



(12) 发明专利

(10) 授权公告号 CN 112553210 B

(45) 授权公告日 2024. 09. 24

(21) 申请号 202011191794.6
(22) 申请日 2018.10.03
(65) 同一申请的已公布的文献号
申请公布号 CN 112553210 A
(43) 申请公布日 2021.03.26
(30) 优先权数据
62/567,311 2017.10.03 US
62/567,310 2017.10.03 US
62/567,301 2017.10.03 US
62/567,319 2017.10.03 US
62/567,296 2017.10.03 US
(62) 分案原申请数据
201880075846.8 2018.10.03
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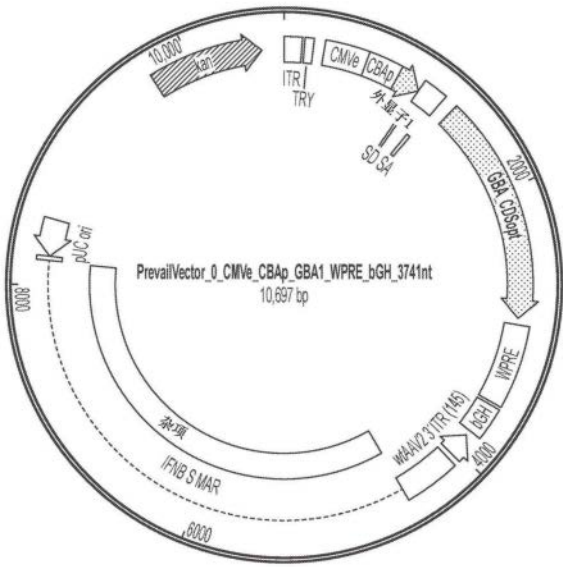
(51) Int.Cl.
C12N 15/12 (2006.01)
C12N 15/864 (2006.01)
C12N 5/10 (2006.01)
A61K 48/00 (2006.01)
A61K 38/47 (2006.01)
A61P 25/28 (2006.01)
(56) 对比文件
WO 2016081927 A2, 2016.05.26
WO 2017151884 A1, 2017.09.08
Teng Jiang等.Upregulation of TREM2
Ameliorates Neuropathology and Rescues
Spatial Cognitive Impairment in a
Transgenic Mouse Model of Alzheimer's
Disease.《Neuropsychopharmacology》.2014,第
39卷第2949-2962页. (续)

审查员 魏应亮

权利要求书3页 说明书30页
序列表173页 附图48页

(54) 发明名称
用于溶酶体障碍的基因疗法

(57) 摘要
本发明涉及用于溶酶体障碍的基因疗法。具体地,本公开在某些方面涉及用于治疗与异常的溶酶体功能相关的疾病例如帕金森氏病(PD)和高雪氏病的组合物和方法。在某些实施方式中,本公开提供了表达构建物,其包含单独的或与一种或多种PD相关基因相组合的编码 β -葡萄糖脑苷脂酶(GBA)或其部分的转入基因。在某些实施方式中,本公开提供了通过将这些表达构建物给药到需要的对象来治疗帕金森氏病的方法。



CN 112553210 B

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(56) 对比文件

Teng Jiang等.Upregulation of TREM2
Ameliorates Neuropathology and Rescues

Spatial Cognitive Impairment in a
Transgenic Mouse Model of Alzheimer's
Disease.《Neuropsychopharmacology》.2014,第
39卷第2949-2962页.

1. 一种分离的核酸,其包含 (i) 包含编码TREM2蛋白和Gcase蛋白的转入基因的表达式构建物,其中所述TREM2蛋白由SEQ ID NO:58中阐述的核酸序列编码,其中所述Gcase蛋白由SEQ ID NO:15中阐述的核酸序列编码;以及 (ii) 在所述表达式构建物两侧的两个腺相关病毒反向末端重复 (ITR) 序列。

2. 根据权利要求1所述的分离的核酸,其中所述转入基因被可操作连接到启动子。

3. 根据权利要求2所述的分离的核酸,其中所述启动子是鸡 β 肌动蛋白 (CBA) 启动子。

4. 根据权利要求2所述的分离的核酸,其还包含CMV增强子。

5. 根据权利要求1所述的分离的核酸,其还包含土拨鼠肝炎病毒转录后调控元件 (WPRE)。

6. 根据权利要求1所述的分离的核酸,其还包含牛生长激素polyA信号尾部。

7. 根据权利要求1所述的分离的核酸,其中每个ITR序列是野生型AAV2 ITR序列。

8. 根据权利要求1所述的分离的核酸,其中每个ITR序列包含位于所述转入基因的近端的序列为SEQ ID NO:27的“D”区。

9. 根据权利要求1所述的分离的核酸,其中所述ITR序列中的至少一者包含相对于所述转入基因位于所述ITR序列外侧的序列为SEQ ID NO:27的“D”区。

10. 根据权利要求1所述的分离的核酸,其中相对于所述转入基因位于5' 的ITR序列包含位于所述转入基因的近端的序列为SEQ ID NO:27的“D”区,并且相对于所述转入基因位于3' 的ITR序列包含相对于所述转入基因位于所述ITR序列外侧的序列为SEQ ID NO:27的“D”区。

11. 根据权利要求10所述的分离的核酸,其中5' ITR的核酸序列是SEQ ID NO:1的第1-145位核苷酸,并且3' ITR的核酸序列是SEQ ID NO:1的第3867-4011位核苷酸。

12. 根据权利要求11所述的分离的核酸,其还包含在所述5' ITR与所述转入基因之间的TRY区,其中所述TRY区具有SEQ ID NO:28中阐述的序列。

13. 一种重组腺相关病毒 (rAAV) 载体,其包括包含两侧带有两个腺相关病毒 (AAV) 反向末端重复序列 (ITR) 的包含编码TREM2蛋白和Gcase蛋白的转入基因的表达式构建物的核酸,其中所述TREM2蛋白由SEQ ID NO:58中阐述的核酸序列编码,并且所述Gcase蛋白由SEQ ID NO:15中阐述的核酸序列编码。

14. 根据权利要求13所述的rAAV载体,其中所述转入基因被可操作连接到启动子。

15. 根据权利要求14所述的rAAV载体,其中所述启动子是鸡 β 肌动蛋白 (CBA) 启动子。

16. 根据权利要求14所述的rAAV载体,其还包含CMV增强子。

17. 根据权利要求13所述的rAAV载体,其还包含土拨鼠肝炎病毒转录后调控元件 (WPRE)。

18. 根据权利要求13所述的rAAV载体,其还包含牛生长激素polyA信号尾部。

19. 根据权利要求13所述的rAAV载体,其中每个ITR序列是野生型AAV2 ITR序列。

20. 根据权利要求13所述的rAAV载体,其中每个ITR序列包含位于所述转入基因的近端的序列为SEQ ID NO:27的“D”区。

21. 根据权利要求13所述的rAAV载体,其中所述ITR序列中的至少一者包含相对于所述转入基因位于所述ITR序列外侧的序列为SEQ ID NO:27的“D”区。

22. 根据权利要求13所述的rAAV载体,其中相对于所述转入基因位于5' 的ITR序列包含

位于所述转入基因的近端的序列为SEQ ID NO:27的“D”区,并且相对于所述转入基因位于3'的ITR序列包含相对于所述转入基因位于所述ITR序列外侧的序列为SEQ ID NO:27的“D”区。

23.根据权利要求13所述的rAAV载体,其中5' ITR的核酸序列是SEQ ID NO:1的第1-145位核苷酸,并且3' ITR的核酸序列是SEQ ID NO:1的第3867-4011位核苷酸。

24.根据权利要求23所述的rAAV载体,其还包含在所述5' ITR与所述转入基因之间的TRY区,其中所述TRY区具有SEQ ID NO:28中阐述的序列。

25.一种重组腺相关病毒(rAAV),其包含:

(i) AAV衣壳蛋白;和

(ii) 根据权利要求13所述的rAAV载体。

26.根据权利要求25所述的rAAV,其中所述AAV衣壳蛋白是AAV9衣壳蛋白。

27.一种重组腺相关病毒(rAAV)载体,其包含核酸,所述核酸以5'至3'顺序包含:

(a) 5' AAV ITR;

(b) CMV增强子;

(c) CBA启动子;

(d) 编码TREM2蛋白和Gcase蛋白的转入基因,其中所述TREM2蛋白由SEQ ID NO:58中阐述的核酸序列编码,其中所述Gcase蛋白由SEQ ID NO:15中阐述的核酸序列编码;

(e) WPRE;

(f) 牛生长激素polyA信号尾部;和

(g) 3' AAV ITR。

28.一种重组腺相关病毒(rAAV),其包含:

(i) AAV衣壳蛋白;和

(ii) 根据权利要求27所述的rAAV载体。

29.根据权利要求28所述的rAAV,其中所述AAV衣壳蛋白是AAV9衣壳蛋白。

30.一种质粒,其包含根据权利要求13所述的rAAV载体。

31.一种杆状病毒载体,其包含SEQ ID NO:58中阐述的核酸序列以及SEQ ID NO:15中阐述的核酸序列。

32.一种细胞,其包含:

(i) 编码一种或多种腺相关病毒rep蛋白和/或一种或多种腺相关病毒cap蛋白的第一载体;和

(ii) 包含SEQ ID NO:58中阐述的核酸序列以及SEQ ID NO:15中阐述的核酸序列的第二载体。

33.根据权利要求32所述的细胞,其中所述第一载体是质粒,并且所述第二载体是质粒。

34.根据权利要求33所述的细胞,其中所述细胞是哺乳动物细胞。

35.根据权利要求34所述的细胞,其中所述哺乳动物细胞是HEK293细胞。

36.根据权利要求32所述的细胞,其中所述第一载体是杆状病毒载体,并且所述第二载体是杆状病毒载体。

37.根据权利要求34所述的细胞,其中所述细胞是昆虫细胞。

38. 根据权利要求37所述的细胞,其中所述昆虫细胞是SF9细胞。

39. 一种生产根据权利要求25、26、28和29中任一项所述的rAAV的方法,所述方法包括:

(i) 将编码一种或多种腺相关病毒rep蛋白和/或一种或多种腺相关病毒cap蛋白的第一载体以及包含含有SEQ ID NO:58的核苷酸序列以及SEQ ID NO:15中阐述的核酸序列的表达盒的重组AAV载体递送至细胞;

(ii) 在允许包装rAAV的条件下培养所述细胞;以及

(iii) 收获培养的宿主细胞或培养基以收集所述rAAV。

40. 根据权利要求1-12中任一项所述的分离的核酸、根据权利要求13-24和27中任一项所述的rAAV载体或根据权利要求25、26、28和29中任一项所述的rAAV在制备用于治疗患有或怀疑患有阿尔茨海默病的对象的药物中的用途。

41. 根据权利要求40所述的用途,其中所述药物被配制成用于直接注射到对象的CNS。

42. 根据权利要求41所述的用途,其中所述直接注射是脑内注射、实质内注射、鞘内注射、大池内(ICM)注射或其任何组合。

43. 根据权利要求41所述的用途,其中所述直接注射到对象的CNS包括对流增强递送(CED)。

44. 根据权利要求40所述的用途,其中所述药物被配制成用于外周注射。

45. 根据权利要求44所述的用途,其中所述外周注射是静脉内注射。

用于溶酶体障碍的基因疗法

[0001] 本申请是国际申请日2018年10月3日、国际申请号PCT/US2018/054227于2020年5月22日进入中国国家阶段、申请号201880075846.8、发明名称“用于溶酶体障碍的基因疗法”的申请的分案申请。

[0002] 相关申请

[0003] 本申请要求2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的美国临时申请系列号62/567,296、2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,311、2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,319、2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,301和2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,310在35U.S.C.119(e)下的利益,每个申请的全部内容通过引用并入本文。

背景技术

[0004] 高雪氏病是由于溶酶体酸性 β -葡萄糖脑苷脂酶(Gcase,“GBA”)缺陷而引起的糖鞘脂代谢的罕见先天性错误。患者遭受非CNS症状和发现,包括肝脾肿大、导致全血细胞减少症的骨髓机能不全、肺部障碍和纤维化以及骨骼缺损。另外,大量患者遭受神经表现,包括有缺陷的扫视性眼球运动和凝视、痫性发作、认知缺陷、发育迟缓以及包括帕金森氏病在内的运动障碍。

[0005] 存在几种针对外周疾病以及造血骨髓和内脏的主要临床表现的疗法,包括如下所述的酶替代疗法,与缺陷型Gcase结合并提高稳定性的伴侣蛋白样小分子药物,以及阻断在高雪氏病中积累、导致症状和发现的底物的生产的底物降低疗法。然而,高雪氏病的其他方面(特别是影响骨骼和脑的方面)似乎难以治疗。

发明内容

[0006] 除了高雪氏病患者(其在GBA1基因的两个染色体等位基因中具有突变)之外,在GBA1的仅仅一个等位基因中具有突变的患者也具有高度增加的帕金森氏病(PD)风险。包括步态困难、静息时震颤、僵硬以及经常抑郁、睡眠困难和认知能力下降在内的PD症状的严重性与酶活性降低的程度有关。因此,高雪氏病患者具有最严重的病程,而在GBA1中具有单个轻度突变的患者通常具有更为良性的病程。突变携带者也具有其他PD相关障碍(包括特征为执行功能障碍、精神病和PD样运动障碍的路易体痴呆症,以及伴有特征性运动和认知障碍的多系统萎缩症)的高风险。不存在改变这些障碍的不可阻挡的病程的疗法。

[0007] 酶例如Gcase(例如GBA1基因的基因产物)的缺陷以及与溶酶体功能或大分子向溶酶体的转运有关的许多基因(例如溶酶体膜蛋白1(LIMP),也被称为SCARB2)中的常见变体,与PD风险提高相关。本公开部分是基于编码一种或多种PD相关基因例如Gcase、GBA2、鞘脂

激活蛋白原 (prosaposin)、颗粒蛋白前体 (progranulin)、LIMP2、GALC、CTSB、SMPD1、GCH1、RAB7、VPS35、IL-34、TREM2、TMEM106B或任何上述基因 (或其部分) 的组合的表达构建物 (例如载体)。在某些实施方式中, 当在对象中表达时, 本文中描述的基因产物的组合一起发挥作用 (例如协同作用), 以减轻PD的一种或多种征兆和症状。

[0008] 因此, 在某些情况下, 本公开提供了一种分离的核酸, 其包含编码Gcase (例如GBA1基因的基因产物) 的表达构建物。在某些实施方式中, 所述分离的核酸包含已被密码子优化 (例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化) 的Gcase编码序列。在某些实施方式中, 所述编码Gcase的核酸序列编码包含SEQ ID NO:14中阐述的 (例如在NCBI参考序列NP_000148.2中阐述的) 氨基酸序列的蛋白质。在某些实施方式中, 所述分离的核酸包含SEQ ID NO:15中阐述的序列。在某些实施方式中, 所述表达构建物包含腺相关病毒 (AAV) 反向末端重复序列 (ITR), 例如在所述编码Gcase蛋白的核酸序列两侧的AAV ITR。

[0009] 在某些情况下, 本公开提供了一种分离的核酸, 其包含编码鞘脂激活蛋白原 (例如PSAP基因的基因产物) 的表达构建物。在某些实施方式中, 所述分离的核酸包含已被密码子优化 (例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化) 的鞘脂激活蛋白原编码序列。在某些实施方式中, 所述编码鞘脂激活蛋白原的核酸序列编码包含SEQ ID NO:16中阐述的 (例如在NCBI参考序列NP_002769.1中阐述的) 氨基酸序列的蛋白质。在某些实施方式中, 所述分离的核酸包含SEQ ID NO:17中阐述的序列。在某些实施方式中, 所述表达构建物包含腺相关病毒 (AAV) 反向末端重复序列 (ITR), 例如在所述编码鞘脂激活蛋白原蛋白的核酸序列两侧的AAV ITR。

[0010] 在某些情况下, 本公开提供了一种分离的核酸, 其包含编码LIMP2/SCARB2 (例如SCARB2基因的基因产物) 的表达构建物。在某些实施方式中, 所述分离的核酸包含已被密码子优化 (例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化) 的SCARB2编码序列。在某些实施方式中, 所述编码LIMP2/SCARB2的核酸序列编码包含SEQ ID NO:18中阐述的 (例如在NCBI参考序列NP_005497.1中阐述的) 氨基酸序列的蛋白质。在某些实施方式中, 所述分离的核酸包含SEQ ID NO:29中阐述的序列。在某些实施方式中, 所述表达构建物包含腺相关病毒 (AAV) 反向末端重复序列 (ITR), 例如在所述编码SCARB2蛋白的核酸序列两侧的AAV ITR。

