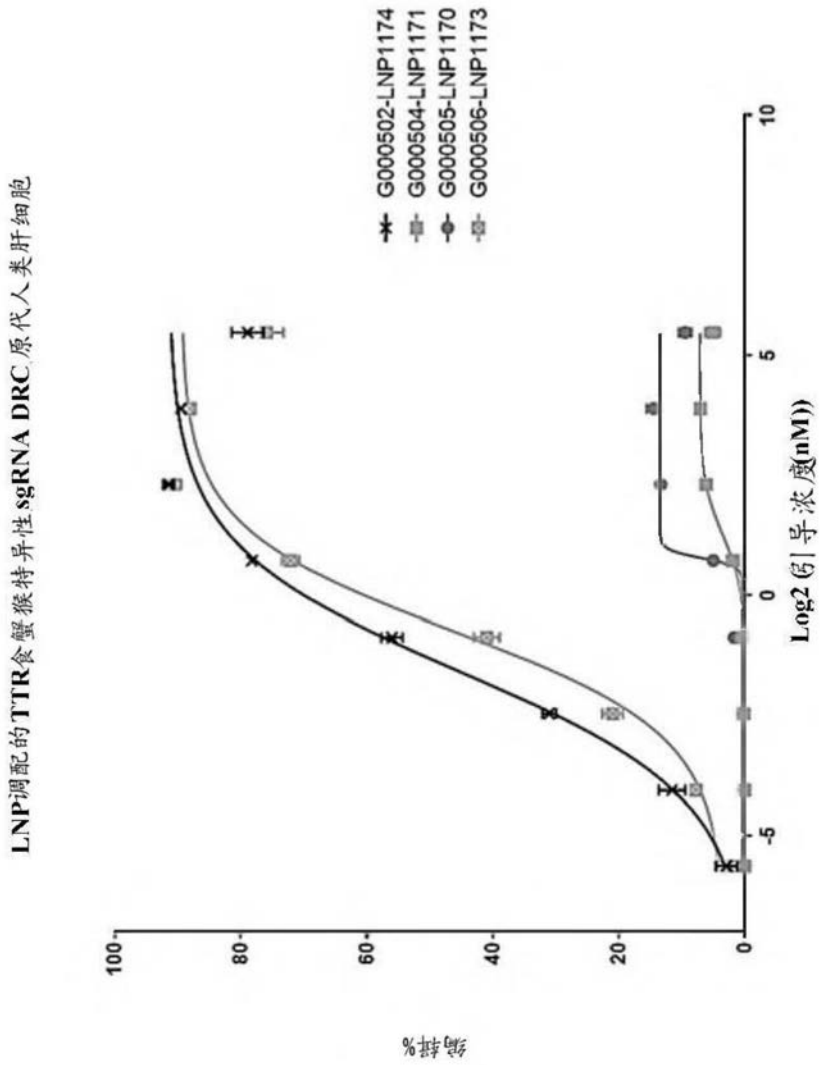

LNP调配的TTR食蟹猴特异性sgRNA DRC原代人类肝细胞
G000502-LNP1174
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
100
80
60
40
20
0
-5
0
5
10
Log2 (引导浓度(nM))
%敲降

图17

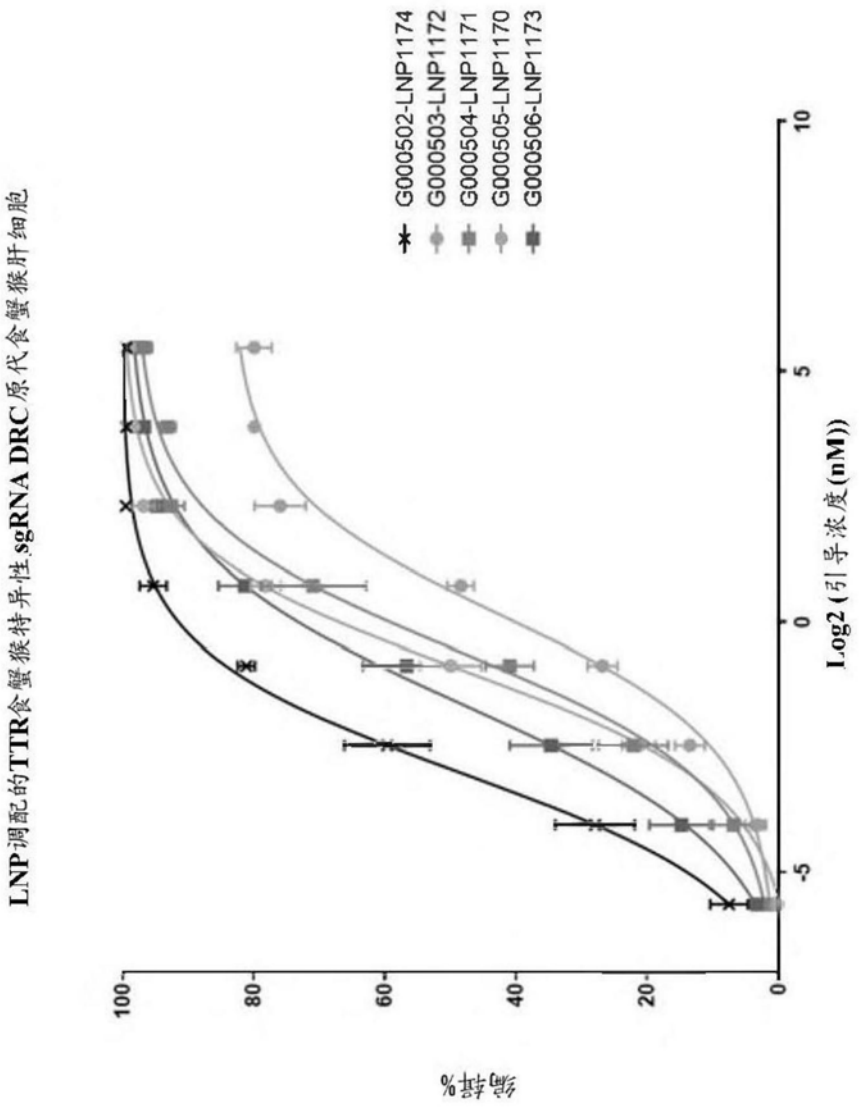

LNP调配的TTR食蟹猴特异性sgRNA DRC原代食蟹猴肝细胞
G000502-LNP1174
G000503-LNP1172
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
Log2(引导浓度(nM))
编辑%
100
80
60
40
20
0
-5
0
5
10

图18

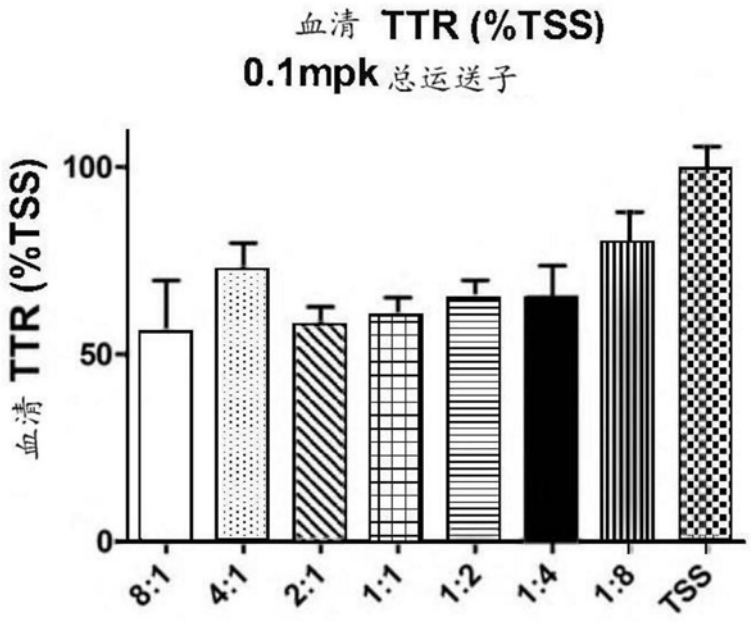

血清 TTR (%TSS)
0.1mpk 总运送子
血清 TTR (%TSS)
100
50
0
8:1
4:1
2:1
1:1
1:2
1:4
1:8
TSS

图19A

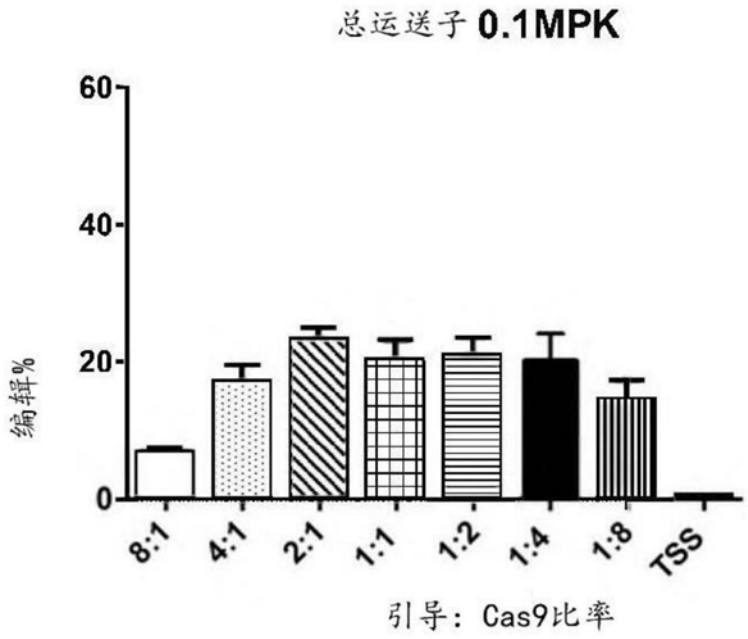

总运送子 0.1MPK
编辑%
60
40
20
0
8:1
4:1
2:1
1:1
1:2
1:4
1:8
TSS
引导：Cas9比率

图19B

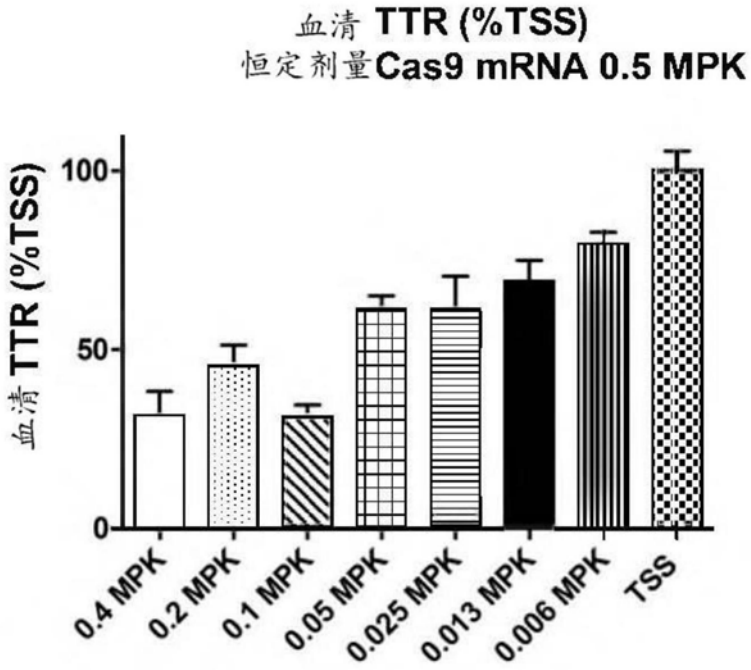

血清 TTR (%TSS)
恒定剂量Cas9 mRNA 0.5 MPK
血清 TTR (%TSS)
100
50
0
0.4 MPK
0.2 MPK
0.1 MPK
0.05 MPK
0.025 MPK
0.013 MPK
0.006 MPK
TSS