[0011] 在某些情况下, 本公开提供了一种分离的核酸, 其包含编码GBA2蛋白 (例如GBA2基因的基因产物) 的表达构建物。在某些实施方式中, 所述分离的核酸包含已被密码子优化 (例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化) 的GBA2编码序列。在某些实施方式中, 所述编码GBA2的核酸序列编码包含SEQ ID NO:30中阐述的 (例如在NCBI参考序列NP_065995.1中阐述的) 氨基酸序列的蛋白质。在某些实施方式中, 所述分离的核酸包含SEQ ID NO:31中阐述的序列。在某些实施方式中, 所述表达构建物包含腺相关病毒 (AAV) 反向末端重复序列 (ITR), 例如在所述编码GBA2蛋白的核酸序列两侧的AAV ITR。

[0012] 在某些情况下, 本公开提供了一种分离的核酸, 其包含编码GALC蛋白 (例如GALC基因的基因产物) 的表达构建物。在某些实施方式中, 所述分离的核酸包含已被密码子优化 (例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化) 的GALC编码序列。在某些实施方式中, 所述编码GALC的核酸序列编码包含SEQ ID NO:33中阐述的 (例如在NCBI参考序列NP_000144.2中阐述的) 氨基酸序列的蛋白质。在某些实施方式中, 所述分离的核酸包

含SEQ ID NO:34中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码GALC蛋白的核酸序列两侧的AAV ITR。

[0013] 在某些情况下,本公开提供了一种分离的核酸,其包含编码CTSB蛋白(例如CTSB基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的CTSB编码序列。在某些实施方式中,所述编码CTSB的核酸序列编码包含SEQ ID NO:35中阐述的(例如在NCBI参考序列NP_001899.1中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:36中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码CTSB蛋白的核酸序列两侧的AAV ITR。

[0014] 在某些情况下,本公开提供了一种分离的核酸,其包含编码SMPD1蛋白(例如SMPD1基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的SMPD1编码序列。在某些实施方式中,所述编码SMPD1的核酸序列编码包含SEQ ID NO:37中阐述的(例如在NCBI参考序列NP_000534.3中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:38中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码SMPD1蛋白的核酸序列两侧的AAV ITR。

[0015] 在某些情况下,本公开提供了一种分离的核酸,其包含编码GCH1蛋白(例如GCH1基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的GCH1编码序列。在某些实施方式中,所述编码GCH1的核酸序列编码包含SEQ ID NO:45中阐述的(例如在NCBI参考序列NP_000534.3中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:46中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码GCH1蛋白的核酸序列两侧的AAV ITR。

[0016] 在某些情况下,本公开提供了一种分离的核酸,其包含编码RAB7L蛋白(例如RAB7L基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的RAB7L编码序列。在某些实施方式中,所述编码RAB7L的核酸序列编码包含SEQ ID NO:47中阐述的(例如在NCBI参考序列NP_003920.1中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:48中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码RAB7L蛋白的核酸序列两侧的AAV ITR。

[0017] 在某些情况下,本公开提供了一种分离的核酸,其包含编码VPS35蛋白(例如VPS35基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的VPS35编码序列。在某些实施方式中,所述编码VPS35的核酸序列编码包含SEQ ID NO:49中阐述的(例如在NCBI参考序列NP_060676.2中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:50中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码VPS35蛋白的核酸序列两侧的AAV ITR。

[0018] 在某些情况下,本公开提供了一种分离的核酸,其包含编码IL-34蛋白(例如IL-34基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化

(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的IL-34编码序列。在某些实施方式中,所述编码IL-34的核酸序列编码包含SEQ ID NO:55中阐述的(例如在NCBI参考序列NP_689669.2中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:56中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码IL-34蛋白的核酸序列两侧的AAV ITR。

[0019] 在某些情况下,本公开提供了一种分离的核酸,其包含编码TREM2蛋白(例如TREM2基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的TREM2编码序列。在某些实施方式中,所述编码TREM2的核酸序列编码包含SEQ ID NO:57中阐述的(例如在NCBI参考序列NP_061838.1中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:58中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码TREM2蛋白的核酸序列两侧的AAV ITR。

[0020] 在某些情况下,本公开提供了一种分离的核酸,其包含编码TMEM106B蛋白(例如TMEM106B基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的TMEM106B编码序列。在某些实施方式中,所述编码TMEM106B的核酸序列编码包含SEQ ID NO:63中阐述的(例如在NCBI参考序列NP_060844.2中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:64中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码TMEM106B蛋白的核酸序列两侧的AAV ITR。

[0021] 在某些情况下,本公开提供了一种分离的核酸,其包含编码颗粒蛋白前体(例如PGRN基因的基因产物)的表达构建物。在某些实施方式中,所述分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的颗粒蛋白前体编码序列。在某些实施方式中,所述编码颗粒蛋白前体(PGRN)的核酸序列编码包含SEQ ID NO:67中阐述的(例如在NCBI参考序列NP_002078.1中阐述的)氨基酸序列的蛋白质。在某些实施方式中,所述分离的核酸包含SEQ ID NO:68中阐述的序列。在某些实施方式中,所述表达构建物包含腺相关病毒(AAV)反向末端重复序列(ITR),例如在所述编码颗粒蛋白前体的核酸序列两侧的AAV ITR。

[0022] 在某些情况下,本公开提供了一种分离的核酸,其包含编码第一基因产物和第二基因产物的表达构建物,其中每个基因产物独立地选自表1中阐述的基因产物或其部分。

[0023] 在某些实施方式中,第一基因产物或第二基因产物是Gcase蛋白或其部分。在某些实施方式中,第一基因产物是Gcase蛋白并且第二基因产物选自GBA2、鞘脂激活蛋白原、颗粒蛋白前体、LIMP2、GALC、CTSB、SMPD1、GCH1、RAB7、VPS35、IL-34、TREM2和TMEM106B。

[0024] 在某些实施方式中,表达构建物还编码干扰核酸(例如shRNA、miRNA、dsRNA等)。在某些实施方式中,干扰核酸抑制 α -突触核蛋白(α -突触核蛋白)的表达。在某些实施方式中,靶向 α -突触核蛋白的干扰核酸包含SEQ ID NO:20-25中的任一者中阐述的序列。在某些实施方式中,靶向 α -突触核蛋白的干扰核酸结合到SEQ ID NO:20-25中的任一者中阐述的序列(例如与所述序列杂交)。

[0025] 在某些实施方式中,干扰核酸抑制TMEM106B的表达。在某些实施方式中,靶向

TMEM106B的干扰核酸包含SEQ ID NO:64或65中阐述的序列。在某些实施方式中,靶向TMEM106B的干扰核酸结合到SEQ ID NO:64或65中阐述的序列(例如与所述序列杂交)。

[0026] 在某些实施方式中,表达构建物还包含一个或多个启动子。在某些实施方式中,启动子是鸡 β 肌动蛋白(CBA)启动子、CAG启动子、CD68启动子或JeT启动子。在某些实施方式中,启动子是RNA pol II启动子(例如)或RNA pol III启动子(例如U6等)。

[0027] 在某些实施方式中,表达构建物还包含内部核糖体进入位点(IRES)。在某些实施方式中,IRES位于第一基因产物与第二基因产物之间。

[0028] 在某些实施方式中,表达构建物还包含自切割肽编码序列。在某些实施方式中,自切割肽是T2A肽。

[0029] 在某些实施方式中,表达构建物包含两个腺相关病毒(AAV)反向末端重复(ITR)序列。在某些实施方式中,ITR序列在第一基因产物和第二基因产物两侧(例如,从5'-末端到3'-末端如下所述排列:ITR-第一基因产物-第二基因产物-ITR)。在某些实施方式中,所述分离的核酸的ITR序列之一缺少有功能的末端解链位点(trs)。例如,在某些实施方式中,所述ITR之一是 Δ ITR。

[0030] 在某些情况下,本公开涉及包含具有修饰的“D”区(例如相对于SEQ ID NO:29的野生型AAV2 ITR修饰的D序列)的ITR的rAAV载体。在某些实施方式中,所述具有修饰的D区的ITR是所述rAAV载体的5' ITR。在某些实施方式中,修饰的“D”区包含“S”序列,例如SEQ ID NO:26中所阐述的。在某些实施方式中,所述具有修饰的“D”区的ITR是所述rAAV载体的3' ITR。在某些实施方式中,修饰的“D”区包含3' ITR,其中所述“D”区位于所述ITR的3'末端处(例如相对于所述载体的转入基因插入片段在所述ITR的外侧或末端上)。在某些实施方式中,修饰的“D”区包含SEQ ID NO:26或27中阐述的序列。

[0031] 在某些实施方式中,分离的核酸(例如rAAV载体)包含TRY区。在某些实施方式中,TRY区包含SEQ ID NO:28中阐述的序列。

[0032] 在某些实施方式中,本公开描述的分离的核酸包含SEQ ID NO:1-78中的任一者中阐述的序列或由所述序列构成,或编码具有所述序列的肽。

[0033] 在某些情况下,本公开提供了一种载体,其包含由本公开描述的分离的核酸。在某些实施方式中,载体是质粒或病毒载体。在某些实施方式中,病毒载体是重组AAV(rAAV)载体或杆状病毒载体。在某些实施方式中,rAAV载体是单链的(例如单链DNA)。

[0034] 在某些情况下,本公开提供了一种宿主细胞,其包含由本公开描述的分离的核酸或由本公开描述的载体。

[0035] 在某些情况下,本公开提供了一种重组腺相关病毒(rAAV),其包含衣壳蛋白和由本公开描述的分离的核酸或载体。

[0036] 在某些实施方式中,衣壳蛋白能够跨过血脑屏障,例如AAV9衣壳蛋白或AAVrh.10衣壳蛋白。在某些实施方式中,rAAV转导中枢神经系统(CNS)的神经元细胞和非神经元细胞。

[0037] 在某些情况下,本公开提供了一种用于治疗患有或怀疑患有帕金森氏病的对象的方法,所述方法包括向所述对象给药由本公开描述的组合物(例如包含分离的核酸或载体或rAAV的组合物)。

[0038] 在某些实施方式中,给药包括直接注射到对象的CNS。在某些实施方式中,直接注

射是脑内注射、实质内注射、鞘内注射、大池内注射或其任何组合。在某些实施方式中,直接注射到对象的CNS包括对流增强递送(CED)。

[0039] 在某些实施方式中,给药包括外周注射。在某些实施方式中,外周注射是静脉内注射。

附图说明

[0040] 图1是描绘了包含编码Gcase (例如GBA1或其部分) 的表达构建物的载体的一个实施方式的示意图。

[0041] 图2是描绘了包含编码Gcase (例如GBA1或其部分) 和LIMP2 (SCARB2) 或其部分的表达构建物的载体的一个实施方式的示意图。所述Gcase和LIMP2的编码序列被内部核糖体进入位点(IRES) 分隔开。

[0042] 图3是描绘了包含编码Gcase (例如GBA1或其部分) 和LIMP2 (SCARB2) 或其部分的表达构建物的载体的一个实施方式的示意图。所述Gcase和LIMP2的编码序列的表达各自由分开的启动子驱动。

[0043] 图4是描绘了包含编码Gcase (例如GBA1或其部分) 、LIMP2 (SCARB2) 或其部分和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。

[0044] 图5是描绘了包含编码Gcase (例如GBA1或其部分) 、鞘脂激活蛋白原(例如PSAP或其部分) 和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。

[0045] 图6是描绘了包含编码Gcase (例如GBA1或其部分) 和鞘脂激活蛋白原(例如PSAP或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和鞘脂激活蛋白原的编码序列被内部核糖体进入位点(IRES) 分隔开。

[0046] 图7是描绘了包含编码Gcase (例如GBA1或其部分) 的表达构建物的载体的一个实施方式的示意图。在这个实施方式中,所述载体包含CBA启动子元件(CBA),其由CMV增强子(CMVe)、CBA启动子(CBAp)、外显子1和内含子(int) 四个部分构成,以组成性表达人类GBA1的密码子优化的编码序列。所述3' 区还含有WPRES调控元件,其后跟有bGH polyA尾部。在所述启动子区的5' 末端处包括三个转录调控激活位点:TATA,RBS和YY1。两侧的ITR允许居间序列的正确包装。评估了5' ITR序列的两种变体(插入图框);它们在野生型AAV2 ITR的20个核苷酸的“D”区内具有几个核苷酸的差异。在某些实施方式中,rAAV载体含有在上方行中示出的“D”结构域核苷酸序列。在某些实施方式中,rAAV载体包含突变的“D”结构域(例如“S”结构域,其具有在下方行中示出的核苷酸变化)。

[0047] 图8是描绘了图6中描述的载体的一个实施方式的示意图。

[0048] 图9示出了在帕金森氏病的CBE小鼠模型中递送包含编码Gcase (例如GBA1或其部分) 的转入基因的rAAV的代表性数据。在P8时开始每日IP递送PBS介质、25mg/kg CBE、37.5mg/kg CBE或50mg/kg CBE(从左至右)。每日两次检查存活率(顶部左侧) 并每日检查体重(顶部右侧)。所有组以n=8开始。行为通过P23时的旷场中的总移动距离(底部左侧) 和P24时的在转棒仪上跌落的等待时间(底部中间) 来评估。在PBS和存在(第3天) 和不存在(第1天) CBE停药的25mg/kg CBE治疗组中,分析小鼠皮层中的GCase底物水平。总计的GluSph和GalSph水平(底部右侧) 被显示为每mg组织湿重的pmol数。呈现了平均值。误差条是SEM。*p<0.05;**p<0.01;***p<0.001,通过线性回归获得的治疗组的名义p-值。

[0049] 图10是描绘了CBE小鼠模型中最高rAAV剂量的研究设计的一个实施方式的示意图。简单来说,rAAV在P3时通过ICV注射来递送,并在P8时开始每日CBE治疗。在P24-25时在旷场和转棒仪测定法中评估行为,并在P36和P38时测量底物水平。

[0050] 图11示出了CBE小鼠模型中最高rAAV剂量的终生评估的代表性数据。在P3时,将小鼠用赋形剂或 8.8×10^9 vg rAAV-GBA1通过ICV递送进行治疗。在P8时开始PBS或25mg/kg CBE的每日IP递送。在研究结束时,将一半的小鼠在P36在它们的最后一次CBE给药后一天(第1天)处死,而剩余的一半经历3天的CBE停药,然后在P38(第3天)时处死。所有治疗组(赋形剂+PBS $n=8$, rAAV-GBA1+PBS $n=7$, 赋形剂+CBE $n=8$, 变体+CBE $n=9$) 每日称重(顶部左侧),并对P36时的体重进行分析(顶部右侧)。行为通过P23时的旷场中的总移动距离(底部左侧)和P24时的在转棒仪上跌落的等待时间(底部右侧)来评估,每只动物被评估为3次试验中的中值。由于死亡,用于行为测定的赋形剂+CBE组 $n=7$,而所有其他组 $n=8$ 。呈现了动物中的平均值。误差条是SEM。 $*p<0.05$; $***p<0.001$,在CBE治疗的动物中通过线性回归获得的治疗组的名义 p -值。

[0051] 图12示出了CBE小鼠模型中最高rAAV剂量的生物化学评估的代表性数据。在CBE停药之前(第1天)或之后(第3天),使用所有治疗组(赋形剂+PBS $n=8$, 变体+PBS $n=7$, 赋形剂+CBE $n=7$, 变体+CBE $n=9$) 的皮层来测量各个组中的GCase活性(顶部左侧)、GluSph水平(顶部右侧)、GluCer水平(底部左侧)和载体基因组(底部右侧)。生物分布被显示为每 $1\mu\text{g}$ 基因组DNA的载体基因组量。呈现了平均值。误差条是SEM。 $(*) p<0.1$; $**p<0.01$; $***p<0.001$,在CBE治疗的动物中通过线性回归获得的治疗组的名义 p -值,并将收集天数和性别作为协变量进行了校正。

[0052] 图13示出了在CBE小鼠模型中,在赋形剂+PBS、赋形剂+CBE和变体+CBE治疗组给药后的行为和生物化学相关性的代表性数据。在各个治疗组中,在转棒仪上的表现与GluCer积累负相关(A,通过线性回归 $p=0.0012$),并且GluSph积累与GCase活性的提高负相关(B,通过线性回归 $p=0.0086$)。

[0053] 图14示出了在CBE小鼠模型中变体的生物分布的代表性数据。对于所有治疗组(赋形剂+PBS $n=8$, 变体+PBS $n=7$, 赋形剂+CBE $n=7$, 变体+CBE $n=9$),评估了肝脏、脾脏、肾脏和性腺中载体基因组的存在。生物分布被显示为每 $1\mu\text{g}$ 基因组DNA的载体基因组数。载体基因组的存在通过定量PCR,使用载体参比标准曲线来定量;基因组DNA浓度通过A260光密度测量来评估。呈现了平均值。误差条是SEM。 $*p<0.05$; $**p<0.01$; $***p<0.001$,在CBE治疗的动物中通过线性回归获得的治疗组的名义 p -值,并将收集天数和性别作为协变量进行了校正。

[0054] 图15示出了在CBE小鼠模型中rAAV剂量范围的终生评估的代表性数据。小鼠在P3时通过ICV递送接受赋形剂或三种不同剂量之一的rAAV-GBA1: 3.2×10^9 vg, 1.0×10^{10} vg或 3.2×10^{10} vg。在P8时,开始25mg/kg CBE的每日IP治疗。接受赋形剂和CBE或赋形剂和PBS的小鼠充当对照。所有治疗组以每组 $n=10$ (5M/5F)开始。所有小鼠在它们的最后一次CBE给药后一天(P38-P40)处死。所有治疗组每日称重,并在P36时分析它们的体重。运动表现通过P24时的在转棒仪上跌落的等待时间和P30时的穿越楔形梁的等待时间进行评估。由于早期死亡,参与行为测定小鼠的数目为:赋形剂+PBS $n=10$, 赋形剂+CBE $n=9$, 3.2×10^9 vg rAAV-GBA1+CBE $n=6$, 1.0×10^{10} vg rAAV-GBA1+CBE $n=10$, 3.2×10^{10} vg rAAV-GBA1+CBE $n=7$ 。呈现

了平均值。误差条是SEM;* $p < 0.05$; ** $p < 0.01$, 在CBE治疗组中通过线性回归获得的名义 p -值, 并将性别作为协变量进行了校正。

[0055] 图16示出了在CBE小鼠模型中rAAV剂量范围的生物化学评估的代表性数据。使用所有治疗组(赋形剂+PBS $n=10$, 赋形剂+CBE $n=9$, 3.2×10^9 vg rAAV-GBA1+CBE $n=6$, 1.0×10^{10} vg rAAV-GBA1+CBE $n=10$, 3.2×10^{10} vg rAAV-GBA1+CBE $n=7$)的皮层来测量GCase活性、GluSph水平、GluCer水平和载体基因组。GCase活性被显示为每mg总蛋白的GCase的ng数。GluSph和GluCer水平被显示为每mg组织湿重的pmol数。生物分布被显示为每1 μ g基因组DNA的载体基因组量。载体基因组的存在通过定量PCR, 使用载体参比标准曲线来定量; 基因组DNA浓度通过A260光密度测量来评估。也在肝脏中测量了载体基因组的存在(E)。呈现了平均值。误差条是SEM。 ** $p < 0.01$; *** $p < 0.001$, 在CBE治疗组中通过线性回归获得的名义 p -值, 并将性别作为协变量进行了校正。

[0056] 图17示出了在遗传小鼠模型中, 在最高剂量rAAV-GBA1下的楔形梁分析的代表性数据。在rAAV-GBA1给药后4周通过梁行走试验来测定治疗组(WT+赋形剂($n=5$), 4L/PS-NA+赋形剂($n=6$)和4L/PS-NA+rAAV-GBA1($n=5$))的运动表现。总滑倒数和活动时间被显示为在不同梁上的5次试验的总和。速度和单位速度的滑倒数被显示为在不同梁上的5次试验的平均值。呈现了平均值。误差条是SEM。

[0057] 图18示出了编码颗粒蛋白前体(PGRN)蛋白的rAAV构建物的体外表达的代表性数据。左图示出了颗粒蛋白前体(PGRN)ELISA测定法的标准曲线。下图示出了在用rAAV转导的HEK293T细胞的细胞裂解物中通过ELISA测定法测量的PGRN表达的剂量响应。MOI = 感染复数(每个细胞的载体基因组数)。

[0058] 图19示出了编码GBA1与鞘脂激活蛋白原(PSAP)、SCARB2和/或一种或多种抑制性核酸的組合的rAAV构建物的体外表达的代表性数据。数据表明用每种构建物转染HEK293细胞相对于模拟转染的细胞引起感兴趣的转入基因的过表达。

[0059] 图20是描绘了包含位于ITR的“外侧”(例如相对于转入基因插入片段或表达构建物在ITR的末端的近端)的“D”区的rAAV载体(上图)和具有在载体的“内侧”(例如在载体的转入基因插入片段的近端)的ITR的野生型rAAV载体的示意图。

[0060] 图21是描绘了包含编码GBA2或其部分和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。

[0061] 图22是描绘了包含编码Gcase(例如GBA1或其部分)和半乳糖基神经酰胺酶(例如GALC或其部分)的表达构建物的载体的一个实施方式的示意图。所述Gcase和半乳糖基神经酰胺酶的编码序列的表达被T2A自切割肽序列分隔开。

[0062] 图23是描绘了包含编码Gcase(例如GBA1或其部分)和半乳糖基神经酰胺酶(例如GALC或其部分)的表达构建物的载体的一个实施方式的示意图。所述Gcase和半乳糖基神经酰胺酶的编码序列的表达被T2A自切割肽序列分隔开。

[0063] 图24是描绘了包含编码Gcase(例如GBA1或其部分)、组织蛋白酶B(Cathepsin B)(例如CTSB或其部分)和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。所述Gcase和组织蛋白酶B的编码序列的表达被T2A自切割肽序列分隔开。

[0064] 图25是描绘了包含编码Gcase(例如GBA1或其部分)、鞘磷脂磷酸二酯酶1(例如SMPD1或其部分)和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。

[0065] 图26是描绘了包含编码Gcase (例如GBA1或其部分) 和半乳糖基神经酰胺酶 (例如GALC或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和半乳糖基神经酰胺酶的编码序列被内部核糖体进入位点 (IRES) 分隔开。

[0066] 图27是描绘了包含编码Gcase (例如GBA1或其部分) 和组织蛋白酶B (例如CTSB或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和组织蛋白酶B的编码序列的表达各自由分开的启动子驱动。

[0067] 图28是描绘了包含编码Gcase (例如GBA1或其部分)、GCH1 (例如GCH1或其部分) 和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。所述Gcase和GCH1的编码序列被T2A自切割肽序列分隔开。

[0068] 图29是描绘了包含编码Gcase (例如GBA1或其部分)、RAB7L1 (例如RAB7L1或其部分) 和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。所述Gcase和RAB7L1的编码序列被T2A自切割肽序列分隔开。

[0069] 图30是描绘了包含编码Gcase (例如GBA1或其部分)、GCH1 (例如GCH1或其部分) 和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。所述Gcase和GCH1的编码序列的表达被内部核糖体进入位点 (IRES) 分隔开。

[0070] 图31是描绘了包含编码VPS35 (例如VPS35或其部分) 和用于 α -Syn和TMEM106B的干扰RNA的表达构建物的载体的一个实施方式的示意图。

[0071] 图32是描绘了包含编码Gcase (例如GBA1或其部分)、IL-34 (例如IL34或其部分) 和用于 α -Syn的干扰RNA的表达构建物的载体的一个实施方式的示意图。所述Gcase和IL-34的编码序列由T2A自切割肽序列分隔开。

[0072] 图33是描绘了包含编码Gcase (例如GBA1或其部分) 和IL-34 (例如IL34或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和IL-34的编码序列被内部核糖体进入位点 (IRES) 分隔开。

[0073] 图34是描绘了包含编码Gcase (例如GBA1或其部分) 和TREM2 (例如TREM2或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和TREM2的编码序列的表达各自由分开的启动子驱动。

[0074] 图35是描绘了包含编码Gcase (例如GBA1或其部分) 和IL-34 (例如IL34或其部分) 的表达构建物的载体的一个实施方式的示意图。所述Gcase和IL-34的编码序列的表达各自由分开的启动子驱动。

[0075] 图36A-36B示出了TREM2和GBA1在HEK293细胞中相对于对照转导细胞中的过表达的代表性数据,正如通过qPCR和ELISA所测量的。图36A示出了TREM2的过表达的数据。图36B示出了来自于同一个构建物的GBA1的过表达的数据。

[0076] 图37示出了通过GFP报告物测定法 (上图) 和 α -Syn测定法 (下图) 获得的代表性数据,指示了在体外成功的SCNA沉默。

[0077] 图38示出了通过GFP报告物测定法 (上图) 和 α -Syn测定法 (下图) 获得的代表性数据,指示了在体外成功的TMEM106B沉默。

[0078] 图39是描绘了包含编码PGRN的表达构建物的载体的一个实施方式的示意图。

[0079] 图40示出了使用具有“D”序列的野生型 (圆圈) 或可选 (例如“外侧”;正方形) 放置的ITR的rAAV转导HEK293细胞的数据。具有放置在“外侧”的ITR的rAAV与具有野生型ITR的

rAAV能够同样有效地转导细胞。

详细描述

[0080] 本公开部分是基于用于在对象中表达PD相关基因产物的组合的组合物和方法。基因产物可以是蛋白质、蛋白质的片段(例如部分)、抑制PD相关基因的干扰核酸等。在某些实施方式中,基因产物是由PD相关基因编码的蛋白质或蛋白质片段。在某些实施方式中,基因产物是抑制PD相关基因的干扰核酸(例如shRNA、siRNA、miRNA、amiRNA等)。

[0081] PD相关基因是指编码的基因产物在遗传、生物化学或功能上与PD相关的基因。例如,已观察到在GBA1基因(其编码蛋白Gcase)中具有突变的个体与在GBA1中没有突变的个体相比具有提高的发生PD的风险。在另一个实例中,PD与包含 α -突触核蛋白(α -Syn)的蛋白聚集体的积累相关;因此,SCNA(其编码 α -Syn)是PD相关基因。在某些实施方式中,本文中描述的表达盒编码野生型或非突变形式的PD相关基因(或其编码序列)。PD相关基因的实例列于表1中。

[0082] 表1:PD相关基因的实例

[0083]

名称	基因	功能	NCBI 登记号
溶酶体膜蛋白 2	<i>SCARB2/LIMP2</i>	葡萄糖神经酰胺酶的溶酶体受体 (靶向 GBA)	NP_005497.1 (同工型 1) , NP_001191184.1 (同工型 2)
鞘脂激活蛋白原	<i>PSAP</i>	鞘脂激活蛋白 A、B、C 和 D 的前体, 其定位到溶酶体区室并促进具有短的寡糖基团的糖鞘脂的分解代谢	AAH01503.1 , AAH07612.1 , AAH04275.1 , AAA60303.1
β -葡萄糖脑苷脂酶	<i>GBA1</i>	切割葡萄糖脑苷脂的 β -葡萄糖苷键	NP_001005742.1 (同工型 1) , NP_001165282.1 (同工型 2) , NP_001165283.1 (同工型 3)
非溶酶体葡萄糖神经酰胺酶	<i>GBA2</i>	催化葡萄糖神经酰胺转变成游离葡萄糖和神经酰胺	NP_065995.1 (同工型 1) , NP_001317589.1 (同工型 2)
半乳糖基神经酰胺酶	<i>GALC</i>	从神经酰胺衍生物除去半乳糖	EAW81359.1 (同工型 CRA_a) , EAW81360.1 (同工型 CRA_b) , EAW81362.1 (同工型 CRA_c)
鞘磷脂磷酸二酯酶 1	<i>SMPD1</i>	将鞘磷脂转变成神经酰胺	EAW68726.1 (同工型 CRA_a) , EAW68727.1 (同工型 CRA_b) , EAW68728.1 (同工型 CRA_c) , EAW68729.1 (同工型 CRA_d)
组织蛋白酶 B	<i>CTSB</i>	据信参与蛋白质的细胞内降解和更新的硫醇蛋白酶; 也与肿瘤侵袭和转移有关	AAC37547.1 , AAH95408.1 , AAH10240.1
RAB7, RAS 癌	<i>RAB7L1</i>	调节囊泡运输	AAH02585.1

[0084]

基因家族样蛋白 1 的成员			
液泡蛋白分选相关蛋白 35	<i>VPS35</i>	逆运体货物蛋白选择性复合体的组分	NP_060676.2
GTP 环水解酶 1	<i>GCH1</i>	负责三磷酸鸟苷的水解以形成 7.8-二氢新蝶呤三磷酸	AAH25415.1
白介素 34	<i>IL34</i>	增加单核细胞的生长或存活；通过结合集落刺激因子 1 受体来引发活性	AAH29804.1
在髓样细胞上表达的触发受体 2	<i>TREM2</i>	与 TYRO 蛋白酪氨酸激酶结合蛋白形成受体信号转导复合物；在免疫应答中起作用，并可能参与慢性炎症	AAF69824.1
颗粒蛋白前体	<i>PGRN</i>	在发育、炎症、细胞增殖和蛋白质体内平衡中发挥作用	NP_002087.1

[0085] 分离的核酸和载体

[0086] 分离的核酸可以是DNA或RNA。在某些情况下,本公开提供了一种分离的核酸(例如rAAV载体),其包含编码一种或多种PD相关基因例如Gcase(例如GBA1基因的基因产物)或其部分的表达构建物。Gcase也被称为 β -葡萄糖脑苷脂酶或GBA,是指一种切割糖脂代谢中的中间体化学物质葡萄糖脑苷脂的 β -葡萄糖苷键的溶酶体蛋白。在人类中,Gcase由位于1号染色体上的GBA1基因编码。在某些实施方式中,GBA1编码由NCBI参考序列NP_000148.2 (SEQ ID NO:14) 表示的肽。在某些实施方式中,分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的Gcase编码序列,例如SEQ ID NO:15中阐述的序列。

[0087] 在某些情况下,本公开提供了一种分离的核酸,其包含编码鞘脂激活蛋白原(例如PSAP基因的基因产物)的表达构建物。鞘脂激活蛋白原是鞘脂活化蛋白(鞘脂激活蛋白)A、B、C和D的前体糖蛋白,其促进具有短的寡糖基团的糖鞘脂的分解代谢。在人类中,所述PSAP基因位于10号染色体上。在某些实施方式中,PSAP编码由NCBI参考序列NP_002769.1 (例如SEQ ID NO:16) 表示的肽。在某些实施方式中,分离的核酸包含已被密码子优化(例如为了在哺乳动物细胞例如人类细胞中表达而被密码子优化)的鞘脂激活蛋白原编码序列,例如SEQ ID NO:17中阐述的序列。

[0088] 本公开的某些方面涉及一种分离的核酸,其包含编码LIMP2/SCARB2(例如SCARB2基因的基因产物)的表达构建物。SCARB2是指一种调控细胞内的溶酶体和内体运输的膜蛋白。在人类中,SCARB2基因位于4号染色体上。在某些实施方式中,所述SCARB2基因编码由NCBI参考序列NP_005497.1 (SEQ ID NO:18) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:19中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的SCARB2编码序列。

[0089] 本公开的某些方面涉及一种分离的核酸,其包含编码GBA2蛋白(例如GBA2基因的基因产物)的表达构建物。GBA2蛋白是指一种非溶酶体葡萄糖神经酰胺酶。在人类中,GBA2基因位于9号染色体上。在某些实施方式中,所述GBA2基因编码由NCBI参考序列NP_065995.1 (SEQ ID NO:30)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:31中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的GBA2编码序列。

[0090] 本公开的某些方面涉及一种分离的核酸,其包含编码GALC蛋白(例如GALC基因的基因产物)的表达构建物。GALC蛋白是指半乳糖基神经酰胺酶(或半乳糖脑苷脂酶),其是一种水解半乳糖脑苷脂、半乳糖基鞘氨醇、乳糖基神经酰胺和单半乳糖基甘油二酸酯的半乳糖酯键的酶。在人类中,GALC基因位于14号染色体上。在某些实施方式中,所述GALC基因编码由NCBI参考序列NP_000144.2 (SEQ ID NO:33)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:34中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的GALC编码序列。

[0091] 本公开的某些方面涉及一种分离的核酸,其包含编码CTSB蛋白(例如CTSB基因的基因产物)的表达构建物。CTSB蛋白是指组织蛋白酶B,其是一种溶酶体半胱氨酸蛋白酶,在细胞内蛋白水解中发挥重要作用。在人类中,CTSB基因位于8号染色体上。在某些实施方式中,所述CTSB基因编码由NCBI参考序列NP_001899.1 (SEQ ID NO:35)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:36中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的CTSB编码序列。

[0092] 本公开的某些方面涉及一种分离的核酸,其包含编码SMPD1蛋白(例如SMPD1基因的基因产物)的表达构建物。SMPD1蛋白是指鞘磷脂磷酸二酯酶1,其是一种参与鞘脂代谢的水解酶。在人类中,SMPD1基因位于11号染色体上。在某些实施方式中,所述SMPD1基因编码由NCBI参考序列NP_000534.3 (SEQ ID NO:37)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:38中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的SMPD1编码序列。

[0093] 本公开的某些方面涉及一种分离的核酸,其包含编码GCH1蛋白(例如GCH1基因的基因产物)的表达构建物。GCH1蛋白是指GTP环水解酶I,其是一种作为叶酸和生物蝶呤生物合成途径的一部分的水解酶。在人类中,GCH1基因位于14号染色体上。在某些实施方式中,所述GCH1基因编码由NCBI参考序列NP_000152.1 (SEQ ID NO:45)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:46中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的GCH1编码序列。

[0094] 本公开的某些方面涉及一种分离的核酸,其包含编码RAB7L蛋白(例如RAB7L基因的基因产物)的表达构建物。RAB7L蛋白是指RAB7, RAS癌基因家族样蛋白1的成员,是一种GTP结合蛋白。在人类中,RAB7L基因位于1号染色体上。在某些实施方式中,所述RAB7L基因编码由NCBI参考序列NP_003920.1 (SEQ ID NO:47)表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:48中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的RAB7L编码序列。