图19C

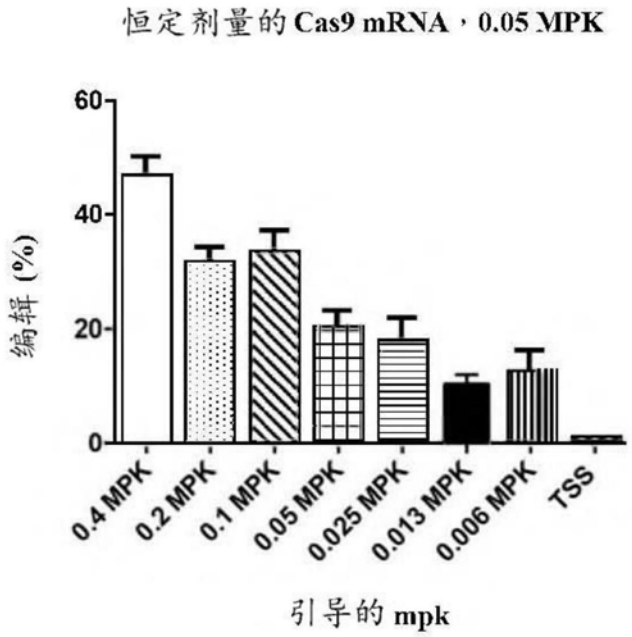

恒定剂量的 Cas9 mRNA，0.05 MPK
编辑 (%)
60
40
20
0
0.4 MPK
0.2 MPK
0.1 MPK
0.05 MPK
0.025 MPK
0.013 MPK
0.006 MPK
TSS
引导的 mpk

图19D

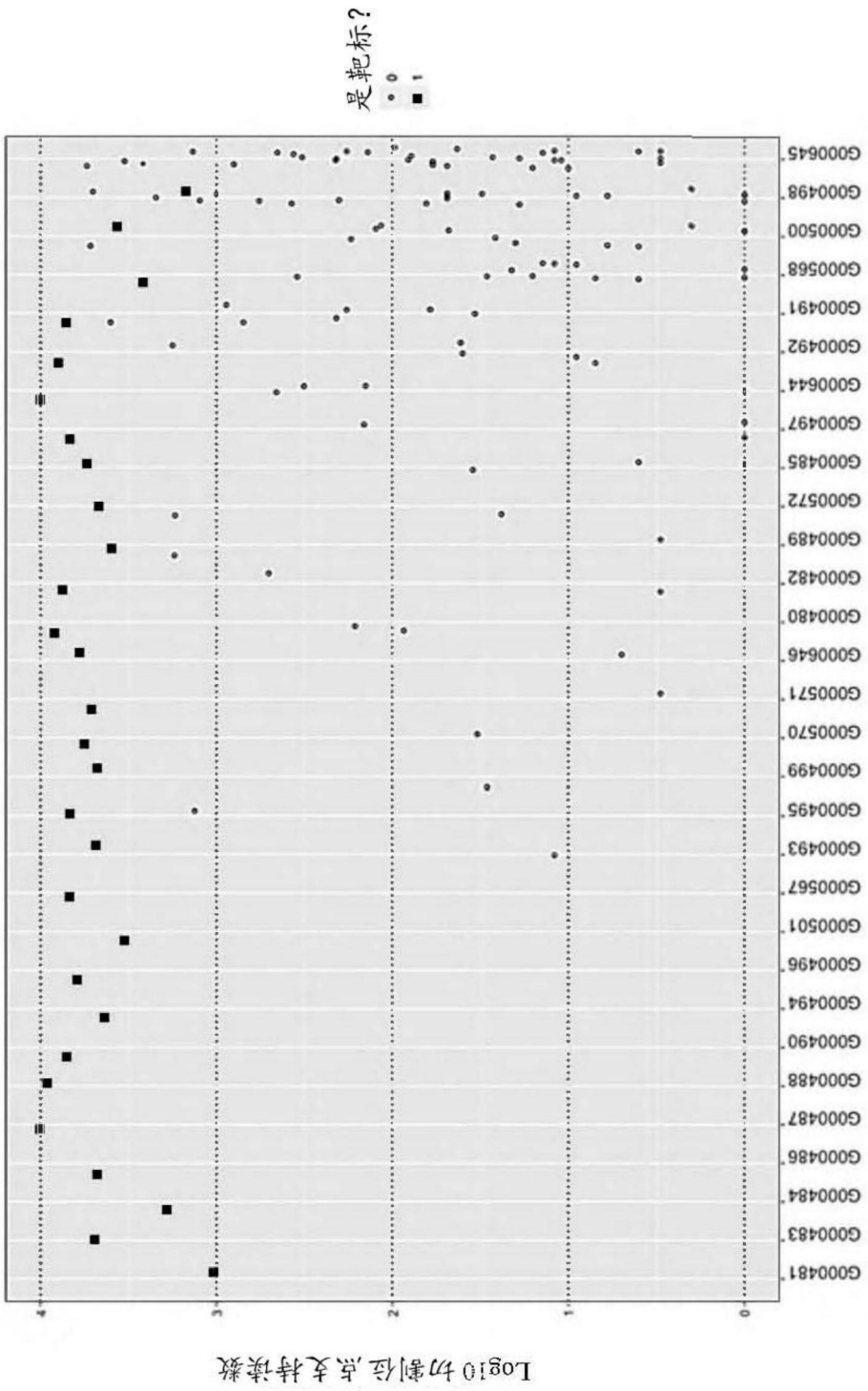

是靶标?
0
1
G0006451
G0004981
G0005001
G0005681
G0004911
G0004921
G0006441
G0004971
G0004851
G0005721
G0004891
G0004821
G0004801
G0006461
G0005711
G0005701
G0004991
G0004951
G0004931
G0005671
G0005011
G0004961
G0004941
G0004901
G0004881
G0004871
G0004861
G0004841
G0004831
4
3
2
1
0
Log10 切割位点支持读数

图20

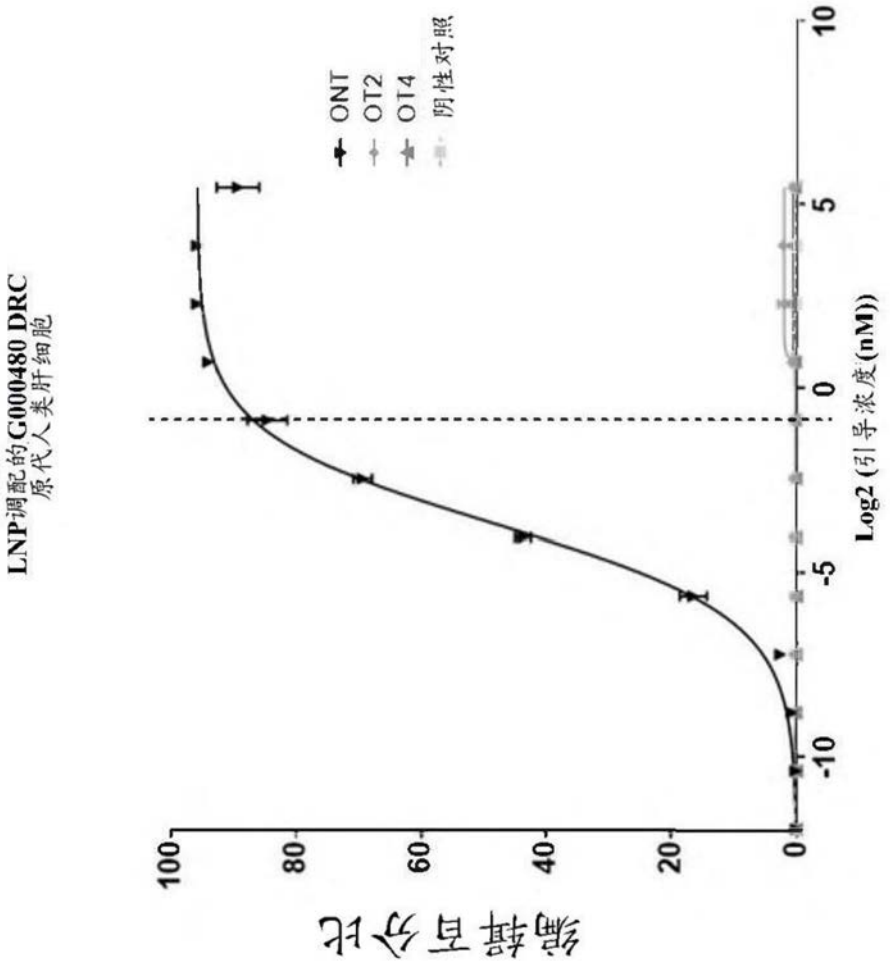

LNP调配的G000480 DRC
原代人类肝细胞
ONT
OT2
OT4
阴性对照
编辑百分比
100
80
60
40
20
0
-10
-5
0
5
10
Log2 (引导浓度(nM))