[0095] 本公开的某些方面涉及一种分离的核酸,其包含编码VPS35蛋白(例如VPS35基因的基因产物)的表达构建物。VPS35蛋白是指液泡蛋白分选相关蛋白35,其是参与蛋白质从内体向反向高尔基体网络的逆行转运的蛋白质复合体的一部分。在人类中,VPS35基因位于

16号染色体上。在某些实施方式中,所述VPS35基因编码由NCBI参考序列NP_060676.2 (SEQ ID NO:49) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:50中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的VPS35编码序列。

[0096] 本公开的某些方面涉及一种分离的核酸,其包含编码IL-34蛋白(例如IL34基因的基因产物)的表达构建物。IL-34蛋白是指白介素34,其是一种增加单核细胞的生长和存活的细胞因子。在人类中,IL34基因位于16号染色体上。在某些实施方式中,所述IL34基因编码由NCBI参考序列NP_689669.2 (SEQ ID NO:55) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:56中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的IL-34编码序列。

[0097] 本公开的某些方面涉及一种分离的核酸,其包含编码TREM2蛋白(例如TREM2基因的基因产物)的表达构建物。TREM2蛋白是指在髓样细胞上表达的触发受体2,其是存在于髓样细胞上的一种免疫球蛋白超家族受体。在人类中,TREM2基因位于6号染色体上。在某些实施方式中,所述TREM2基因编码由NCBI参考序列NP_061838.1 (SEQ ID NO:57) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:58中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的TREM2编码序列。

[0098] 本公开的某些方面涉及一种分离的核酸,其包含编码TMEM106B蛋白(例如TMEM106B基因的基因产物)的表达构建物。TMEM106B蛋白是指跨膜蛋白106B,其是参与树突形态发生和溶酶体运输的调控的一种蛋白质。在人类中,TMEM106B基因位于7号染色体上。在某些实施方式中,所述TMEM106B基因编码由NCBI参考序列NP_060844.2 (SEQ ID NO:62) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:63中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的TMEM106B编码序列。

[0099] 本公开的某些方面涉及一种分离的核酸,其包含编码颗粒蛋白前体蛋白(例如PRGN基因的基因产物)的表达构建物。PGRN蛋白是指颗粒蛋白前体,其是参与发育、炎症、细胞增殖和蛋白质体内平衡的一种蛋白质。在人类中,PRGN基因位于17号染色体上。在某些实施方式中,所述PRGN基因编码由NCBI参考序列NP_002078.1 (SEQ ID NO:67) 表示的肽。在某些实施方式中,分离的核酸包含SEQ ID NO:68中阐述的序列。在某些实施方式中,所述分离的核酸包含已被密码子优化的PGRN编码序列。

[0100] 在某些情况下,本公开提供了一种分离的核酸,其包含编码第一基因产物和第二基因产物的表达构建物,其中每个基因产物独立地选自表1中阐述的基因产物或其部分。

[0101] 在某些实施方式中,基因产物由天然存在的基因的编码部分(例如cDNA) 编码。在某些实施方式中,第一基因产物是由GBA1基因编码的蛋白质(或其片段)。在某些实施方式中,基因产物是由表1中列出的另一个基因例如SCARB2/LIMP2基因或PSAP基因编码的蛋白质(或其片段)。然而,专业技术人员认识到第一基因产物(例如Gcase) 和第二基因产物(例如LIMP2等) 的表达顺序通常可以颠倒(例如LIMP2是第一基因产物,并且Gcase是第二基因产物)。在某些实施方式中,基因产物是表1中列出的基因的片段(例如部分)。蛋白质片段可以包含由表1中列出的基因编码的蛋白质的约50%、约60%、约70%、约80%、约90%或约99%。在某些实施方式中,蛋白质片段包含由表1中列出的基因编码的蛋白质的50%至99.9%之间(例如50%至99.9%之间的任何值)。

[0102] 在某些实施方式中,表达构建物是单顺反子的(例如所述表达构建物编码包含第

一基因产物和第二基因产物的单一融合蛋白)。在某些实施方式中,表达构建物是多顺反子的(例如所述表达构建物编码两个不同的基因产物,例如两个不同的蛋白质或蛋白质片段)。

[0103] 多顺反子表达载体可以包含一个或多个(例如1、2、3、4、5个或更多个)启动子。可以使用任何适合的启动子,例如组成型启动子、诱导型启动子、内源启动子、组织特异性启动子(例如CNS特异性启动子)等。在某些实施方式中,启动子是鸡 β -肌动蛋白启动子(CBA启动子)、CAG启动子(例如由Alexopoulou等,(2008)BMC Cell Biol.9:2;doi:10.1186/1471-2121-9-2所描述的)、CD68启动子或JeT启动子(例如由Tornøe等,(2002)Gene 297 (1-2):21-32所描述的)。在某些实施方式中,启动子被可操作连接到编码第一基因产物、第二基因产物或第一基因产物和第二基因产物的核酸序列。在某些实施方式中,表达盒包含一个或多个另外的调控序列,包括但不限于转录因子结合序列、内含子剪接位点、poly(A)添加位点、增强子序列、阻遏蛋白结合位点或上述序列的任何组合。

[0104] 在某些实施方式中,编码第一基因产物的核酸序列和编码第二基因产物的核酸序列被编码内部核糖体进入位点(IRES)的核酸序列分隔开。IRES位点的实例被例如Mokrejs等,(2006)Nucleic Acids Res.34(Database issue):D125-30描述。在某些实施方式中,编码第一基因产物的核酸序列和编码第二基因产物的核酸序列被编码自切割肽的核酸序列分隔开。自切割肽的实例包括但不限于T2A、P2A、E2A、F2A、BmCPV 2A和BmIFV 2A,以及被Liu等,(2017)Sci Rep.7:2193所描述的那些。在某些实施方式中,所述自切割肽是T2A肽。

[0105] 在病理学上,诸如PD和高雪氏病的障碍与主要由 α -突触核蛋白(α -Syn)构成的蛋白聚集体的积累相关。因此,在某些实施方式中,本文中描述的分离的核酸包含降低或阻止 α -Syn蛋白的表达的抑制性核酸。编码抑制性核酸的序列可以被放置在表达载体的非翻译区(例如内含子、5' UTR、3' UTR等)中。

[0106] 在某些实施方式中,抑制性核酸被放置在表达构建物的内含子中,例如在编码第一基因产物的序列上游的内含子中。抑制性核酸可以是双链RNA(dsRNA)、siRNA、micro RNA(miRNA)、人工miRNA(amiRNA)或RNA适体。通常,抑制性核酸结合到靶RNA(例如mRNA)的约6至约30个之间(例如6至30之间的任何整数(含))的连续核苷酸(例如与所述连续核苷酸杂交)。在某些实施方式中,所述抑制性核酸核酸是miRNA或amiRNA,例如靶向SNCA(编码 α -Syn蛋白的基因)或TMEM106B(例如编码TMEM106B蛋白的基因)的miRNA。在某些实施方式中,所述miRNA不包含与它所杂交的SNCA mRNA的区域的任何错配(例如所述miRNA是“完美的”)。在某些实施方式中,所述抑制性核酸是shRNA(例如靶向SNCA或TMEM106B的shRNA)。在某些实施方式中,抑制性核酸是包括miR-155支架和SNCA或TMEM106B靶向序列的人工miRNA(amiRNA)。

[0107] 本文中描述的分离的核酸可以自身存在或作为载体的一部分存在。通常,载体可以是质粒、粘粒、噬菌粒、细菌人工染色体(BAC)或病毒载体(例如腺病毒载体、腺相关病毒(AAV)载体、反转录病毒载体、杆状病毒载体等)。在某些实施方式中,所述载体是质粒(例如包含本文中描述的分离的核酸的质粒)。在某些实施方式中,rAAV载体是单链的(例如单链DNA)。在某些实施方式中,所述载体是重组AAV(rAAV)载体。在某些实施方式中,载体是杆状病毒载体(例如苜蓿银纹夜蛾(Autographa californica)核多角体病毒(AcNPV)载体)。

[0108] 通常,rAAV载体(例如rAAV基因组)包含两侧带有两个AAV反向末端重复(ITR)序列

的转入基因(例如包含一个或多个下述每一者的表达构建物:启动子,内含子,增强子序列,蛋白编码序列,抑制性RNA编码序列,polyA尾部序列等)。在某些实施方式中,所述rAAV载体的转入基因包含本公开所描述的分离的核酸。在某些实施方式中,所述rAAV载体的两个ITR序列中的每一者是全长ITR(例如长度为约145bp,并含有有功能的Rep结合位点(RBS)和末端解链位点(trs))。在某些实施方式中,所述rAAV载体的ITR之一是截短的(例如缩短或非全长的)。在某些实施方式中,截短的ITR缺少有功能的末端解链位点(trs)并被用于生产自我互补的AAV载体(scAAV载体)。在某些实施方式中,截短的ITR是 Δ ITR,例如由McCarty等,(2003)Gene Ther. 10(26):2112-8所描述的。

[0109] 本公开的某些方面涉及分离的核酸(例如rAAV载体),其包含相对于野生型AAV ITR,例如相对于野生型AAV2 ITR(例如SEQ ID NO:29)具有一个或多个修饰(例如核酸添加、缺失、替换等)的ITR。野生型AAV2 ITR的结构示出在图20中。通常,野生型ITR包含125个核苷酸的区域,其自我退火形成回文双链T形发夹结构,所述发夹结构由两个横臂(分别由被称为B/B'和C/C'的序列形成)、较长的茎区(由序列A/A'形成)和被称为“D”区的单链末端区域构成(图20)。通常,所述ITR的“D”区位于所述由A/A'序列形成的茎区与含有所述rAAV载体的转入基因的插入片段之间(例如相对于ITR的末端位于所述ITR的“内侧”或位于所述rAAV载体的转入基因插入片段或表达构建物的近端)。在某些实施方式中,“D”区包含SEQ ID NO:27中阐述的序列。已观察到所述“D”区在rAAV载体被衣壳蛋白的衣壳化中发挥重要作用,例如由Ling等,(2015)J Mol Genet Med 9(3)所公开的。

[0110] 本公开部分是基于下述令人吃惊的发现:包含位于ITR的“外侧”(例如相对于转入基因插入片段或表达构建物位于ITR的末端的近端)的“D”区的rAAV载体,与ITR具有未修饰的(例如野生型)ITR的rAAV载体相比高效地被AAV衣壳蛋白衣壳化。在某些实施方式中,具有修饰的“D”序列(例如在“外侧”位置中的“D”序列)的rAAV载体相对于具有野生型ITR序列的rAAV载体具有降低的毒性。

[0111] 在某些实施方式中,修饰的“D”序列相对于野生型“D”序列(例如SEQ ID NO:27)包含至少一个核苷酸替换。修饰的“D”序列相对于野生型“D”序列(例如SEQ ID NO:27)可以具有至少1、2、3、4、5、6、7、8、9、10个或超过10个核苷酸替换。在某些实施方式中,修饰的“D”序列相对于野生型“D”序列(例如SEQ ID NO:27)包含至少10、11、12、13、14、15、16、17、18或19个核酸替换。在某些实施方式中,修饰的“D”序列与野生型“D”序列(例如SEQ ID NO:27)具有约10%至约99%之间(例如10%、15%、20%、25%、30%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或99%)的同一性。在某些实施方式中,修饰的“D”序列包含SEQ ID NO:26中阐述的序列,其也被称为“S”序列,正如在Wang等,(1995)J Mol Biol 250(5):573-80中所描述的。

[0112] 本公开描述的分离的核酸或rAAV载体还可以包含“TRY”序列,例如SEQ ID NO:28中所阐述的或由Francois等,2005,细胞的TATA结合蛋白是最小腺相关病毒2型p5元件的Rep依赖性复制所需的(The Cellular TATA Binding Protein Is Required for Rep-Dependent Replication of a Minimal Adeno-Associated Virus Type 2p5 Element),J Virol所描述的。在某些实施方式中,TRY序列位于分离的核酸或rAAV载体的ITR(例如5' ITR)与表达构建物(例如编码转入基因的插入片段)之间。

[0113] 在某些情况下,本公开涉及杆状病毒载体,其包含本公开所描述的分离的核酸或

rAAV载体。在某些实施方式中,所述杆状病毒载体是苜蓿银纹夜蛾核多角体病毒(AcNPV)载体,例如由Urabe等,(2002)Hum Gene Ther13(16):1935-43和Smith等,(2009)Mol Ther17(11):1888-1896所描述的。

[0114] 在某些情况下,本公开提供了一种宿主细胞,其包含本文中所描述的分离的核酸或载体。宿主细胞可以是原核细胞或真核细胞。例如,宿主细胞可以是哺乳动物细胞、细菌细胞、酵母细胞、昆虫细胞等。在某些实施方式中,宿主细胞是哺乳动物细胞例如HEK293T细胞。在某些实施方式中,宿主细胞是细菌细胞例如大肠杆菌(E.coli)细胞。

[0115] rAAV

[0116] 在某些情况下,本公开涉及包含编码本文中所描述的核酸的转入基因的重组AAV(rAAV)(例如本文中所描述的rAAV载体)。术语“rAAV”通常是指包含被一种或多种AAV衣壳蛋白衣壳化的rAAV载体的病毒粒子。本公开描述的rAAV可以包含具有选自AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9和AAV10的血清型的衣壳蛋白。在某些实施方式中,rAAV包含来自于非人类宿主的衣壳蛋白,例如恒河猴的AAV衣壳蛋白例如AAVrh.10、AAVrh.39等。在某些实施方式中,本公开描述的rAAV包含作为野生型衣壳蛋白的变体的衣壳蛋白,例如相对于它所源自的野生型AAV衣壳蛋白包括至少1、2、3、4、5、6、7、8、9、10个或超过10个(例如15、20、25、50、100个等)氨基酸替换(例如突变)的衣壳蛋白变体。

[0117] 在某些实施方式中,本公开描述的rAAV容易地通过CNS传播,特别是当被引入到CSF空间或直接引入到脑实质中时。因此,在某些实施方式中,本公开描述的rAAV包含能够跨过血脑屏障(BBB)的衣壳蛋白。例如,在某些实施方式中,rAAV包含具有AAV9或AAVrh.10血清型的衣壳蛋白。rAAV的生产被例如Samulski等,(1989)J Virol.63(9):3822-8和Wright(2009)Hum Gene Ther.20(7):698-706描述。

[0118] 在某些实施方式中,本公开描述的rAAV(例如包含被AAV衣壳蛋白衣壳化以形成rAAV衣壳粒子的重组rAAV基因组)在杆状病毒载体表达系统(BEVS)中生产。例如Urabe等,(2002)Hum Gene Ther13(16):1935-43,Smith等,(2009)Mol Ther 17(11):1888-1896,美国专利号8,945,918,美国专利号9,879,282和国际PCT公布W02017/184879描述了使用BEVS生产rAAV。然而,rAAV可以使用任何适合的方法(例如使用重组rep和cap基因)来生产。

[0119] 药物组合物

[0120] 在某些情况下,本公开提供了包含本文中所描述的分离的核酸或rAAV和可药用载体的药物组合物。当在本文中使用,术语“可药用”是指材料例如载体或稀释剂不废除化合物的生物活性或性质,并且是相对无毒性的,例如所述材料可以被给药到个体而不引起不想要的生物学效应或不以有害的方式与包含它的组合物的任何组分相互作用。

[0121] 当在本文中使用,术语“可药用载体”意味着可药用材料、组合物或载体例如液体或固体填充剂、稳定剂、分散剂、悬浮剂、稀释剂、赋形剂、增稠剂、溶剂或包封材料,它们参与将本发明中的有用化合物携带到患者或在患者内运输,使得所述化合物可以执行其目标功能。可以被包括在本发明的实践中使用的药物组合物中的其他成分在本领域中是已知的,并描述在例如《Remington制药学》(Remington's Pharmaceutical Sciences)(Genaro主编,Mack Publishing Co.,1985,Easton,PA)中,其通过引用并入本文。

[0122] 本文中提供的组合物(例如药物组合物)可以通过任何途径给药,包括肠(例如口服)、肠胃外、静脉内、肌肉内、动脉内、髓内、鞘内、皮下、脑室内、透皮、真皮内、直肠、阴道

内、腹膜内、局部(如通过粉剂、软膏、霜剂和/或滴剂)、粘膜、鼻、颊、舌下,通过气管内滴注、支气管滴注和/或吸入,和/或作为口喷剂、鼻喷剂和/或气溶胶。具体设想的途径是口服给药、静脉内给药(例如全身性静脉内注射)、通过血液和/或淋巴供应的区域性给药和/或向患病部位的直接给药。通常,最适合的给药途径取决于各种不同因素,包括所述药剂的本质(例如它在胃肠道环境中的稳定性)和/或所述对象的状况(例如所述对象是否能够耐受口服给药)。在某些实施方式中,本文中描述的化合物或药物组合物适合于局部给药到对象的眼。

[0123] 方法

[0124] 本公开部分是基于用于在对象中表达一起(例如协同)起作用以治疗帕金森氏病的PD相关基因产物的组合的组合物。当在本文中使用时,“治疗”是指(a)阻止或延迟帕金森氏病的发作;(b)减轻帕金森氏病的严重性;(c)减轻或阻止帕金森氏病特有的症状的发生;(d)和/或阻止帕金森氏病特有的症状的恶化。帕金森氏病的症状包括例如运动功能障碍(例如摇晃、僵硬、运动缓慢、行走困难)、认知功能障碍(例如痴呆、抑郁、焦虑)、情绪和行为功能障碍。

[0125] 因此,在某些情况下,本公开提供了一种用于治疗患有或怀疑患有帕金森氏病的对象的方法,所述方法包括向所述对象给药本公开所描述的组合物(例如包含分离的核酸或载体或rAAV的组合物)。

[0126] 在某些实施方式中,组合物被直接给药到所述对象的CNS,例如通过直接注射到所述对象的脑和/或脊髓中。CNS直接给药方式的实例包括但不限于脑内注射、脑室内注射、脑池内注射、实质内注射、鞘内注射和上述方式的任何组合。在某些实施方式中,直接注射到对象的CNS中引起转入基因在所述对象的中脑、纹状体和/或大脑皮层中的表达(例如第一基因产物、第二基因产物以及如果适用的话第三基因产物的表达)。在某些实施方式中,直接注射到CNS中引起转入基因在所述对象的脊髓和/或CSF中的表达(例如第一基因产物、第二基因产物以及如果适用的话第三基因产物的表达)。

[0127] 在某些实施方式中,直接注射到对象的CNS包括对流增强递送(CED)。对流增强递送是一种治疗策略,其包括将脑手术暴露并将小直径的导管直接放置在脑的靶区域中,然后将治疗剂(例如本文中所描述的组合物或rAAV)直接输注到所述对象的脑中。CED被例如Debinski等,(2009)Expert Rev Neurother.9(10):1519-27描述。

[0128] 在某些实施方式中,组合物被外周给药到对象,例如通过外周注射。外周注射的实例包括皮下注射、静脉内注射、动脉内注射、腹膜内注射或上述方式的任何组合。在某些实施方式中,所述外周注射是动脉内注射,例如注射到对象的颈动脉中。

[0129] 在某些实施方式中,本公开所描述的组合物(例如包含分离的核酸或载体或rAAV的组合物)通过外周和直接两种方式被给药到对象的CNS。例如,在某些实施方式中,对象通过动脉内注射(例如注射到颈动脉中)并通过实质内注射(例如通过CED的实质内注射)来给药组合物。在某些实施方式中,所述直接注射到CNS和外周注射是同时的(例如在同一时间发生)。在某些实施方式中,所述直接注射发生在所述外周注射之前(例如外周注射之前1分钟至1周或更长时间)。在某些实施方式中,所述直接注射发生在所述外周注射之后(例如外周注射之后1分钟至1周或更长时间)。

[0130] 本公开所描述的组合物(例如包含分离的核酸或载体或rAAV的组合物)的给药到

对象的量将随着给药方法而变。例如,在某些实施方式中,本文中所描述的rAAV以约 10^9 个基因组拷贝(GC)/kg至约 10^{14} GC/kg之间(例如约 10^9 GC/kg、约 10^{10} GC/kg、约 10^{11} GC/kg、约 10^{12} GC/kg、约 10^{13} GC/kg或约 10^{14} GC/kg)的滴度给药到对象。在某些实施方式中,对象通过注射到CSF空间或通过实质内注射被高滴度给药(例如 $>10^{12}$ 个基因组拷贝GC/kg的rAAV)。

[0131] 本公开所描述的组合物(例如包含分离的核酸或载体或rAAV的组合物)可以被一次或多次(例如2、3、4、5、6、7、8、9、10、20次或更多次)给药到对象。在某些实施方式中,组合物被连续(例如长期)给药到对象,例如通过输注泵。

实施例

[0132] 实施例1:rAAV载体

[0133] AAV载体使用细胞例如用于三质粒转染的HEK293细胞来产生。ITR序列在表达构建物的两侧,所述表达构建物包含用于每个感兴趣的转入基因的启动子/增强子元件、3' polyA信号和翻译后信号例如WPPE元件。多个基因产物例如GBA1和LIMP2和/或鞘脂激活蛋白原可以被同时表达,所述同时表达是通过蛋白质序列的融合,或使用由于阻止肽键的生成而产生具有添加的氨基酸的2个肽片段的2A肽连接物例如T2A或P2A,或使用IRES元件,或通过用2个分开的表达盒表达来实现的。在被表达基因上游的高效剪接的短内含子序列的存在可以提高表达水平。shRNA和其他调控性RNA可以潜在地包含在这些序列中。本公开所描述的表达构建物的实例示出在图1-8和21-35以及下面的表2中。

[0134] 表2

[0135]

名称	启动子 1	shRNA	CDS1	PolyA 1	双顺反子元件	启动子 2	CDS2	PolyA2	ITR 之间的长度
CMVe_CBAp_GBA1_WP RE bGH	CBA		GBA1	WPPE-bGH					3741
LT1s_JetLong_mRNAiaSYn_SCARB2-T2A-GBA1_bGH	JetLong	aSyn	SCARB2	bGH	T2A		GBA1		4215
LI1_JetLong_SCARB2-IRES-GBA1 bGH	JetLong		SCARB2	bGH	IRES		GBA1		4399
FP1_JetLong_GBA1 bGH_JetLong_SCARB2 SV40L	JetLong		GBA1	bGH		JetLong	SCARB2	SV40L	4464
PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-T2A-GBA1 bGH 4353nt	JetLong	aSyn	PSAP	bGH	T2A	-	GBA1	-	4353
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1 Symt heticpolyA 4337nt	JetLong	-	PSAP	合成的 pA	IRES	-	GBA1	-	4337

[0136]

PrevailVector_10s_JetLong_mRNAiaSyn_GBA2_WPRE_bGH_4308nt	JetLong	aSyn	GBA2	WPRE_bGH	-	-	-	-	4308
PrevailVector_FT4_JetLong_GBA1_T2A_GALC_SynteticpolyA_4373nt	JetLong	-	GBA1	合成的pA	T2A	-	GALC	-	4373
PrevailVector_LT4_JetLong_GALC_T2A_GBA1_SynteticpolyA_4373nt	JetLong	-	GALC	合成的pA	T2A	-	GBA1	-	4373
PrevailVector_LT5s_JetLong_mRNAiaSyn_CTSB-T2A-GBA1_WPRE_bGH_4392nt	JetLong	aSyn	CTSB	WPRE_bGH	T2A	-	GBA1	-	4392
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_4477nt	JetLong	aSyn	GBA1	合成的pA	T2A	-	SMPD1	-	4477
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SyntheticpolyA_4820nt	JetLong	-	GALC	合成的pA	IRES	-	GBA1	-	4820
PrevailVector_FP5_JetLong_GBA1_bGH_JetLong_CTSB_SV40L_4108nt	JetLong	-	GBA1	bGH	-	JetLong	CTSB	SV40L	4108
PrevailVector_FT6s_JetLong_mRNAiaSyn_GBA1-T2A-GCH1_WPRE_bGH_4125nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	GCH1	-	4125
PrevailVector_LT7s_JetLong_mRNAiaSyn_RAB7L1-T2A-GBA1_WPRE_bGH_3984nt	JetLong	aSyn	RAB7L1	WPRE_bGH	T2A	-	GBA1	-	3984
PrevailVector_FI6s_JetLong_mRNAiaSyn_GBA1-IRES-GCH1_bGH_3978nt	JetLong	aSyn	GBA1	bGH	IRES	-	GCH1	-	3978
PrevailVector_9st_JetLong_mRNAiaSyn_mRNAiTME_M106B_VPS35_WPRE_bGH_4182nt	JetLong	aSyn & TMEM106B	VPS35	WPRE_bGH	-	-	-	-	4182
PrevailVector_FT12s_JetLong_mRNAiaSyn_GBA1-T2A-IL34_WPRE_bGH_4104nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	IL34	-	4104
PrevailVector_FI12s_JetLong_mRNAiaSyn_GBA1-IRES-IL34_bGH_3957nt	JetLong	aSyn	GBA1	bGH	IRES	-	IL34	-	3957
PrevailVector_FP8_JetLong_GBA1_bGH_CD68_TRE_M2_SV40L_4253nt	JetLong	-	GBA1	bGH	-	CD68	TREM2	SV40L	4253
PrevailVector_FP12_CMVe_CBA_GBA1_bGH_JetLong_IL34_SV40L_4503nt	CBA		GBA1	bGH		JetLong	IL34	SV40L	4503

[0137] 实施例2:病毒在GBA缺陷细胞中的转导的基于细胞的测定法

[0138] GBA1缺陷的细胞作为例如来自于GD患者的成纤维细胞、单核细胞或hES细胞或患者来源的诱导多能干细胞(iPSC)获得。这些细胞积累底物例如葡萄糖神经酰胺和葡萄糖鞘氨醇(GlcCer和GlcSph)。用Gcase抑制剂例如CBE处理野生型或突变的培养细胞系,也被用于获得GBA缺陷细胞。

[0139] 使用这些细胞模型,根据蛋白聚集体例如 α -突触核蛋白聚集体的积累,使用针对这种蛋白或磷酸化 α Syn的抗体,然后使用荧光显微镜通过成像来定量溶酶体缺陷。也通过针对蛋白质标志物例如LAMP1、LAMP2、LIMP1、LIMP2或使用染料例如Lysotracker的ICC,或通过荧光葡聚糖或其他标志物经内吞区室的摄入,对溶酶体异常进行了成像。也可以对由于与溶酶体的融合的缺陷造成的自吞噬标志物例如LC3的积累进行成像。使用Western印迹和/或ELISA来定量这些标志物的异常积累。另外,GBA1的糖脂底物和产物的积累使用标准方法来测量。

[0140] 在AAV载体的转导的表达的背景下测量治疗终点(例如PD相关病理的减轻),以确

认并定量活性和功能。Gcase也可以使用蛋白质ELISA测量或通过标准的Gcase活性测定法来定量。

[0141] 实施例3:使用突变小鼠的体内测定法

[0142] 本实施例描述了使用突变小鼠进行的AAV载体的体内测定法。如上所述的AAV载体在突变小鼠中的体内研究,使用例如由Liou等,(2006)J.Biol.Chem.281(7):4242-4253,Sun等,(2005)J.Lipid Res.46:2102-2113和Farfel-Becker等,(2011)Dis.Model Mech.4(6):746-752描述的测定法来进行。

[0143] 介质对照和AAV载体(例如以 2×10^{11} vg/小鼠的剂量)的鞘内或脑室内递送使用浓缩的AAV储用液,例如以5-10 μ L之间的注射体积来进行。实质内递送通过对流增强递送来进行。

[0144] 治疗在症状出现之前或出现之后开始。测量的终点是底物在CNS和CSF中的积累,通过ELISA和酶活性确定的Gcase酶的积累,运动和认知终点,溶酶体功能障碍,和 α -突触核蛋白单体、原纤丝或纤丝的积累。

[0145] 实施例4:疾病的化学模型

[0146] 本实施例描述了使用高雪氏病的化学诱导的小鼠模型(例如CBE小鼠模型)进行的AAV载体的体内测定法。这些AAV载体的体内研究在高雪氏病的化学诱导的小鼠模型中进行,例如由Vardi等,(2016)J Pathol.239(4):496-509所描述的。

[0147] 介质对照和AAV载体(例如以 2×10^{11} vg/小鼠的剂量)的鞘内或脑室内递送使用浓缩的AAV储用液,例如以5-10 μ L之间的注射体积来进行。实质内递送通过对流增强递送来进行。外周递送通过尾静脉注射来实现。

[0148] 治疗在症状出现之前或出现之后开始。测量的终点是底物在CNS和CSF中的积累,通过ELISA和酶活性确定的Gcase酶的积累,运动和认知终点,溶酶体功能障碍,和 α -突触核蛋白单体、原纤丝或纤丝的积累。

[0149] 实施例5:PD、LBD、高雪氏病患者中的临床试验

[0150] 在某些实施方式中,患有某些形式的高雪氏病(例如GD1)的患者具有提高的发生帕金森氏病(PD)或路易体痴呆症(LBD)的风险。本实施例描述了临床试验,用于评估本公开所描述的rAAV在患有高雪氏病、PD和/或LBD的患者中的安全性和功效。

[0151] 这些载体用于治疗高雪氏病、PD和/或LBD的临床试验使用与Grabowski等,(1995)Ann.Intern.Med.122(1):33-39中所描述的相似的研究设计来进行。

[0152] 实施例6:外周疾病的治疗

[0153] 在某些实施方式中,患有某些形式的高雪氏病的患者表现出外周神经病的症状,例如在Biegstraaten等,(2010)Brain 133(10):2909-2919中所描述的。

[0154] 本实施例描述了本文中所描述的AAV载体用于治疗与高雪氏病(例如1型高雪氏病)相关的外周神经病的体内测定法。简单来说,向被鉴定为具有外周神经病的征兆或症状的1型高雪氏病患者给药本公开所描述的rAAV。在某些实施方式中,在所述rAAV给药后,使用例如在Biegstraaten等人的文献中描述的方法来监测所述对象的外周神经病的征兆和症状。

[0155] 在患者中(例如在患者的血清中,在患者的外周组织(例如肝组织、脾组织等)中)存在的本公开所描述的转导的基因产物的水平,通过例如Western印迹分析、酶功能测定法

或成像研究来测定。

[0156] 实施例7:CNS形式的治疗

[0157] 本实施例描述了本文中所描述的rAAV用于治疗高雪氏病的CNS形式的体内测定法。简单来说,向被鉴定为具有高雪氏病的CNS形式(例如2型或3型高雪氏病)的高雪氏病患者给药本公开所描述的rAAV。在患者的CNS中(例如在患者的CNS的血清中,在患者的脑脊液(CSF)中,或在患者的CNS组织中)存在的本公开所描述的转导的基因产物的水平,通过例如Western印迹分析、酶功能测定法或成像研究来测定。

[0158] 实施例8:在GBA1中具有突变的对象中帕金森氏病的基因疗法

[0159] 本实施例描述了向以GBA1基因中的突变为特征的患有帕金森氏病的对象给药编码GBA1的重组腺相关病毒(rAAV)。

[0160] 所述rAAV-GBA1载体插入片段含有CBA启动子元件(CBA),其由四个部分构成:CMV增强子(CMVe),CBA启动子(CBAp),外显子1和内含子(int),以组成性表达人类GBA1(maroon)的密码子优化的编码序列(CDS)。3'区还含有土拨鼠肝炎病毒转录后调控元件(WPRE),后面跟有牛生长激素polyA信号(bGH polyA)尾部。两侧的ITR允许居间序列的正确包装。评估了5' ITR序列的两种变体(图7,插入图框,下方序列);这些变体在ITR的20个核苷酸的“D”区内具有几个核苷酸差异,其据信将影响包装和表达的效率。所述rAAV-GBA1载体产物含有图7中示出的“D”结构域核苷酸序列(插入图框,上方序列)。变体载体带有突变的“D”结构域(在本文中被称作“S”结构域,具有由阴影示出的核苷酸变化),在临床前研究中的表现类似。骨架含有赋予对卡那霉素的抗性的基因以及防止反向包装的填充序列。描绘了rAAV-GBA1载体的示意图示出在图8中。使用AAV9血清型衣壳蛋白将所述rAAV-GBA1载体包装在rAAV中。

[0161] rAAV-GBA1作为单剂通过荧光镜引导的枕骨下注射到大池中(大池内;ICM)而被给药到对象。rAAV-GBA1给药方案研究的一个实施方式如下:

[0162] 将单剂rAAV-GBA1以两种剂量水平(3×10^{13} vg(低剂量), 1×10^{14} vg(高剂量)等)之一给药到患者(N=12),所述剂量水平是基于非临床药理学和毒理学研究的结果而确定的。

[0163] 初始研究在每日递送GCase的抑制剂环己烯四醇-b-环氧化物(CBE)的化学小鼠模型中进行,以评估rAAV-GBA1载体和rAAV-GBA1 S-变体构建物(如下文进一步描述)的的功效和安全性。此外,初始研究在带有纯合的GBA1突变并且鞘脂激活蛋白部分缺陷(4L/PS-NA)的遗传小鼠模型中进行。另外在小鼠和非人类灵长动物(NHP)中进行剂量范围研究,以进一步评估载体的安全性和功效。

[0164] 测试了AAV骨架中两种略微不同版本的5' 反向末端重复序列(ITR),以评估可制造性和转入基因表达(图7)。145bp 5' ITR内的20bp的“D”结构域被认为对病毒载体的最佳生产来说是必需的,但已报道所述“D”结构域内的突变在某些情况下提高转入基因的表达。因此,除了带有完整的“D”结构域的病毒载体rAAV-GBA1之外,也评估了具有突变的D结构域(在本文中被称作“S”结构域)的第二种载体形式。rAAV-GBA1和所述变体两者都表达相同的转入基因。尽管正如下文详述的,两种载体产生在体内有效的病毒,但含有野生型“D”结构域的rAAV-GBA1被选择用于进一步开发。