图21A

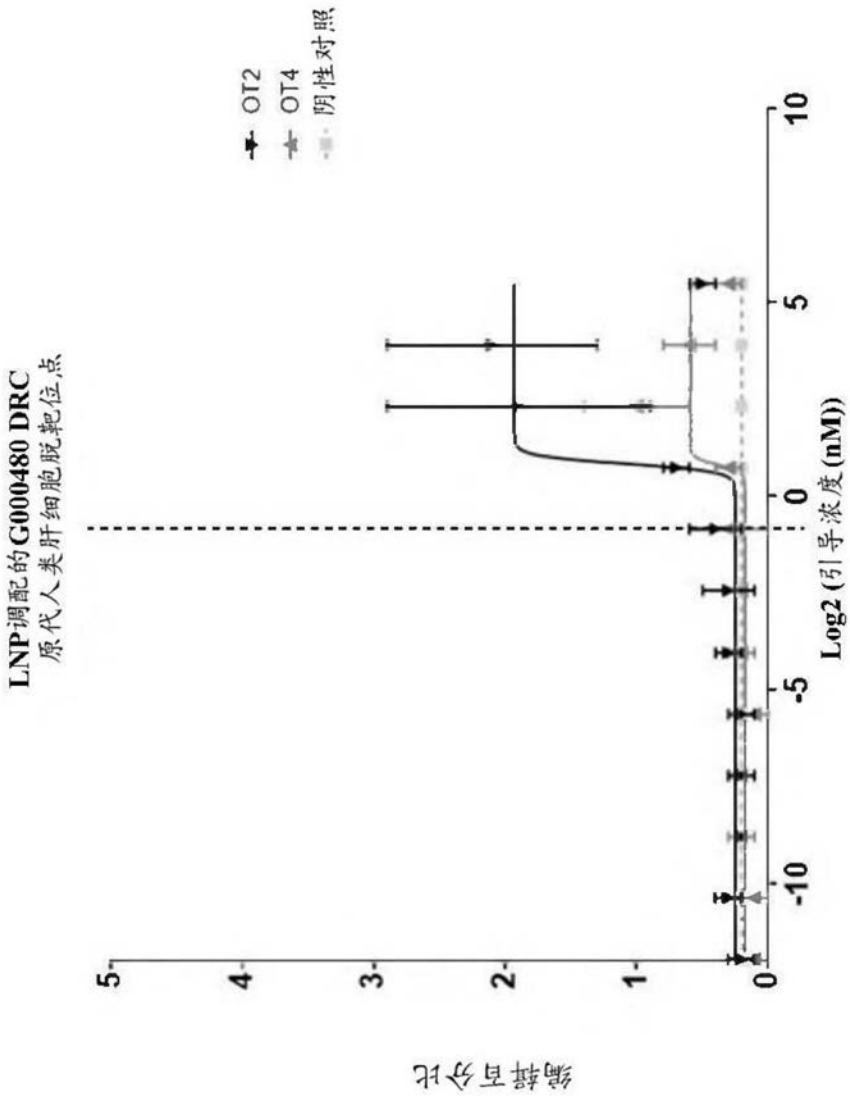

LNP调配的G000480 DRC
原代人类肝细胞脱靶位点
OT2
OT4
阴性对照
编辑百分比
Log2 (引导浓度 (nM))
0
1
2
3
4
5
-10
-5
0
5
10

图21B

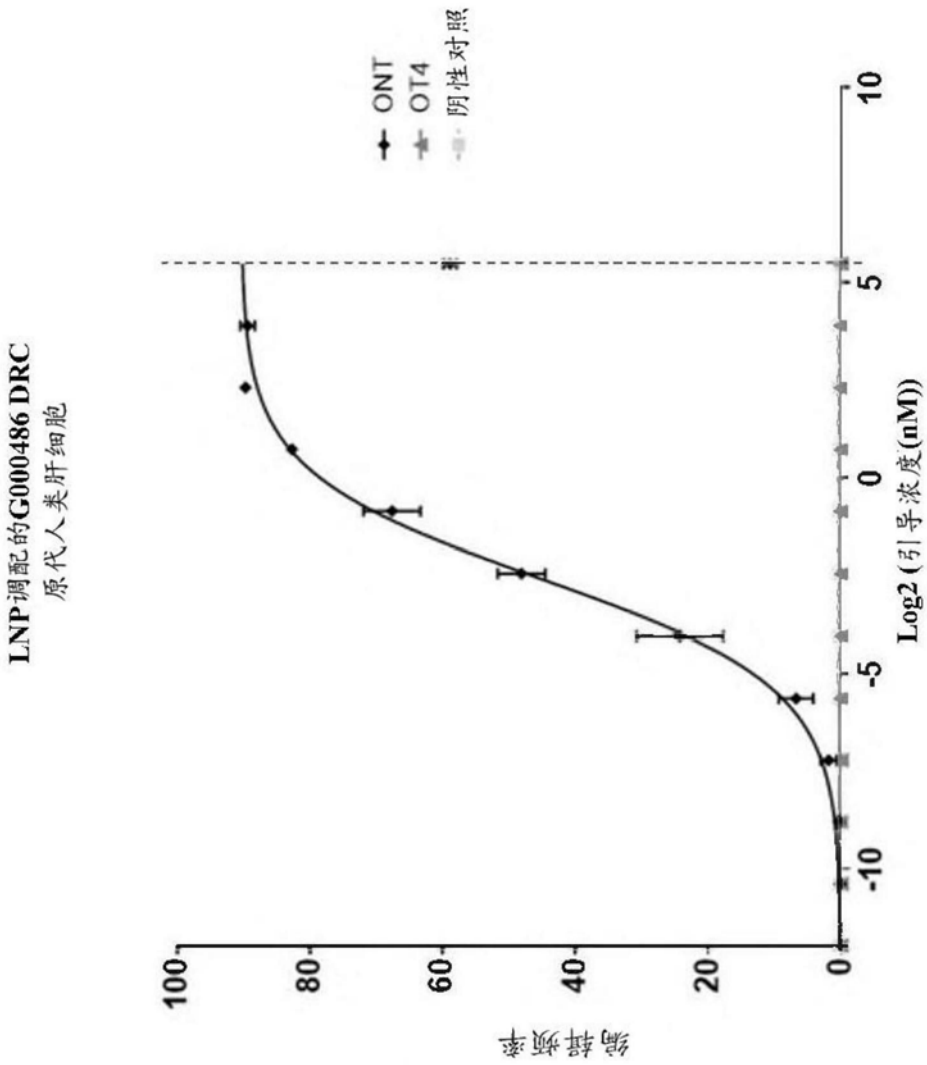

LNP调配的G000486 DRC
原代人类肝细胞
ONT
OT4
阴性对照
编辑频率
100
80
60
40
20
0
-10
-5
0
5
10
Log2 (引导浓度(nM))

图22A

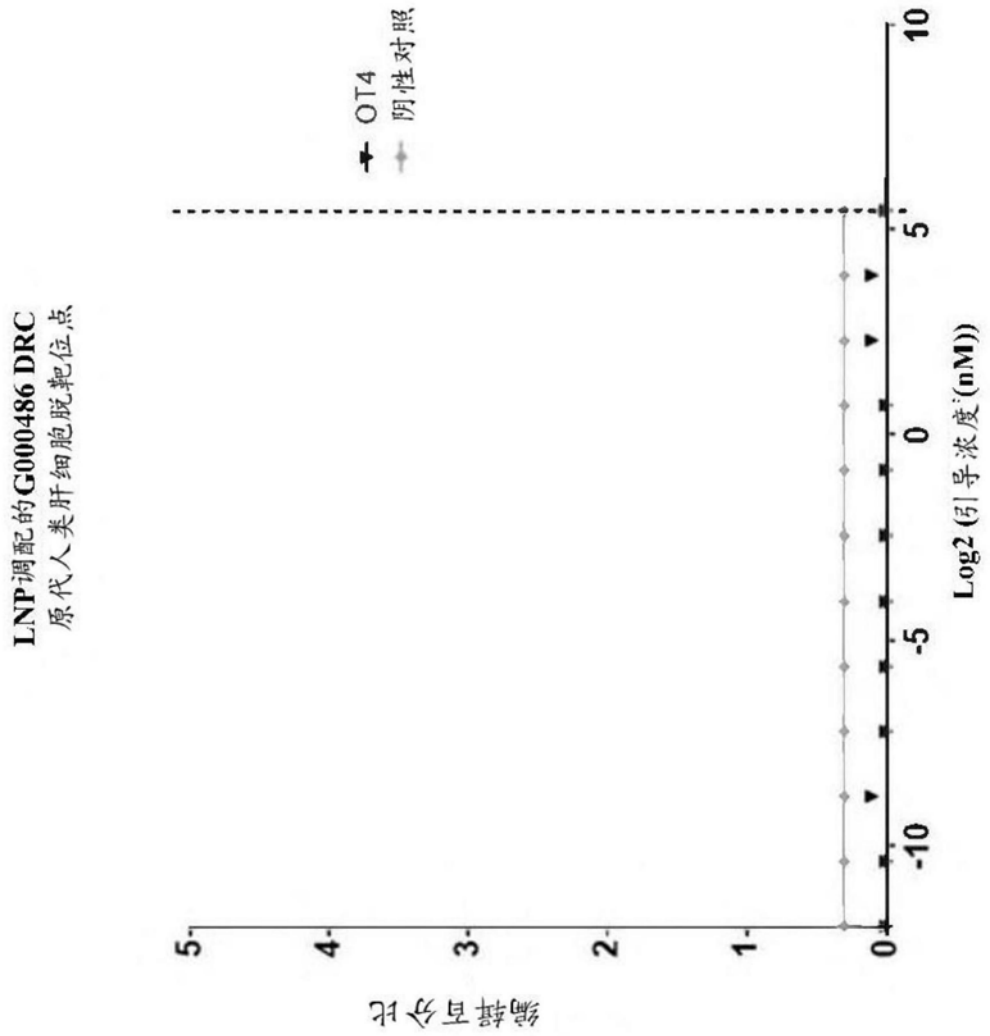

LNP调配的G000486 DRC
原代人类肝细胞脱靶位点
OT4
阴性对照
编辑百分比
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (引导浓度(nM))