[0165] 为了建立GCase缺陷的CBE模型,将幼年小鼠用GCase的特异性抑制剂CBE给药。小鼠在出生后第8天(P8)开始每天通过IP注射给药CBE。测试了三种不同的CBE剂量(25mg/kg、

37.5mg/kg、50mg/kg) 和PBS来建立表现出行为表型的模型(图9)。较高剂量的CBE以剂量依赖性方式导致死亡。用50mg/kg CBE处理的所有小鼠到P23时死亡,用37.5mg/kg CBE处理的8只小鼠中的5只到P27时死亡。在用25mg/kg CBE处理的小鼠中没有死亡。尽管CBE注射的小鼠在旷场测定法中没有显示出总体运动缺陷(与给药PBS的小鼠移动相同的距离并具有相同的速度),但通过转棒仪测定法测量时CBE处理的小鼠表现出运动协调和平衡的缺陷。

[0166] 将存活到研究结束的小鼠在它们的最后一次CBE给药后当天(P27,“第1天”)或在CBE停药三天后(P29,“第3天”)处死。在第1天和第3天两个组群中对给药25mg/kg CBE的小鼠的皮层进行脂质分析,以评估GCCase底物的积累。与PBS处理的对照相比,在CBE处理的小鼠中GluSph和GalSph水平(在本实施例中以聚集体进行测量)显著积累,与GCCase不足相符。

[0167] 在上述研究的基础上选择了25mg/kg CBE剂量,因为它产生行为缺陷而不影响存活。为了在CBE处理期间获得GBA1在整个脑中的广泛分布和转入基因表达,在出生后第3天(P3)通过脑室内(ICV)注射递送rAAV-GBA1或赋形剂,随后在P8时开始每日进行IP CBE或PBS处理(图10)。

[0168] 接受rAAV-GBA1的CBE处理的小鼠与接受赋形剂的小鼠相比在转棒仪上具有统计学显著的更好表现(图11)。变体治疗组中的小鼠与赋形剂治疗的小鼠在其他行为度量例如试验期间的总移动距离方面没有差异(图11)。

[0169] 在终生研究完成时,将一半的小鼠在最后一次CBE给药后当天(P36,“第1天”)或在CBE停药三天后(P38,“第3天”)处死,用于生物化学分析(图12)。使用在三份生物学平行样中进行的荧光测量酶测定法,评估皮层中的GCCase活性。在用rAAV-GBA1治疗的小鼠中GCCase活性提高,而CBE处理降低GCCase活性。此外,接受CBE和rAAV-GBA1两者的小鼠具有与PBS处理组相近的GCCase活性水平,表明rAAV-GBA1的递送能够克服由CBE处理引起的GCCase活性的抑制。对小鼠的运动皮层进行脂质分析,以检查底物GluCer和GluSph的水平。在给药CBE的小鼠的脑中两种脂质积累,并且rAAV-GBA1治疗显著减少底物积累。

[0170] 在不同治疗组中,脂质水平与GCCase活性和在转棒仪上的表现两者负相关。在rAAV-GBA1给药后GCCase活性的提高与底物减少和运动功能增强相关(图13)。正如在图14中所示,初步生物分布通过载体基因组的存在来评估,正如通过qPCR所测量的(每1 μ g基因组DNA>100个载体基因组被定义为阳性)。接受rAAV-GBA1的使用和不使用CBE的两种小鼠在皮层中对rAAV-GBA1载体基因组呈阳性,表明ICV递送导致rAAV-GBA1递送到皮层。此外,载体基因组在肝脏中检测到,在脾脏中很少,在心脏、肾脏或性腺中没有。对于所有度量来说,在第1天与第3天的组之间不存在统计学显著的差异。

[0171] 在CBE模型中的更大型研究进一步探索了CBE模型中rAAV-GBA1的有效剂量。使用25mg/kg CBE剂量模型,在P3时通过ICV递送赋形剂或rAAV-GBA1,并在P8时开始每天的IP PBS或CBE处理。考虑到在以前的研究中观察到的CBE停药和不停药的组之间的相似性,所有小鼠在最后一次CBE给药后一天(P38-40)处死。评估了三种不同rAAV-GBA1剂量的效果,产生了下述5个组,其中每组10只小鼠(5M/5F):

[0172] 赋形剂ICV+PBS IP

[0173] 赋形剂ICV+25mg/kg CBE IP

[0174] 3.2e9 vg(2.13e10 vg/g脑)rAAV-GBA1 ICV+25mg/kg CBE IP

[0175] 1.0e10 vg(6.67e10 vg/g脑)rAAV-GBA1 ICV+25mg/kg CBE IP

[0176] 3.2e10 vg (2.13e11 vg/g脑) rAAV-GBA1 ICV+25mg/kg CBE IP。

[0177] 最高剂量的rAAV-GBA1挽救了与CBE处理相关的在P37时未能增加体重。另外,与赋形剂+CBE处理组相比,这个剂量引起在转棒仪和楔形梁上的表现的统计学显著的提高(图15)。在几个组中观察到致死性,包括赋形剂治疗和rAAV-GBA1治疗的组两者(赋形剂+PBS:0;赋形剂+25mg/kg CBE:1;3.2e9 vg rAAV-GBA1+25mg/kg CBE:4;1.0e10 vg rAAV-GBA1+25mg/kg CBE:0;3.2e10 vg rAAV-GBA1+25mg/kg CBE:3)。

[0178] 在终生研究完成后,将小鼠处死用于生物化学分析(图16)。在三份生物学平行样中通过荧光测量测定法评估皮层中的GCase活性。CBE处理的小鼠显示出降低的GCase活性,而接受高rAAV-GBA1剂量的小鼠与CBE处理相比显示出统计学显著的GCase活性提高。CBE处理的小鼠还具有GluCer和GluSph的积累,这两者通过给药高剂量的rAAV-GBA1得以挽救。

[0179] 除了已建立的化学CBE模型之外,rAAV-GBA1还在4L/PS-NA遗传模型中进行了评估,所述模型对Gba1中的V394L GD突变来说是纯合的,并且也是鞘脂激活蛋白中部分缺陷的,这影响了GCase的定位和活性。这些小鼠表现出运动强度、协调性和平衡的缺陷,正如它们在梁行走、转棒仪和悬丝测定法中的表现所证实的。通常,这些小鼠的寿命短于22周。在初始研究中,在P23时通过ICV递送3 μ l最高滴度的病毒,最终剂量为2.4e10 vg (6.0e10 vg/g脑)。每个组6只小鼠,治疗组为:

[0180] WT+赋形剂ICV

[0181] 4L/PS-NA+赋形剂ICV

[0182] 4L/PS-NA+2.4e10 vg (6.0e10 vg/g脑) rAAV-GBA1 ICV

[0183] 在rAAV-GBA1递送后4周通过梁行走试验评估运动表现。当与用赋形剂治疗的突变体小鼠相比时,接受rAAV-GBA1的突变体小鼠组显示出更少的总滑倒数和更少的单位速度的滑倒数的趋势,将运动功能恢复到接近WT水平(图17)。由于随着这些小鼠的衰老运动表型变得更加严重,因此在更晚的时间点评估了它们在这个和其他行为试验中的表现。在终生研究完成时,评估这些小鼠中的脂质水平、GCase活性和生物分布。

[0184] 其他较低剂量的rAAV-GBA1目前正在使用CBE模型进行测试,对应于所提出的1期高临床剂量的0.03x、0.1x和1x。每个组包括10只小鼠(5M/5F)/组:

[0185] 赋形剂ICV

[0186] 赋形剂ICV+25mg/kg CBE IP

[0187] 3.2e8 vg (2.13e9 vg/g脑) rAAV-GBA1 ICV+25mg/kg CBE IP

[0188] 1.0e9 vg (6.67e9 vg/g脑) rAAV-GBA1 ICV+25mg/kg CBE IP

[0189] 1.0e10 vg (6.67e10 vg/g脑) rAAV-GBA1 ICV+25mg/kg CBE IP。

[0190] 除了运动表型之外,评估了皮层中的脂质水平和GCase活性。还进行了治疗和分析的时间过程评估。

[0191] 启动了更大型的剂量范围研究以评估功效和安全性数据。将10只4L/PS-NA小鼠(每组5M/5F)用10 μ l rAAV-GBA1注射。使用异速生长脑重量计算,剂量与所述提出的1期高临床剂量的0.15x、1.5x、4.4x和14.5x相关。注射组由如下构成:

[0192] WT+赋形剂ICV

[0193] 4L/PS-NA+赋形剂ICV

[0194] 4L/PS-NA+4.3e9 vg (1.1e10 vg/g脑) rAAV-GBA1 ICV

[0195] 4L/PS-NA+4.3e10 vg (1.1e11 vg/g/脑) rAAV-GBA1 ICV

[0196] 4L/PS-NA+1.3e11 vg (3.2e11 vg/g/脑) rAAV-GBA1 ICV

[0197] 4L/PS-NA+4.3e11 vg (1.1e12 vg/g/脑) rAAV-GBA1 ICV。

[0198] CBE模型中的非临床研究的概述示出在下面的表3中。

[0199] 表3:CBE小鼠模型中的结果的概述

	试验材料	研究编号	剂量组群	行为变化			脂质	酶	BD	
				转染效率	运动	平衡			脑	肝
[0200]	rAAV-GBA1	PRV-2018-005 在 CBE 模型中 rAAV-GBA1 的 剂量范围	3.2e9 vg (2.13e10 vg/g 脑)	NS	NS	NS	NS	NS	+	-
			1.10e10 vg (6.67e10 vg/g 脑)	T	NS	NS	T/S	NS	+	+
			2.3e10vg (2.13e11 vg/g 脑)	S	S	NS	S	S	+	+
	变体	PRV-2018-005 在 CBE 模型中变 体的剂量范围	8.8e9 vg (5.9e10 vg/g 脑)	S	N/A	NS	S	S	+	+

[0201] 注意,阳性生物分布被定义为>100vg/1μg基因组DNA。

[0202] 缩略语:BD=生物分布;NS=不显著;T=趋势;S=显著;N/A=不适用;+=阳性;-=阴性。

[0203] 实施例9:rAAV载体的体外分析

[0204] 在体外和体内对rAAV构建物进行了测试。图18示出了编码颗粒蛋白前体 (PGRN) 蛋白的rAAV构建物的体外表达的代表性数据。左图示出了颗粒蛋白前体 (PGRN) ELISA测定法的标准曲线。下图示出了在用rAAV转导的HEK293T细胞的细胞裂解物中通过ELISA测定法测量的PGRN表达的剂量响应。MOI=感染复数(每个细胞的载体基因组数)。

[0205] 进行了初步研究以评估编码单独的或与GBA1和/或一种或多种抑制性RNA组合的鞘脂激活蛋白原 (PSAP) 和SCARB2的rAAV载体的体外活性。还测试了一种编码PSAP和颗粒蛋白前体 (PGRN) 的构建物。测试的载体包括表4中示出的那些载体。“Opt”是指为了在哺乳动物细胞(例如人类细胞)中表达而密码子优化的核酸序列。图19示出的代表性数据表明用每种构建物转染HEK293细胞与模拟转染的细胞相比导致相应的基因产物的过表达。

[0206] 进行了初步研究以评估编码单独的或与一种或多种抑制性RNA组合的TREM2的rAAV载体的体外活性。测试的载体包括表4中示出的那些载体。“Opt”是指为了在哺乳动物细胞(例如人类细胞)中表达而密码子优化的核酸序列。图36A-36B示出的代表性数据表明用每种构建物转染HEK293细胞与模拟转染的细胞相比导致相应的基因产物的过表达。

[0207] 表4

ID	启动子	抑制性RNA	启动子	转入基因
I00015	JL_intronic	SCNA	JetLong	Opt-PSAP_GBA1
I00039	-	-	JetLong	Opt-PSAP-GRN
I00046	-	-	CBA	Opt-PSAP
I00014	JetLong	SCNA	JetLong	Opt-SCARB2_GBA1

I00040			JL, CD68	opt-GBA1, TREM2
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[0209] 实施例10: SCNA和TMEM106B shRNA构建物的试验

[0210] HEK293细胞

[0211] 在本研究中使用了人类胚胎肾293细胞系(HEK293) (#85120602, Sigma-Aldrich)。将HEK293细胞维持在含有100单位/ml青霉素和100 μ g/ml链霉素(#15140122, Thermo Fisher Scientific)的培养基(增补有10%胎牛血清[FBS] [#10082147, Thermo Fisher Scientific]的D-MEM[#11995065, Thermo Fisher Scientific])中。

[0212] 质粒转染

[0213] 使用Lipofectamine 2000转染试剂(#11668019, Thermo Fisher Scientific), 按照制造商的说明书进行质粒转染。简单来说, 将HEK293细胞(#12022001, Sigma-Aldrich)以 3×10^5 个细胞/ml的密度在不含抗生素的培养基中铺板。第二天, 将质粒和Lipofectamine 2000试剂合并并在Opti-MEM溶液(#31985062, Thermo Fisher Scientific)中。在5分钟后, 将所述混合物添加到HEK293培养物。72小时后, 收获细胞用于RNA或蛋白质提取, 或进行成像分析。对于成像分析来说, 在细胞铺板之前将板用0.01%聚L-赖氨酸溶液(P8920, Sigma-Aldrich)预包被。通过定量实时PCR(qRT-PCR)进行的基因表达分析

[0214] 相对基因表达水平通过定量实时PCR(qRT-PCR), 使用Power SYBR Green Cells-to-CT试剂盒(#4402955, Thermo Fisher Scientific), 按照制造商的说明书来确定。将候选质粒使用Lipofectamine 2000转染试剂(0.5 μ g质粒和1.5 μ l试剂在50 μ l Opti-MEM溶液中)瞬时转染到铺于48孔板上的HEK293细胞(7.5×10^4 个细胞/孔)中。72小时后, 从所述细胞提取RNA, 并按照制造商的说明书用于反转录以合成cDNA。对于定量PCR分析来说, 将2~5 μ l cDNA产物使用基因特异性引物对(终浓度为250nM)和Power SYBR Green PCR主混合物(#4367659, Thermo Fisher Scientific)一式两份扩增。用于SNCA、TMEM106B和GAPDH基因的引物序列是: 用于SNCA的5'-AAG AGG GTG TTC TCT ATG TAG GC-3' (SEQ ID NO:71), 5'-GCT CCT CCA ACA TTT GTC ACT T-3' (SEQ ID NO:72); 用于TMEM106B的5'-ACA CAG TAC CTA CCG TTA TAG CA-3' (SEQ ID NO:73), 5'-TGT TGT CAC AGT AAC TTG CAT CA-3' (SEQ ID NO:74); 和用于GAPDH的5'-CTG GGC TAC ACT GAG CAC C-3' (SEQ ID NO:75), 5'-AAG TGG TCG TTG AGG GCA ATG-3' (SEQ ID NO:76)。定量PCR在QuantStudio 3实时PCR系统(Thermo Fisher Scientific)中进行。表达水平通过看家基因GAPDH进行归一化并使用比较CT方法来计算。

[0215] 荧光成像分析

[0216] 在EGFP编码区的下游含有人类SNCA基因的3'-UTR的EGFP报告质粒, 被用于SNCA和TMEM106B敲落质粒的验证。将EGFP报告质粒和候选敲落质粒使用Lipofectamine 2000转染试剂(0.04 μ g报告质粒、0.06 μ g敲落质粒和0.3 μ l试剂在10 μ l Opti-MEM溶液中)同时转染到铺于聚L-赖氨酸包被的96孔板上的HEK293细胞(3.0×10^4 个细胞/孔)中。72小时后, 使用Varioskan LUX多模式读板器(Thermo Fisher Scientific)在激发488nm/发射512nm下测量EGFP信号的荧光强度。将细胞在RT下用4%PFA固定10分钟, 并与含有40 μ g/ml 7-氨基放线菌素D(7-AAD)的D-PBS在RT温育30min。在用D-PBS洗涤后, 使用Varioskan读板器在激发546nm/发射647nm下测量7-AAD信号的荧光强度, 以定量细胞数目。将针对7-AAD信号水平归一化的EGFP信号与对照敲落样品进行比较。

[0217] 酶联免疫吸附测定法 (ELISA)

[0218] 将在SNCA编码区的下游含有人类SNCA基因或TMEM106B基因的3'-UTR的 α -突触核蛋白报告质粒用于敲落质粒在蛋白质水平上的验证。 α -突触核蛋白的水平使用从HEK293细胞提取的裂解物,通过ELISA(#KHB0061,Thermo Fisher Scientific)来确定。将候选质粒使用Lipofectamine 2000转染试剂(0.1 μ g报告质粒、0.15 μ g敲落质粒和0.75 μ l试剂在25 μ l Opti-MEM溶液中)瞬时转染到铺于48孔板上的HEK293细胞(7.5×10^4 个细胞/孔)中。72小时后,将细胞在增补有蛋白酶抑制剂混合物(#P8340,Sigma-Aldrich)的放射免疫沉淀测定(RIPA)缓冲液(#89900,Thermo Fisher Scientific)中裂解,并超声几秒钟。在冰上温育30min后,将裂解物在4℃下以 $20,000 \times g$ 离心15min,并收集上清液。对蛋白质水平进行定量。在Varioskan读板器中在450nm下读板,并使用SoftMax Pro 5软件计算浓度。将测量到的蛋白质浓度归一化到使用二辛可宁酸测定法(#23225,Thermo Fisher Scientific)确定的总蛋白浓度。