图22B

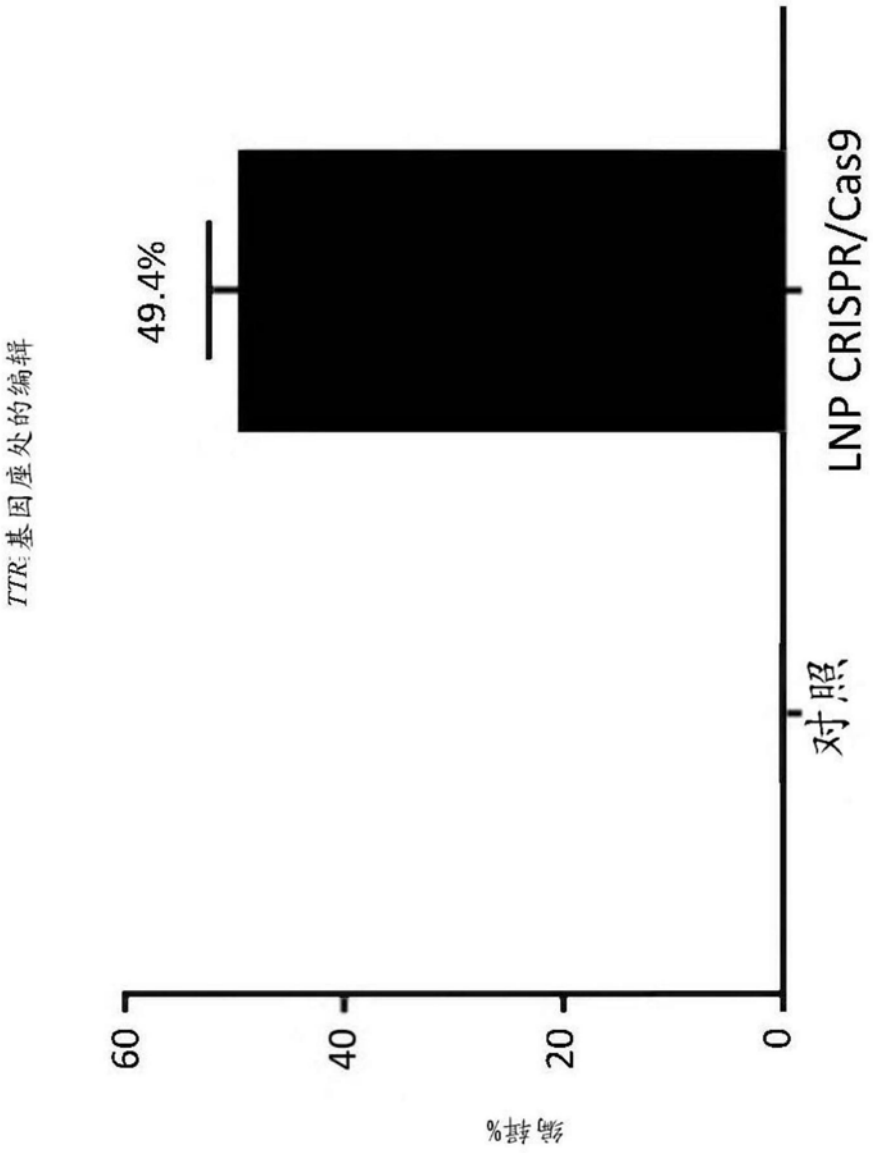


图23A

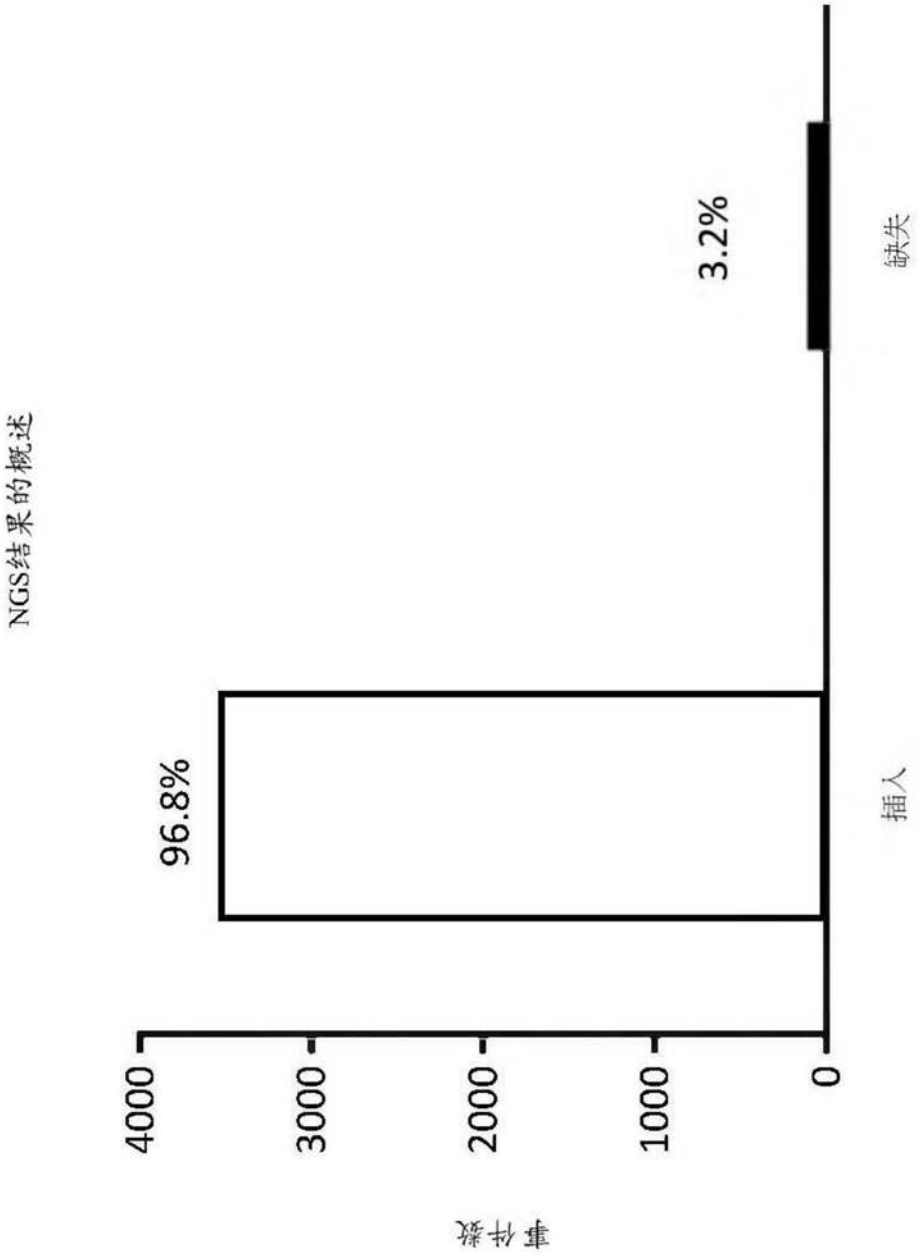


图23B

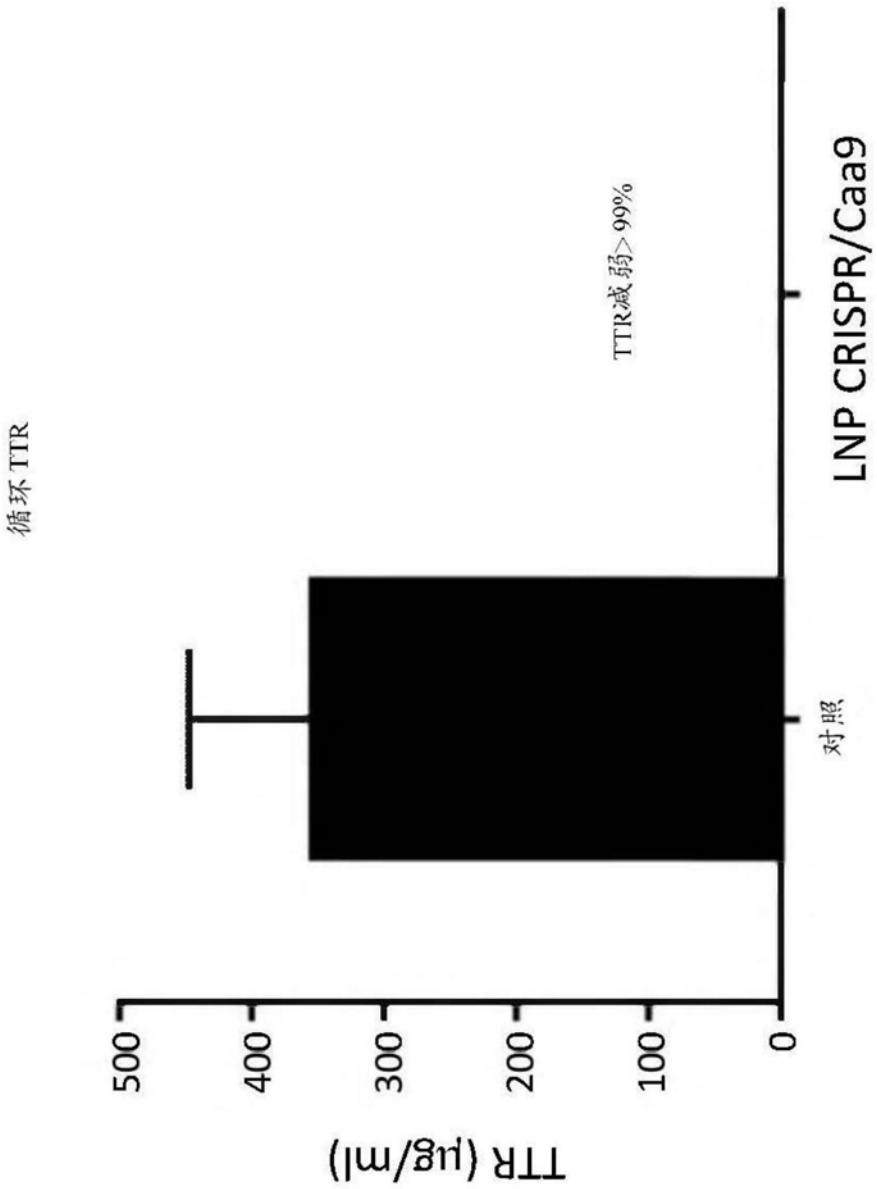

循环 TTR
TTR减弱>99%
LNP CRISPR/Caa9
对照
TTR (μg/ml)
500
400
300
200
100
0

图24A

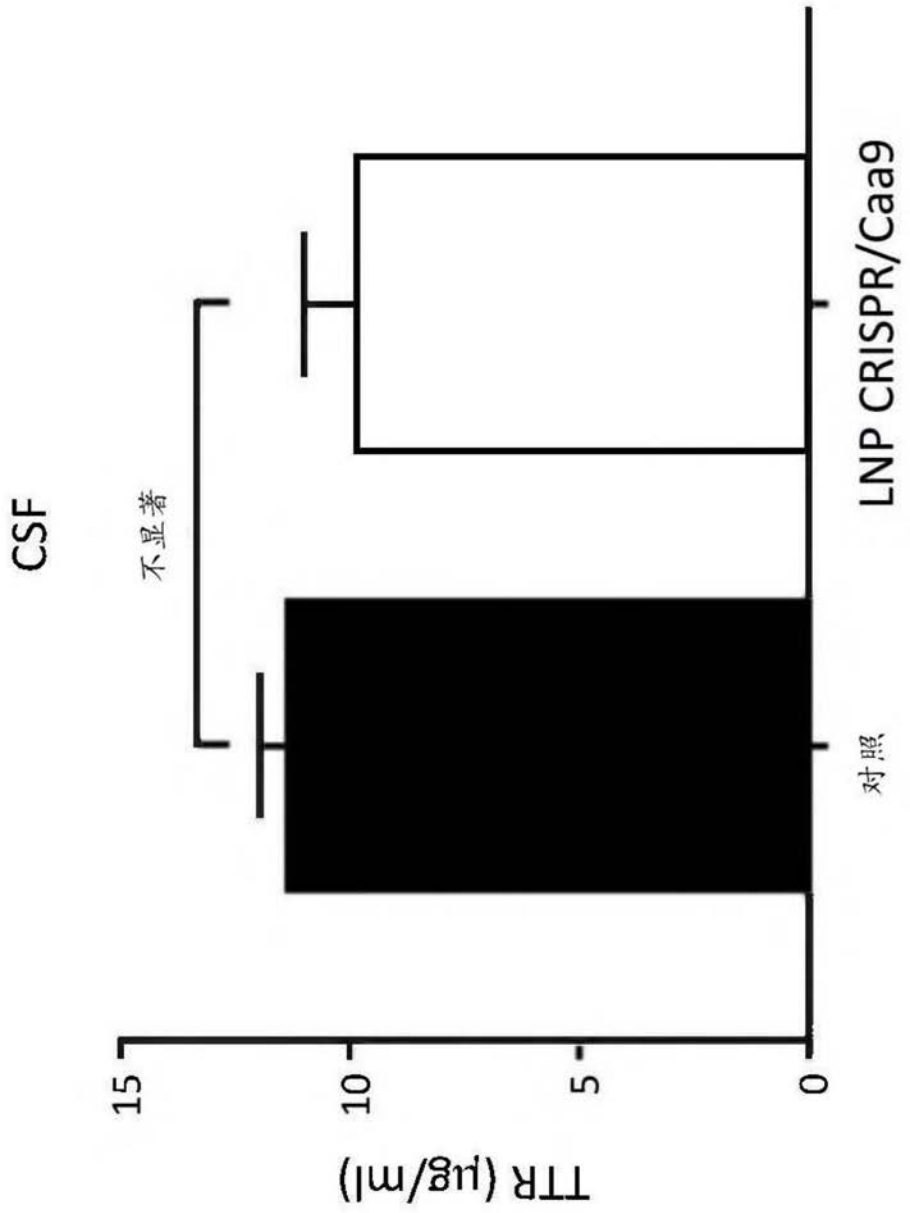
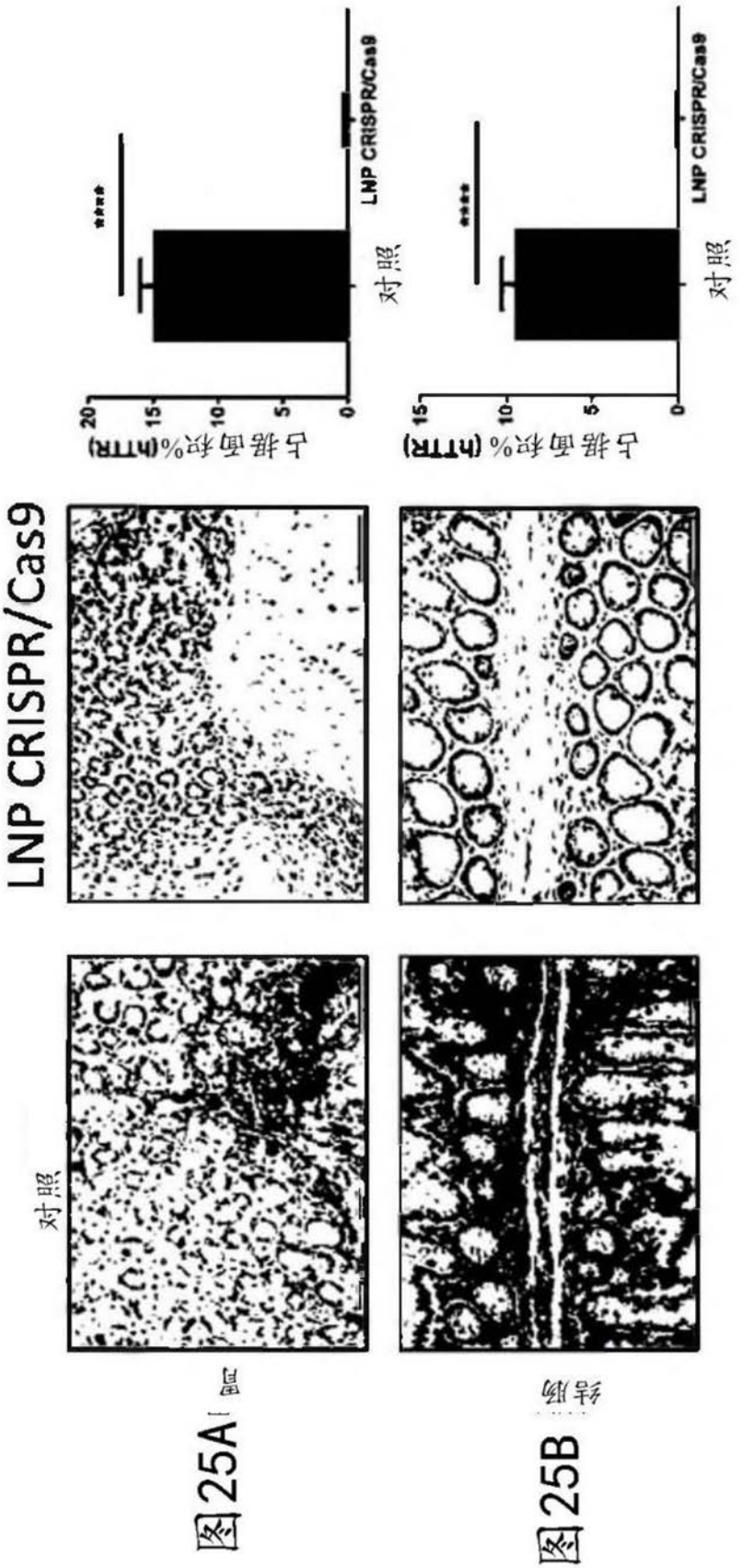


图24B



胃

图25A

结肠

图25B

对照
LNP CRISPR/Cas9
坐骨神经
DRG
占据面积%(hTTR)
0
1
2
3
4
****
对照
LNP CRISPR/Cas9

图25C

图25D