[0219] 图37和表5示出了通过GFP报告物测定法(上图)和 α -Syn测定法(下图)获得的代表性数据,指示了在体外成功的SCNA沉默。图38和表6示出了通过GFP报告物测定法(上图)和 α -Syn测定法(下图)获得的代表性数据,指示了在体外成功的TMEM106沉默。

[0220] 表5

[0221]

ID	启动子	敲落	启动子	过表达
I00007	CMV_intronic	SNCA_mi	CMV	opt-GBA1
I00008	H1	SNCA_sh	CMV	opt-GBA1
I00009	H1	SNCA_Pubsh4	CMV	opt-GBA1
I00014	JL_intronic	SNCA_mi	JetLong	opt-SCARB2_GBA
I00015	JL_intronic	SNCA_mi	JetLong	opt-PSAP_GBA
I00016	JL_intronic	SNCA_mi	JetLong	opt-CTSB_GBA
I00019	JL_intronic	SNCA_TMEM_mi	JetLong	opt-VPS35
I00023	JL_intronic	SNCA_mi	JetLong	opt-GBA1_IL34
I00024	JL_intronic	SNCA_mi	JetLong	opt-GBA2
I00028	intronic	SNCA_Broadsh	CMV	opt-GBA1
I00029	intronic	SNCA_Pubsh4	CMV	opt-GBA1

[0222] 表6

[0223]

ID	启动子	敲落	启动子	过表达
I00010	H1	TMEM_Pubsh	CMV	opt-GRN
I00011	JL_intronic	TMEM_mi	JetLong	opt-GBA1_GRN
I00012	H1	TMEM_sh	CMV	opt-GRN
I00019	JL_intronic	SNCA_TMEM_mi	JetLong	opt-VPS35

[0224] 实施例11: ITR“D”序列的放置和细胞转导

[0225] 调查了ITR“D”序列的放置对rAAV载体的细胞转导的影响。将HEK 293细胞用编码Gcase的rAAV转导,所述rAAV具有1)野生型ITR(例如在转入基因插入片段的近端和所述ITR的末端的远端的“D”序列)或2)具有位于所述载体的“外侧”的“D”序列(例如位于所述ITR的末端的近端和所述转入基因插入片段的远端的“D”序列)的ITR,正如在图20中所示。令人吃

惊的是,数据表明具有位于“外侧”位置中的“D”序列的rAAV保留了高效包装和转导细胞的能力(图40)。

[0226] 实施例12:颗粒蛋白前体rAAV的体外测试

[0227] 图39是描绘了包含编码PGRN的表达构建物的载体的一个实施方式的示意图。将颗粒蛋白前体在对于GRN缺失来说杂合或纯合的GRN缺陷的啮齿动物的CNS中,通过实质内或鞘内注射将编码PGRN(例如密码子优化的PGRN)的rAAV载体注射到例如大池中进行过表达。

[0228] 小鼠在2月龄或6月龄时注射,生长到6个月或12个月,并分析下述一者或多者:在RNA和蛋白质水平上GRN的表达水平,行为测定(例如运动改善),存活分析(例如存活率提高),小胶质细胞和炎症标志物,神经胶质增生,神经元缺失,脂褐质沉积和/或溶酶体标志物例如LAMP1积累的挽救。在PGRN缺陷型小鼠上的测定法由例如下述文献描述:Arrant等(2017) Brain 140:1477-1465;Arrant等(2018) J.Neuroscience 38(9):2341-2358;和Amado等(2018) doi:<https://doi.org/10.1101/30869>;所述文献的全部内容通过引用并入本文。

[0229] 等同性

[0230] 本申请通过引用整体并入了下述文献的内容:2018年10月3日提交的代理人案号为P1094.70002W000的国际PCT申请;2018年10月3日提交的代理人案号为P1094.70004W000的国际PCT申请;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的临时申请系列号62/567,296;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,311;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,319;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,301;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,310;2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,303;和2017年10月3日提交的题为“用于溶酶体障碍的基因疗法”(GENE THERAPIES FOR LYSOSOMAL DISORDERS)的62/567,305。

[0231] 因此,已经描述了本发明的至少一个实施方式的几个方面,但应当理解,本领域技术人员将会容易地做出各种不同的改变、修改和改进。这样的改变、修改和改进打算作为本公开的一部分,并且打算落入本发明的精神和范围之内。因此,前面的描述和附图仅作为示例。

[0232] 尽管已经在本文中描述和说明了本发明的几个实施方式,但本领域普通技术人员将会容易想到用于执行本文中描述的功能和/或获得本文中描述的结果和/或一种或多种优点的各种其他手段和/或结构,并且每个这样的变化和/或修改都被视为在本发明的范围之内。更一般而言,本领域技术人员将会容易地理解,本文中描述的所有参数、尺寸、材料和构造均打算是示例性的,并且实际的参数、尺寸、材料和/或构造将取决于本发明的教导将用于的一种或多种具体应用。本领域技术人员将会认识到或仅使用常规实验就能确定本文中描述的本发明的具体实施方式的许多等同方案。因此应当理解,前述实施方式仅作为示例给出,并且在随附的权利要求书及其等同物的范围之内,本发明可以被具体描述和要求保护的之外的其他方式来实践。本发明针对本文中描述的每个单独的特点、系统、物

品、材料和/或方法。另外,如果这样的特点、系统、物品、材料和/或方法不是互相矛盾的,则两个或更多个这样的特点、系统、物品、材料和/或方法的任何组合都被包括在本发明的范围之内。

[0233] 当在本说明书和权利要求书中使用时,除非有相反的确指示,否则没有具体数目的指称应该被理解为意味着“至少一个”。

[0234] 当在本说明书和权利要求书中使用时,短语“和/或”应该被理解为是指如此连接的要素中的“任一者或两者”,即要素在某些情况下共同存在而在另一些情况中分开存在。除非有相反的确指示,否则在由“和/或”子句具体指定的要素之外的其他要素可以任性地存在,无论与那些具体指定的要素相关还是无关。因此,作为非限制性示例,当与诸如“包含”的开放式语言相结合使用时,对“A和/或B”的指称在一个实施方式中可以是指A但没有B(任性地包括B之外的要素),在另一个实施方式中可以是指B但没有A(任性地包括A之外的要素),在又一个实施方式中可以是指A和B两者(任性地包括其他要素),等等。

[0235] 当在本说明书和权利要求书中使用时,“或”应该被理解为具有与上文所定义的“和/或”相同的含义。例如,当在名单中将项目分隔开时,“或”或“和/或”应该被解释为是包含性的,即包括大量要素或要素名单中的至少一者,但是也包括其中的超过一者,并任性地包括另外的未列出的项目。只有明确指示相反的术语例如“仅仅一者”或“只有一者”或当在权利要求书中使用时的“由……构成”,才是指包括大量要素或要素名单中的仅仅一个要素。通常,当在本文中使用时,术语“或”当在前面具有排他性术语例如“任一”、“之一”、“仅仅一者”或“只有一者”时,应该仅仅被解释为指示排他性可选项(即“一者或另一者但不是两者”)。当在权利要求书中使用时,“基本上由……构成”应该具有它在专利法领域中使用时的通常含义。

[0236] 当在本说明书和权利要求书中使用时,短语“至少一者”在指称一个或多个要素的名单时,应该被理解为意味着选自所述要素名单中的任一个或多个要素的至少一个要素,但不必定包括至少一个所述要素名单内具体列出的每一个要素,并且不排除所述要素名单中的要素的任何组合。这个定义也允许在短语“至少一者”所指称的要素名单内具体指明的要素之外的要素可以任性地存在,不论其与那些具体指明的要素相关还是无关。因此,作为非限制性实例,“A和B中的至少一者”(或等同地“A或B中的至少一者”或等同地“A和/或B中的至少一者”)在一个实施方式中可以是指至少一个、任性地包括超过一个A且B不存在(并且任性地包括B之外的要素),在另一个实施方式中可以是指至少一个、任性地包括超过一个B且A不存在(并且任性地包括A之外的要素),在又一个实施方式中是指至少一个、任性地包括超过一个A和至少一个、任性地包括超过一个B(并且任性地包括其他要素),等等。

[0237] 在权利要求书中以及在上面的说明书中,所有过渡性短语例如“包含”、“包括”、“带有”、“具有”、“含有”、“涉及”、“保有”等应该被理解为是开放性的,即意味着包括但不限于。只有过渡性术语“由……构成”和“基本上由……构成”应分别是封闭或半封闭的过渡性术语,正如在美国专利局专利审查程序手册第2111.03节中所阐述的。

[0238] 在权利要求书中使用诸如“第一”、“第二”、“第三”等的序数术语来修饰权利要求要素,本身并不意味着一个权利要求要素相对于另一个要素的任何优先性、优先级或顺序,或执行方法的行动的时间顺序,而是仅用作区分具有某个名称的一个权利要求要素与具有相同名称(但使用序数术语)的另一个要素的标签,用于区分所述权利要求要素。

[0239] 还应该理解,除非有相反的明确指示,否则在本文中要求保护的包括超过一个步骤或行动的任何方法中,所述方法的步骤或行动的顺序不必限于所述方法的步骤或行动被叙述的顺序。

[0240] 序列

[0241] 在某些实施方式中,编码一个或多个基因产物(例如第一、第二和/或第三基因产物)的表达盒包含SEQ ID NO:1-78中的任一者中阐述的序列,或由所述序列构成(或编码具有所述序列的多肽)。在某些实施方式中,基因产物由SEQ ID NO:1-78中的任一者的部分(例如片段)编码。

- [0001] 序列表
- [0002] <110> 普利维尔治疗公司(Prevail Therapeutics, Inc.)
- [0003] <120> 用于溶酶体障碍的基因疗法
- [0004] <130> SPI204763-71
- [0005] <140> 尚未分配
- [0006] <141> 2018-10-03
- [0007] <150> US 62/567,310
- [0008] <151> 2017-10-03
- [0009] <150> US 62/567,296
- [0010] <151> 2017-10-03
- [0011] <150> US 62/567,311
- [0012] <151> 2017-10-03
- [0013] <150> US 62/567,319
- [0014] <151> 2017-10-03
- [0015] <150> US 62/567,301
- [0016] <151> 2017-10-03
- [0017] <160> 78
- [0018] <170> PatentIn version 3.5
- [0019] <210> 1
- [0020] <211> 10697
- [0021] <212> DNA
- [0022] <213> 人工序列
- [0023] <220>
- [0024] <223> 合成的多核苷酸
- [0025] <400> 1
- [0026] ttggccactc cctctctgcg cgctcgctcg ctactgagg cgggcgacc aaaggtcgcc 60
- [0027] cgacgcccg gctttgccg ggcggcctca gtgagcgagc gagcgcgag agagggagt 120
- [0028] gccaaactcca tactagggg ttctgctag ctctgggtat ttaagcccga gtgagcacgc 180
- [0029] agggctccca ttttgaagcg ggaggttacg cgttcgtcga ctactagtgg gtaccagagc 240
- [0030] tcctagggtt ctagaaccgg tgacgtctcc catggtgaag cttggatctg aattcggtac 300
- [0031] cctagttatt aatagtaatc aattacgggg tcattagttc atagcccata tatggagtgc 360
- [0032] cgcggtacat aacttacggt aaatggcccg cctggctgac cgcccaacga ccccgccca 420
- [0033] ttgacgtcaa taatgacgta tgttcccata gtaacgcaa tagggacttt ccattgacgt 480
- [0034] caatgggtgg actatttacg gtaaaactgcc cacttggcag tacatcaagt gtatcatatg 540
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[2654]	Ser Lys Asn Val Ile Pro Ala Leu Glu Leu Val Glu Pro Ile Lys Lys
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[2656]	His Glu Val Pro Ala Lys Ser Asp Val Tyr Cys Glu Val Cys Glu Phe
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[2658]	Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys
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[2666]	Leu His Leu Cys Ser Gly Thr Arg Leu Pro Ala Leu Thr Val His Val
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[2668]	Thr Gln Pro Lys Asp Gly Gly Phe Cys Glu Val Cys Lys Lys Leu Val
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[2670]	Gly Tyr Leu Asp Arg Asn Leu Glu Lys Asn Ser Thr Lys Gln Glu Ile
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[2672]	Leu Ala Ala Leu Glu Lys Gly Cys Ser Phe Leu Pro Asp Pro Tyr Gln
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[2674]	Lys Gln Cys Asp Gln Phe Val Ala Glu Tyr Glu Pro Val Leu Ile Glu
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[2676]	Ile Leu Val Glu Val Met Asp Pro Ser Phe Val Cys Leu Lys Ile Gly
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[2678]	Ala Cys Pro Ser Ala His Lys Pro Leu Leu Gly Thr Glu Lys Cys Ile
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[2680]	Trp Gly Pro Ser Tyr Trp Cys Gln Asn Thr Glu Thr Ala Ala Gln Cys
[2681]	500 505 510
[2682]	Asn Ala Val Glu His Cys Lys Arg His Val Trp Asn
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[2687]	<213> 智人(Homo sapiens)

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[2735]	Ser Asn Lys Ala Tyr Val Phe Glu Arg Asp Gln Ser Val Gly Asp Pro			
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[2739]	Glu Trp Ser Gln Val His Phe Leu Arg Glu Ile Ile Glu Ala Met Leu			
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[2741]	Lys Ala Tyr Gln Gln Lys Leu Phe Val Thr His Thr Val Asp Glu Leu			
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[2743]	Leu Trp Gly Tyr Lys Asp Glu Ile Leu Ser Leu Ile His Val Phe Arg			
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[2745]	Pro Asp Ile Ser Pro Tyr Phe Gly Leu Phe Tyr Glu Lys Asn Gly Thr			
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[2747]	Asn Asp Gly Asp Tyr Val Phe Leu Thr Gly Glu Asp Ser Tyr Leu Asn			
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[2749]	Phe Thr Lys Ile Val Glu Trp Asn Gly Lys Thr Ser Leu Asp Trp Trp			
[2750]	225	230	235	240
[2751]	Ile Thr Asp Lys Cys Asn Met Ile Asn Gly Thr Asp Gly Asp Ser Phe			
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[2753]	His Pro Leu Ile Thr Lys Asp Glu Val Leu Tyr Val Phe Pro Ser Asp			
[2754]		260	265	270
[2755]	Phe Cys Arg Ser Val Tyr Ile Thr Phe Ser Asp Tyr Glu Ser Val Gln			
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[2757]	Gly Leu Pro Ala Phe Arg Tyr Lys Val Pro Ala Glu Ile Leu Ala Asn			
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[2759]	Thr Ser Asp Asn Ala Gly Phe Cys Ile Pro Glu Gly Asn Cys Leu Gly			
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[2761]	Ser Gly Val Leu Asn Val Ser Ile Cys Lys Asn Gly Ala Pro Ile Ile			
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[2763]	Met Ser Phe Pro His Phe Tyr Gln Ala Asp Glu Arg Phe Val Ser Ala			
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[2767]	Ile Asn Pro Leu Thr Gly Ile Ile Leu Lys Ala Ala Lys Arg Phe Gln			
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[2769]	Ile Asn Ile Tyr Val Lys Lys Leu Asp Asp Phe Val Glu Thr Gly Asp			
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[2771]	Ile Arg Thr Met Val Phe Pro Val Met Tyr Leu Asn Glu Ser Val His			