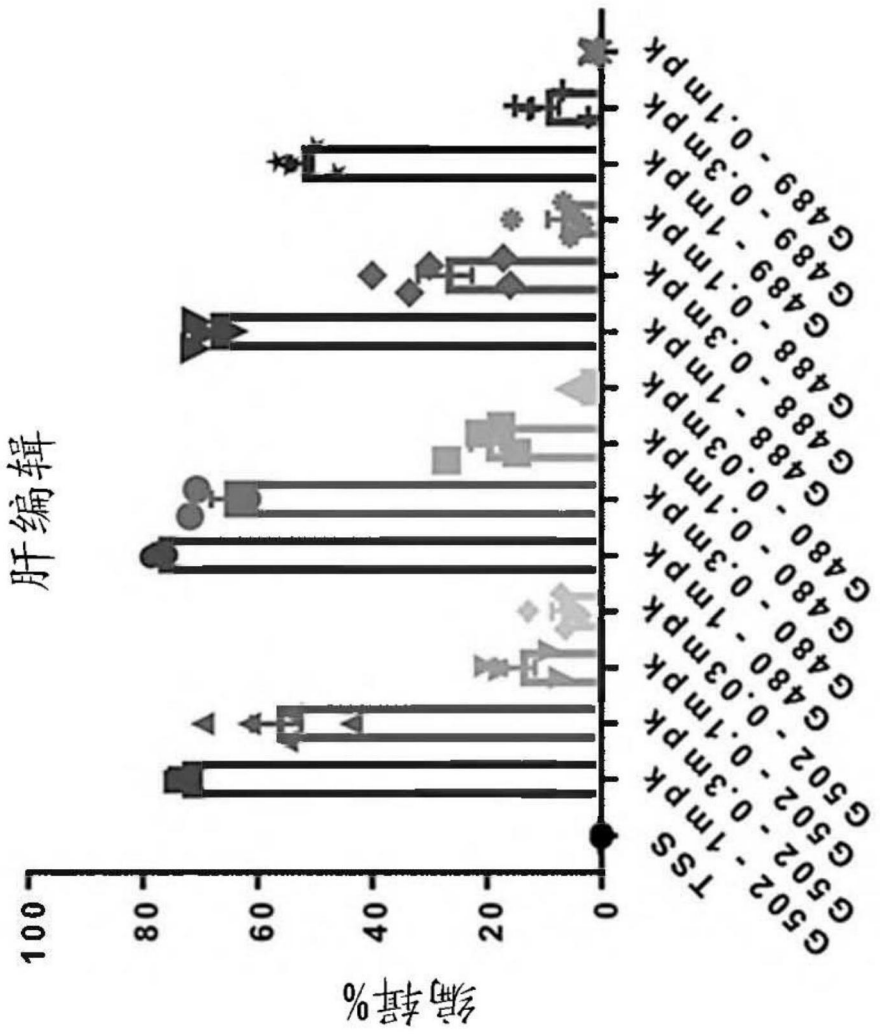

肝编辑
编辑%
100
80
60
40
20
0
TSS
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 1mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

图26A

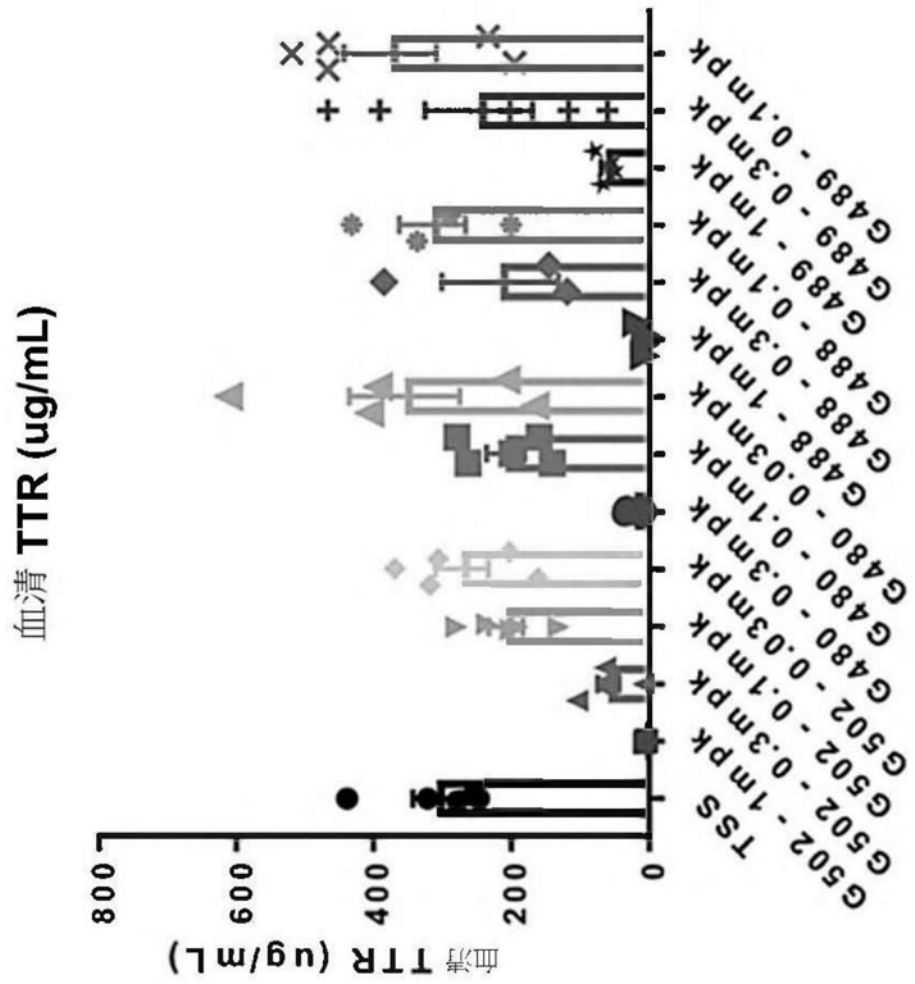

血清 TTR (ug/mL)
800
600
400
200
0
TSS - 1mpk
G502 - 0.1mpk
G502 - 0.3mpk
G502 - 1mpk
G480 - 0.1mpk
G480 - 0.3mpk
G480 - 1mpk
G488 - 0.1mpk
G488 - 0.3mpk
G488 - 1mpk
G489 - 0.1mpk
G489 - 0.3mpk
G489 - 1mpk

图26B

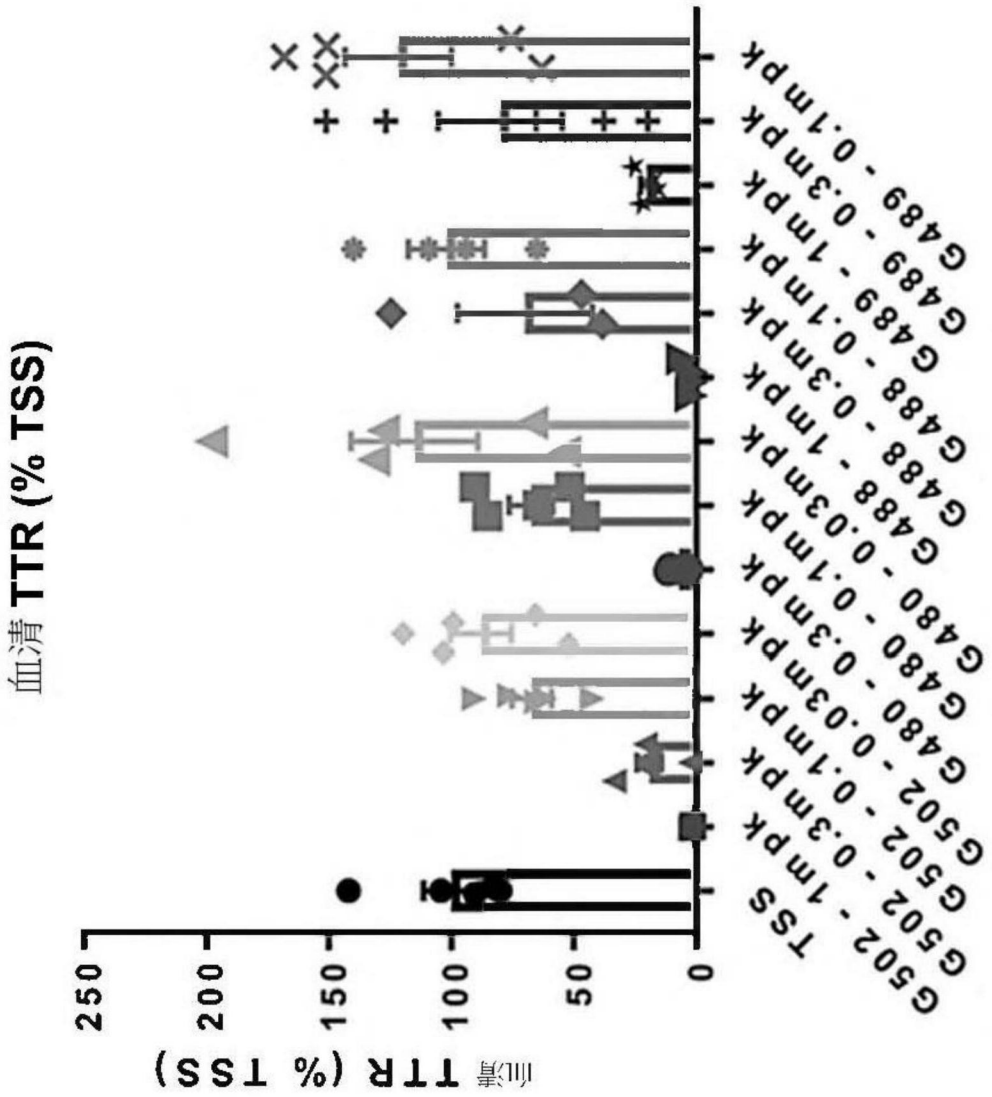

血清 TTR (% TSS)
250
200
150
100
50
0
TSS - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

图26C

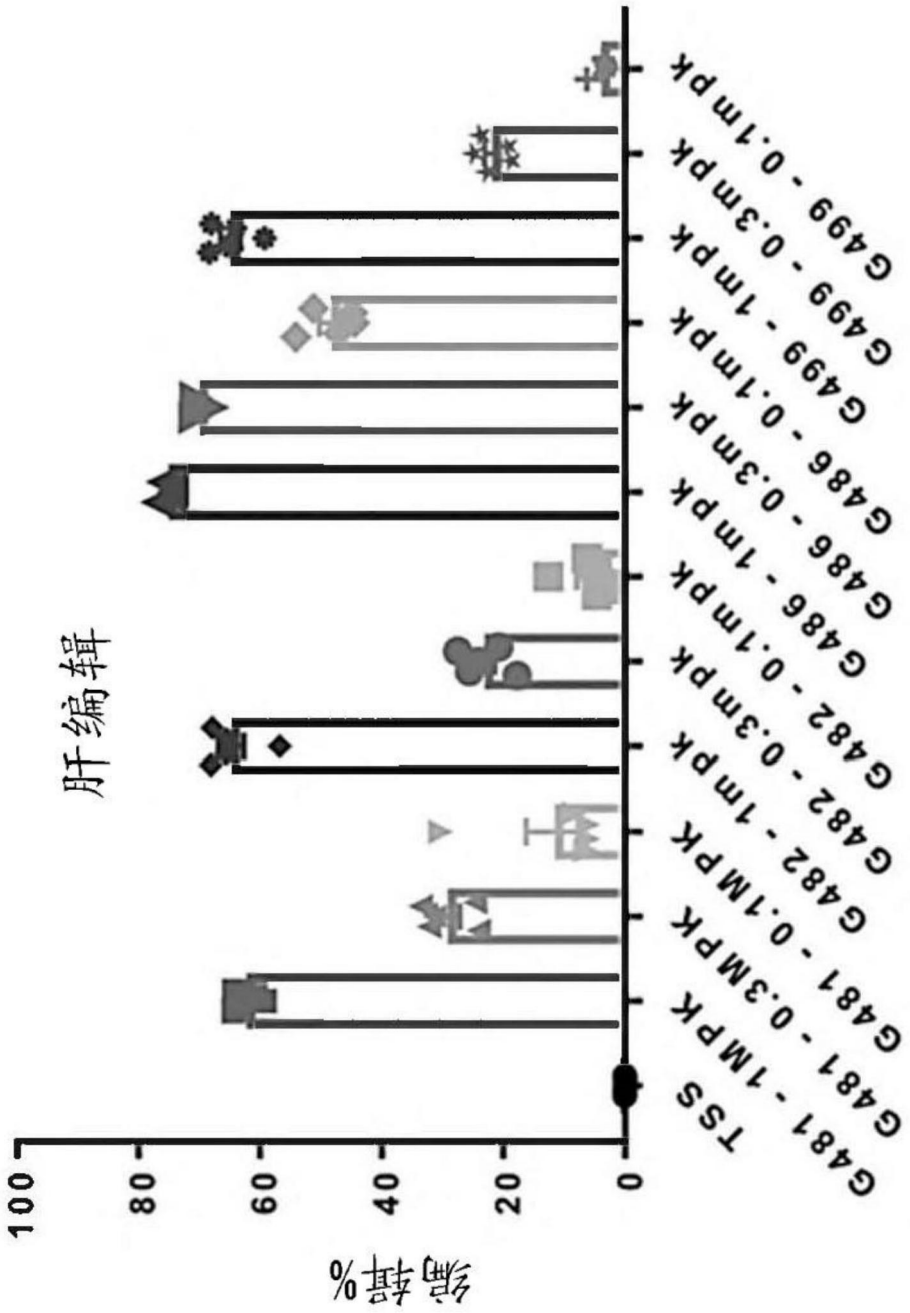

肝编辑
编辑%
100
80
60
40
20
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

图27A

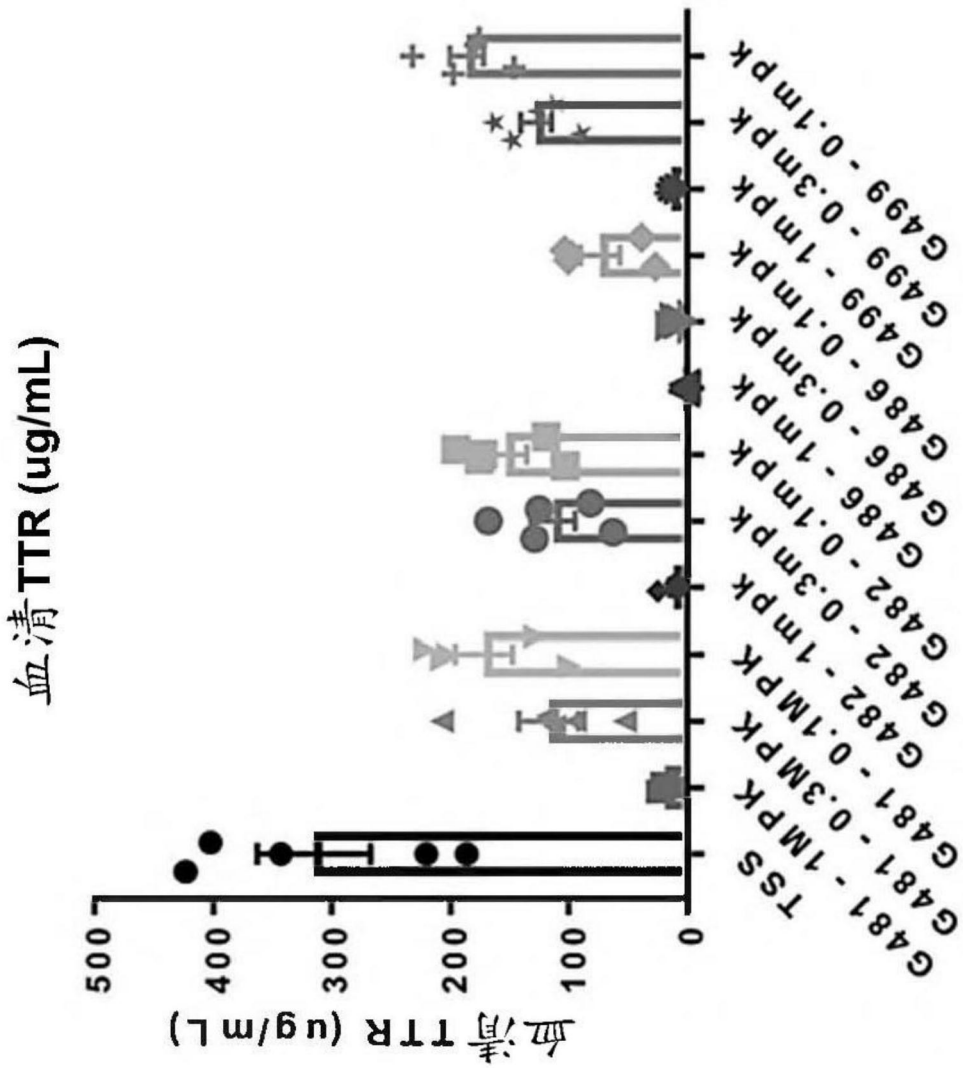

血清TTR (ug/mL)
500
400
300
200
100
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G482 - 1MPK
G482 - 0.3MPK
G486 - 1MPK
G486 - 0.3MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK
+

图27B

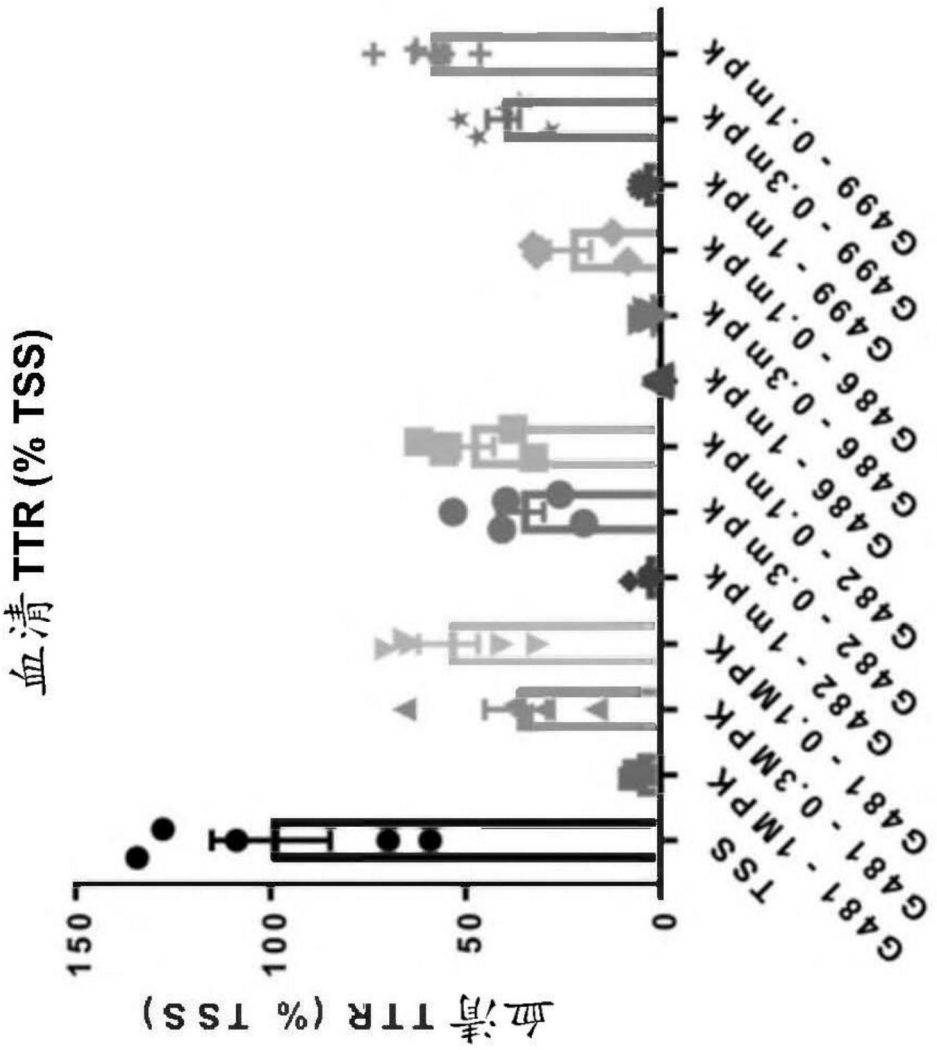

血清 TTR (% TSS)
150
100
50
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G482 - 1MPK
G482 - 0.3MPK
G486 - 1MPK
G486 - 0.3MPK
G499 - 1MPK
G499 - 0.3MPK

图27C

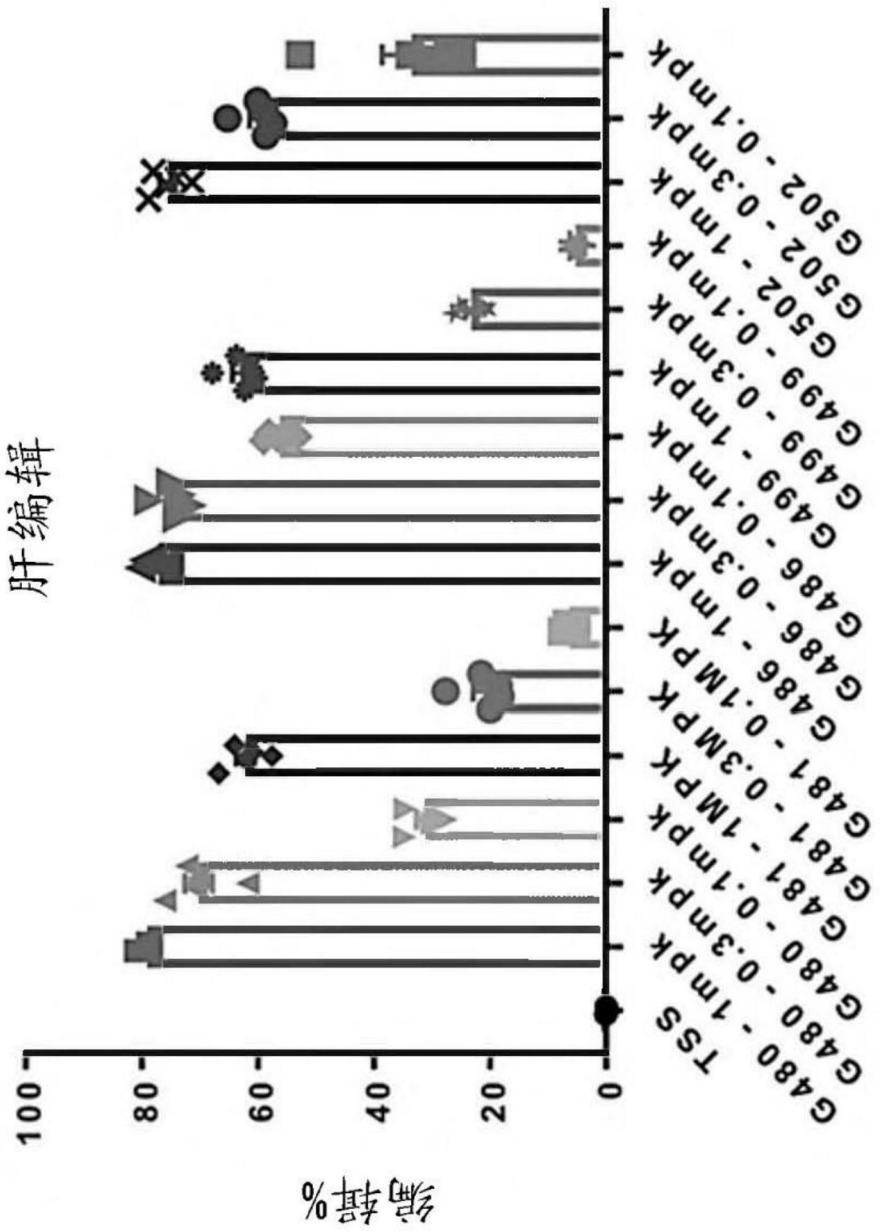

肝编辑
编辑%
100
80
60
40
20
0
TSS
G480 - 1mPk
G480 - 0.3mPk
G480 - 0.1mPk
G481 - 1mPk
G481 - 0.3mPk
G481 - 0.1mPk
G486 - 1mPk
G486 - 0.3mPk
G486 - 0.1mPk
G499 - 1mPk
G499 - 0.3mPk
G499 - 0.1mPk
G502 - 1mPk
G502 - 0.3mPk
G502 - 0.1mPk

图28A

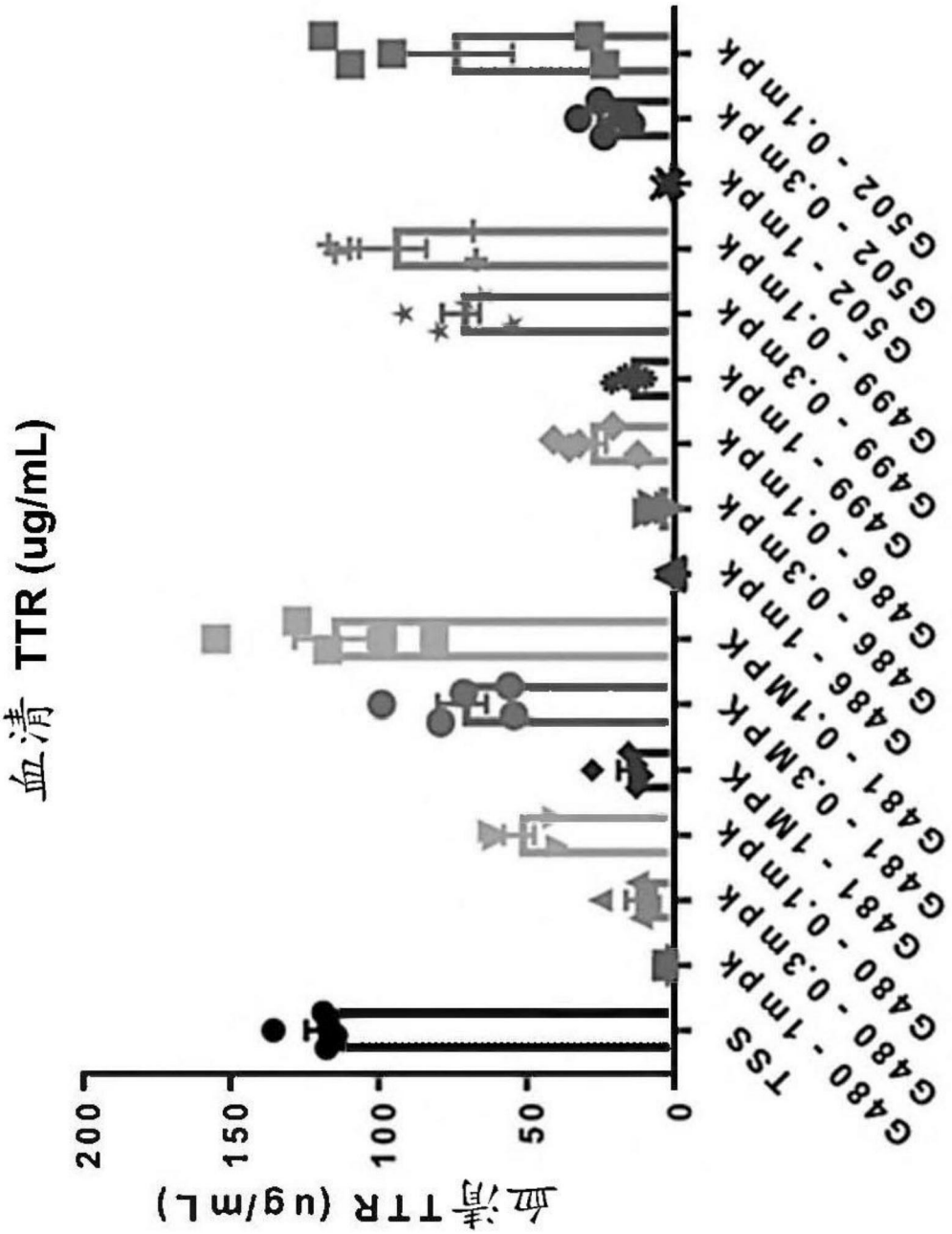

血清 TTR (ug/mL)
200
150
100
50
0
TSS
G480 - 1mpk
G480 - 0.3mpk
G481 - 0.1mpk
G481 - 1mpk
G481 - 0.3mpk
G486 - 0.1mpk
G486 - 1mpk
G486 - 0.3mpk
G499 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G502 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

图28B

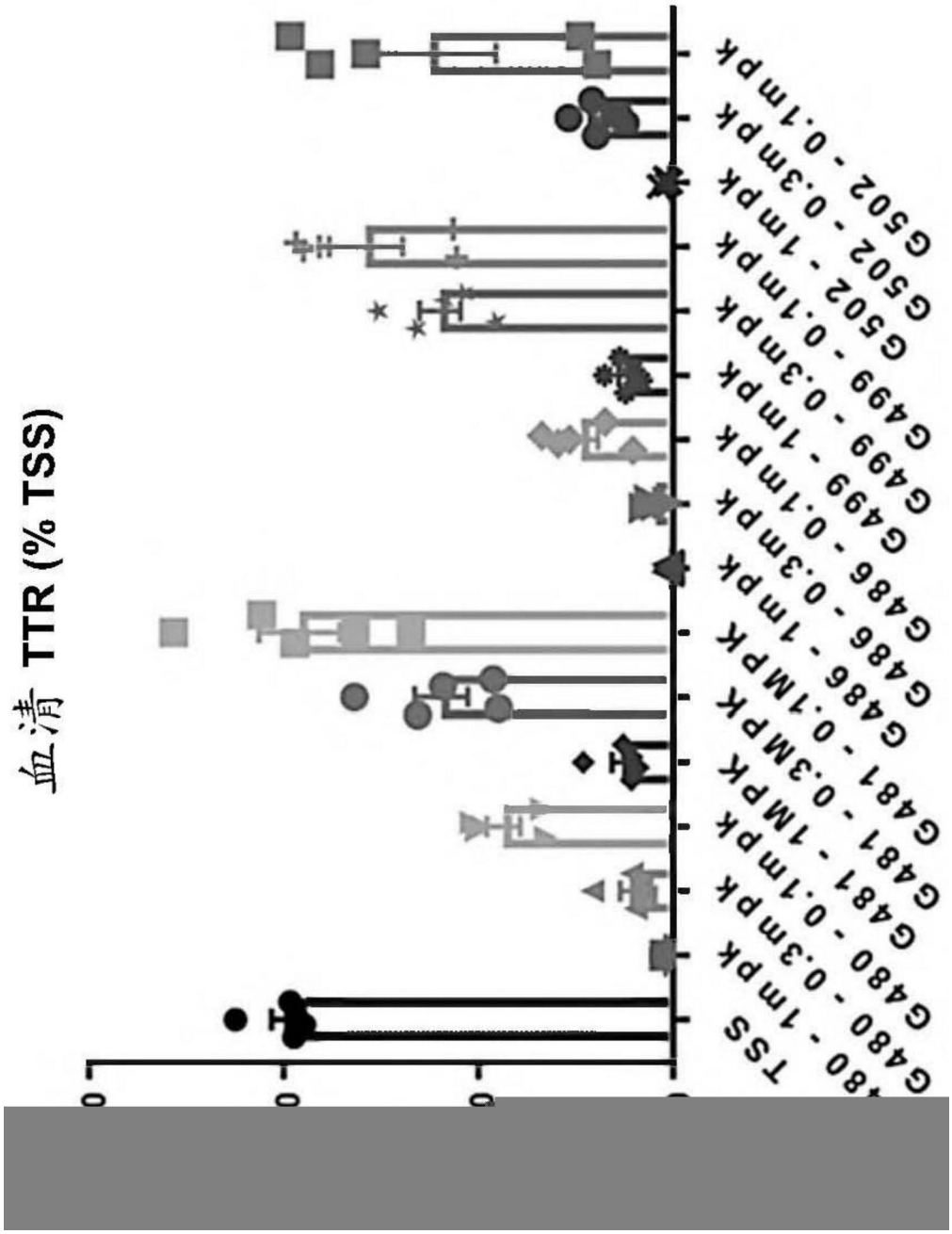

血清 TTR (% TSS)
TSS - 1mpk
G480 - 0.3mpk
G480 - 1mpk
G481 - 0.1mpk
G481 - 1mpk
G481 - 0.3mpk
G486 - 0.1mpk
G486 - 1mpk
G486 - 0.3mpk
G499 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G502 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

图28C

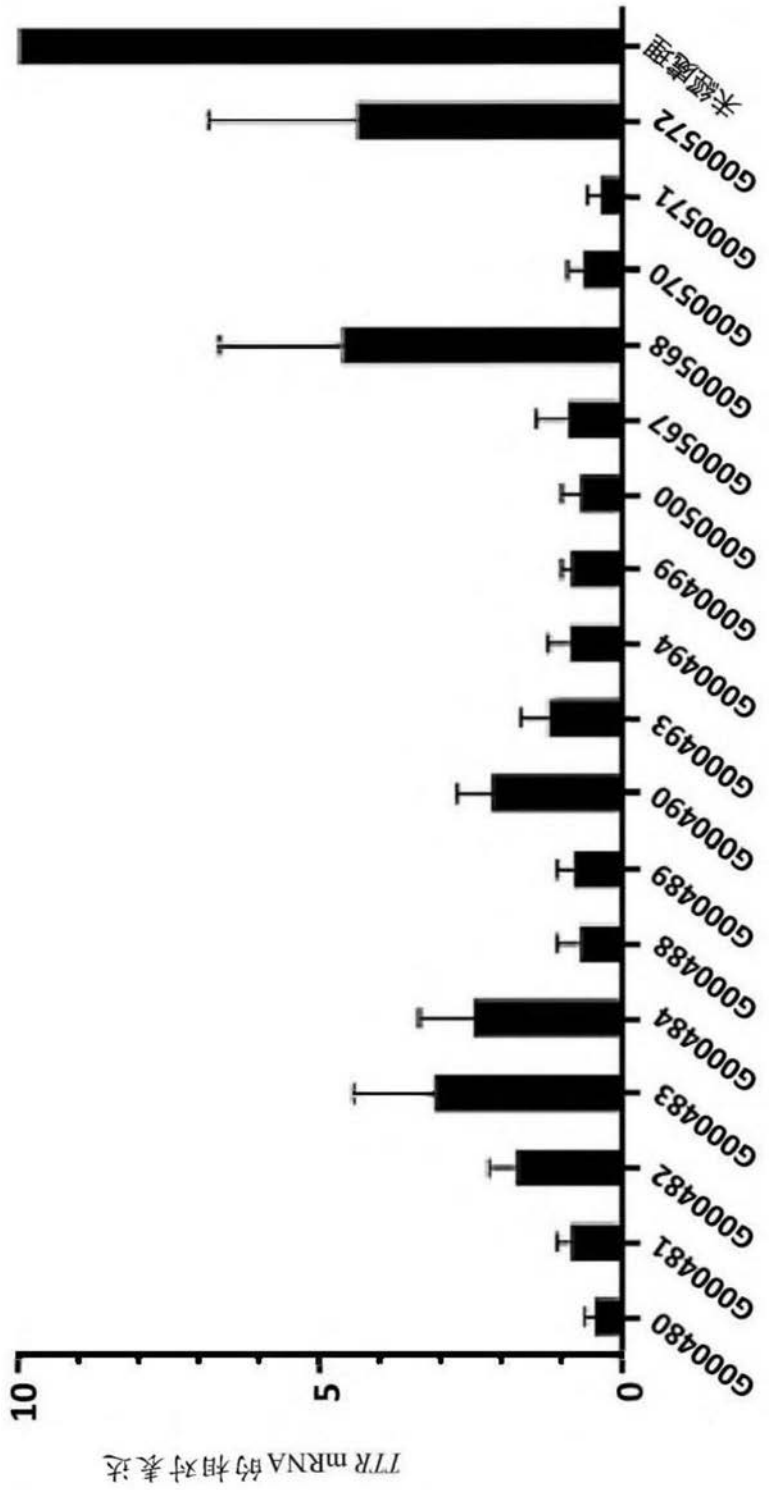

未感染组
G000572
G000571
G000570
G000568
G000567
G000500
G000499
G000494
G000493
G000490
G000489
G000488
G000484
G000483
G000482
G000481
G000480
10
5
0
TTR mRNA的相对表达

图29

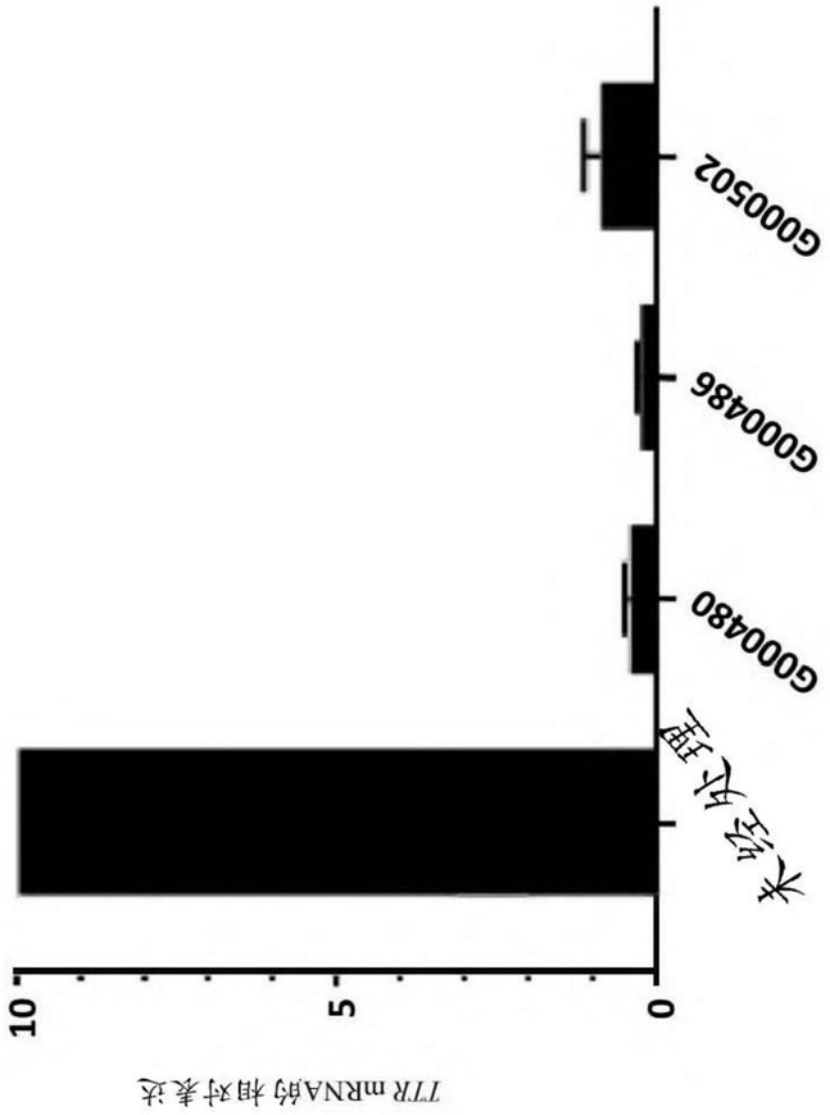


图30