[2772]	405	410	415
[2773]	Ile Asp Lys Glu Thr Ala Ser Arg Leu Lys Ser Met Ile Asn Thr Thr		
[2774]	420	425	430
[2775]	Leu Ile Ile Thr Asn Ile Pro Tyr Ile Ile Met Ala Leu Gly Val Phe		
[2776]	435	440	445
[2777]	Phe Gly Leu Val Phe Thr Trp Leu Ala Cys Lys Gly Gln Gly Ser Met		
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[2779]	Asp Glu Gly Thr Ala Asp Glu Arg Ala Pro Leu Ile Arg Thr		
[2780]	465	470	475
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[2784]	<213> 智人(Homo sapiens)		
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[2895]	<213> 智人(Homo sapiens)
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[2904]	50	55	60	
[2905]	Ser Gly Gln Leu Met Val Ser Tyr Glu Gly Lys Ala Met Gly Tyr Gln			
[2906]	65	70	75	80
[2907]	Val Pro Pro Phe Gly Trp Arg Ile Cys Leu Ala His Glu Phe Thr Glu			
[2908]	85	90	95	
[2909]	Lys Arg Lys Pro Phe Gln Ala Asn Asn Val Ser Leu Ser Asn Met Ile			
[2910]	100	105	110	
[2911]	Lys His Ile Gly Met Gly Leu Arg Tyr Leu Gln Trp Trp Tyr Arg Lys			
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[2913]	Thr His Val Glu Lys Lys Thr Pro Phe Ile Asp Met Ile Asn Ser Val			
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[2915]	Pro Leu Arg Gln Ile Tyr Gly Cys Pro Leu Gly Gly Ile Gly Gly Gly			
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[2917]	Thr Ile Thr Arg Gly Trp Arg Gly Gln Phe Cys Arg Trp Gln Leu Asn			
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[2919]	Pro Gly Met Tyr Gln His Arg Thr Val Ile Ala Asp Gln Phe Thr Val			
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[2921]	Cys Leu Arg Arg Glu Gly Gln Thr Val Tyr Gln Gln Val Leu Ser Leu			
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[2923]	Glu Arg Pro Ser Val Leu Arg Ser Trp Asn Trp Gly Leu Cys Gly Tyr			
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[2925]	Phe Ala Phe Tyr His Ala Leu Tyr Pro Arg Ala Trp Thr Val Tyr Gln			
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[2927]	Leu Pro Gly Gln Asn Val Thr Leu Thr Cys Arg Gln Ile Thr Pro Ile			
[2928]	245	250	255	
[2929]	Leu Pro His Asp Tyr Gln Asp Ser Ser Leu Pro Val Gly Val Phe Val			
[2930]	260	265	270	
[2931]	Trp Asp Val Glu Asn Glu Gly Asp Glu Ala Leu Asp Val Ser Ile Met			
[2932]	275	280	285	
[2933]	Phe Ser Met Arg Asn Gly Leu Gly Gly Gly Asp Asp Ala Pro Gly Gly			
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[2935]	Leu Trp Asn Glu Pro Phe Cys Leu Glu Arg Ser Gly Glu Thr Val Arg			
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[2937]	Gly Leu Leu Leu His His Pro Thr Leu Pro Asn Pro Tyr Thr Met Ala			
[2938]	325	330	335	
[2939]	Val Ala Ala Arg Val Thr Ala Ala Thr Thr Val Thr His Ile Thr Ala			

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[2943]	Asp Gly Gln Leu Asp Ser Pro Thr Gly Gln Ser Thr Pro Thr Gln Lys		
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[2945]	Gly Val Gly Ile Ala Gly Ala Val Cys Val Ser Ser Lys Leu Arg Pro		
[2946]	385	390	395
[2947]	Arg Gly Gln Cys Arg Leu Glu Phe Ser Leu Ala Trp Asp Met Pro Arg		
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[2949]	Ile Met Phe Gly Ala Lys Gly Gln Val His Tyr Arg Arg Tyr Thr Arg		
[2950]	420	425	430
[2951]	Phe Phe Gly Gln Asp Gly Asp Ala Ala Pro Ala Leu Ser His Tyr Ala		
[2952]	435	440	445
[2953]	Leu Cys Arg Tyr Ala Glu Trp Glu Glu Arg Ile Ser Ala Trp Gln Ser		
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[2955]	Pro Val Leu Asp Asp Arg Ser Leu Pro Ala Trp Tyr Lys Ser Ala Leu		
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[2957]	Phe Asn Glu Leu Tyr Phe Leu Ala Asp Gly Gly Thr Val Trp Leu Glu		
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[2959]	Val Leu Glu Asp Ser Leu Pro Glu Glu Leu Gly Arg Asn Met Cys His		
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[2963]	Gln Glu Tyr Arg Met Tyr Asn Thr Tyr Asp Val His Phe Tyr Ala Ser		
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[2965]	Phe Ala Leu Ile Met Leu Trp Pro Lys Leu Glu Leu Ser Leu Gln Tyr		
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[2967]	Asp Met Ala Leu Ala Thr Leu Arg Glu Asp Leu Thr Arg Arg Arg Tyr		
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[2973]	Tyr Leu Ile His Asp Thr Ala Asp Trp Lys Asp Leu Asn Leu Lys Phe		
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[2975]	Val Leu Gln Val Tyr Arg Asp Tyr Tyr Leu Thr Gly Asp Gln Asn Phe		
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[2981]	Asp Gln Thr Tyr Asp Gly Trp Val Thr Thr Gly Pro Ser Ala Tyr Cys		

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[2987]	Arg Gly Gln Glu Ala Tyr Glu Arg Leu Leu Trp Asn Gly Arg Tyr Tyr		
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[2989]	Asn Tyr Asp Ser Ser Ser Arg Pro Gln Ser Arg Ser Val Met Ser Asp		
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[2991]	Gln Cys Ala Gly Gln Trp Phe Leu Lys Ala Cys Gly Leu Gly Glu Gly		
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[2993]	Asp Thr Glu Val Phe Pro Thr Gln His Val Val Arg Ala Leu Gln Thr		
[2994]	770	775	780
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[2997]	Val Asn Gly Met Gln Pro His Gly Val Pro Asp Lys Ser Ser Val Gln		
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[2999]	Ser Asp Glu Val Trp Val Gly Val Val Tyr Gly Leu Ala Ala Thr Met		
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[3001]	Ile Gln Glu Gly Leu Thr Trp Glu Gly Phe Gln Thr Ala Glu Gly Cys		
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[3003]	Tyr Arg Thr Val Trp Glu Arg Leu Gly Leu Ala Phe Gln Thr Pro Glu		
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[3005]	Ala Tyr Cys Gln Gln Arg Val Phe Arg Ser Leu Ala Tyr Met Arg Pro		
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[3007]	Leu Ser Ile Trp Ala Met Gln Leu Ala Leu Gln Gln Gln Gln His Lys		
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[3009]	Lys Ala Ser Trp Pro Lys Val Lys Gln Gly Thr Gly Leu Arg Thr Gly		
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[3016]	<213> 智人 (Homo sapiens)		
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[3020]	cagtgaccg actgcaagag ccccgaggac agccgcccc ccaaggagac cgactgctgc	180	
[3021]	aaccccgagg acagcggcca gctgatggtg agctacgagg gcaaggccat gggctaccag	240	
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[4797]	Ser Arg Pro Ala Glu Lys Pro Pro Arg Pro Glu Ala Lys Ser Ala Gln
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[4801]	Glu Leu Asn Leu Pro Asn Leu Ala Ala Ala Tyr Ser Ser Ile Leu Ser
[4802]	65 70 75 80
[4803]	Ser Leu Gly Glu Asn Pro Gln Arg Gln Gly Leu Leu Lys Thr Pro Trp
[4804]	85 90 95
[4805]	Arg Ala Ala Ser Ala Met Gln Phe Phe Thr Lys Gly Tyr Gln Glu Thr
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[4807]	Ile Ser Asp Val Leu Asn Asp Ala Ile Phe Asp Glu Asp His Asp Glu
[4808]	115 120 125
[4809]	Met Val Ile Val Lys Asp Ile Asp Met Phe Ser Met Cys Glu His His
[4810]	130 135 140
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[4813]	Gln Val Leu Gly Leu Ser Lys Leu Ala Arg Ile Val Glu Ile Tyr Ser
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[4819]	Thr His Met Cys Met Val Met Arg Gly Val Gln Lys Met Asn Ser Lys
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[4821]	Thr Val Thr Ser Thr Met Leu Gly Val Phe Arg Glu Asp Pro Lys Thr
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[4856]	Ser Lys His Tyr Lys Ser Thr Val Gly Val Asp Phe Ala Leu Lys Val
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[4858]	Leu Gln Trp Ser Asp Tyr Glu Ile Val Arg Leu Gln Leu Trp Asp Ile
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[4860]	Ala Gly Gln Glu Arg Phe Thr Ser Met Thr Arg Leu Tyr Tyr Arg Asp
[4861]	65 70 75 80
[4862]	Ala Ser Ala Cys Val Ile Met Phe Asp Val Thr Asn Ala Thr Thr Phe
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[4864]	Ser Asn Ser Gln Arg Trp Lys Gln Asp Leu Asp Ser Lys Leu Thr Leu
[4865]	100 105 110
[4866]	Pro Asn Gly Glu Pro Val Pro Cys Leu Leu Leu Ala Asn Lys Cys Asp
[4867]	115 120 125
[4868]	Leu Ser Pro Trp Ala Val Ser Arg Asp Gln Ile Asp Arg Phe Ser Lys
[4869]	130 135 140
[4870]	Glu Asn Gly Phe Thr Gly Trp Thr Glu Thr Ser Val Lys Glu Asn Lys
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[4908]	35 40 45
[4909]	Met Leu Gly Glu Leu Arg Thr Ser Met Leu Ser Pro Lys Ser Tyr Tyr
[4910]	50 55 60
[4911]	Glu Leu Tyr Met Ala Ile Ser Asp Glu Leu His Tyr Leu Glu Val Tyr
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[4913]	Leu Thr Asp Glu Phe Ala Lys Gly Arg Lys Val Ala Asp Leu Tyr Glu

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[4937]	Glu Phe His Leu Gln Thr Leu Asn Pro Phe Leu Arg Ala Cys Ala Glu					
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[4939]	Leu His Gln Asn Val Asn Val Lys Asn Ile Ile Ile Ala Leu Ile Asp					
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[4947]	Ser Leu Ile Asn Leu Ala Met Lys Cys Tyr Pro Asp Arg Val Asp Tyr					
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[4953]	Leu Leu Lys Ile Pro Val Asp Thr Tyr Asn Asn Ile Leu Thr Val Leu					
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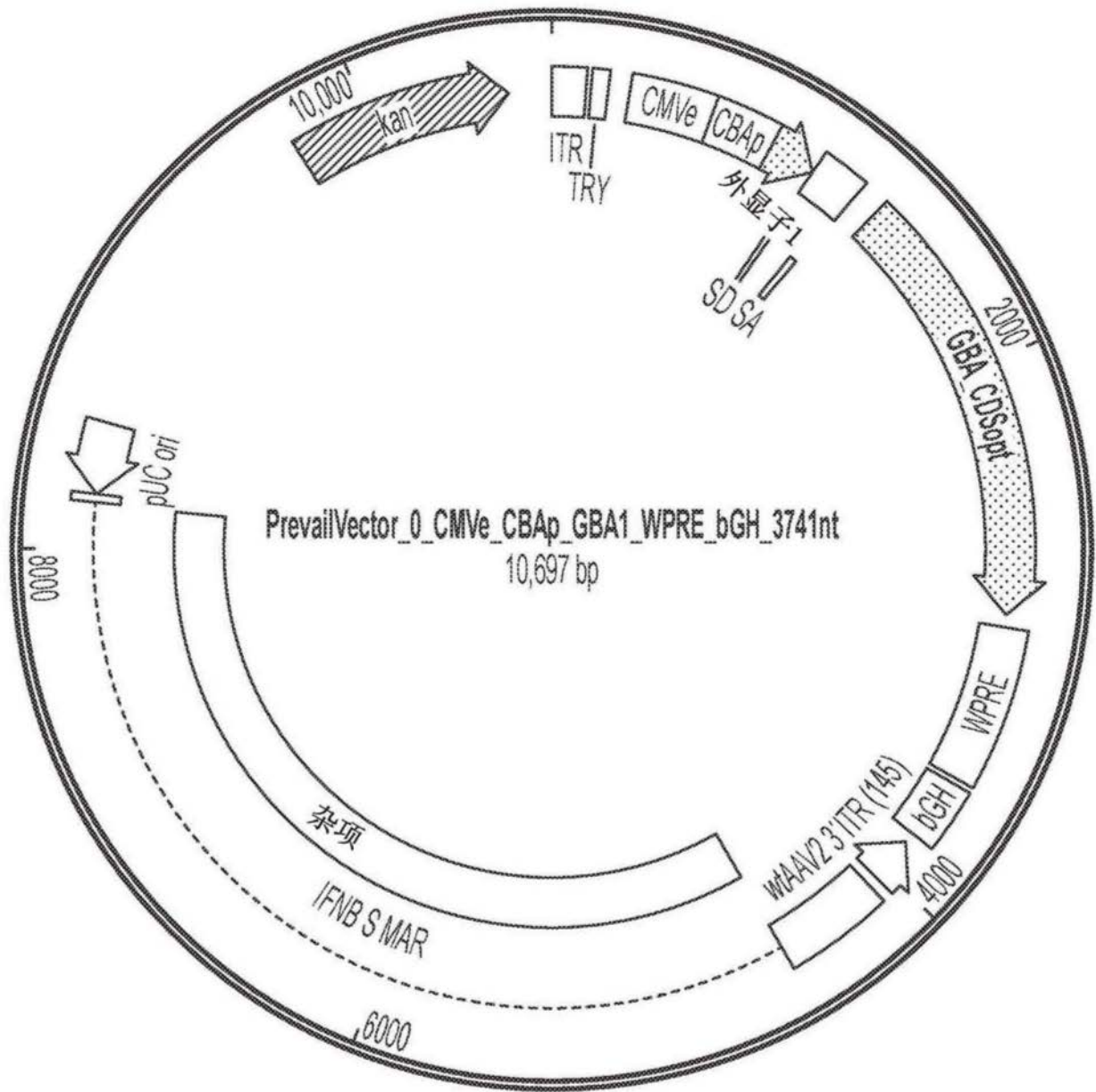


图1

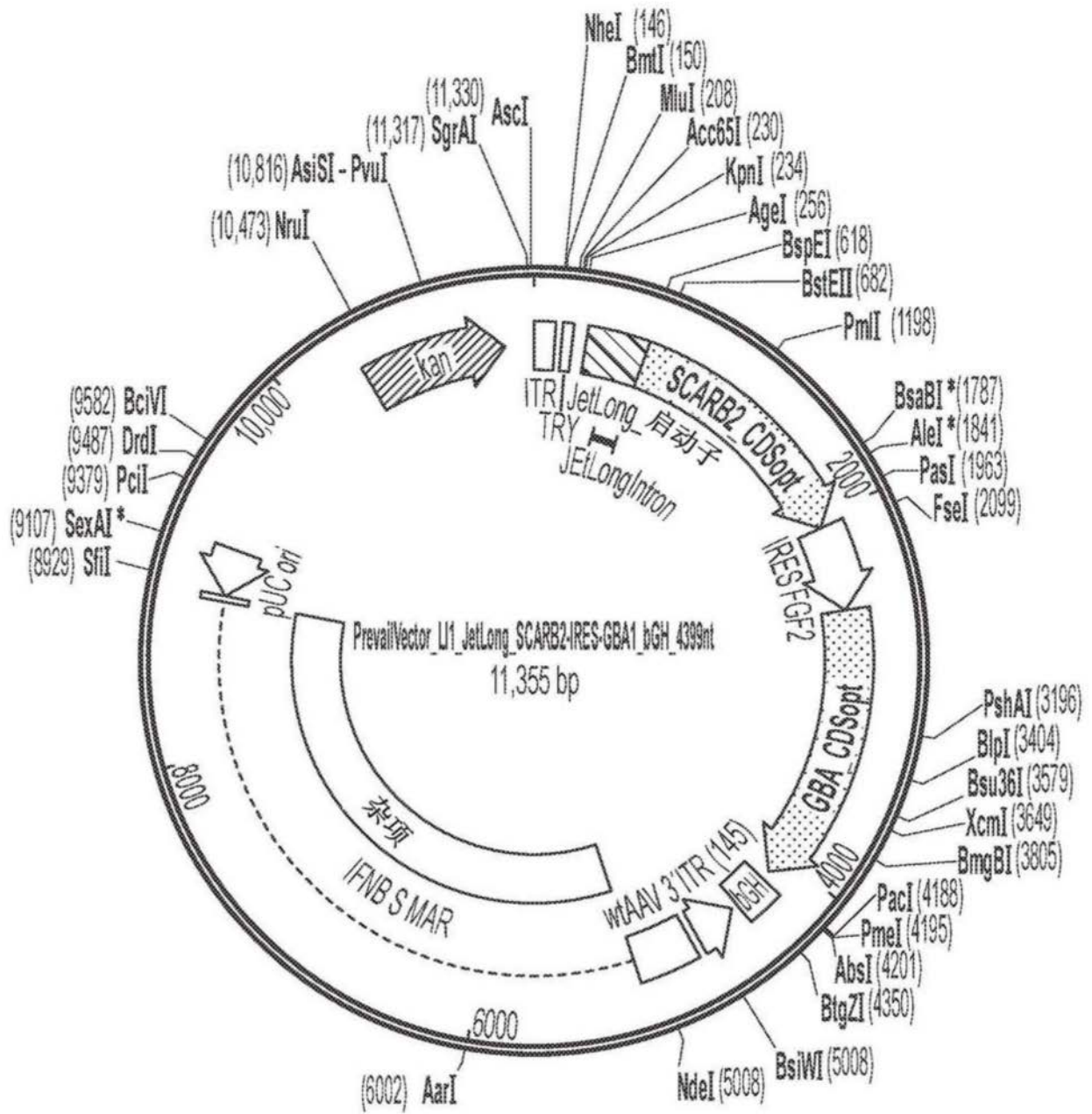


图2

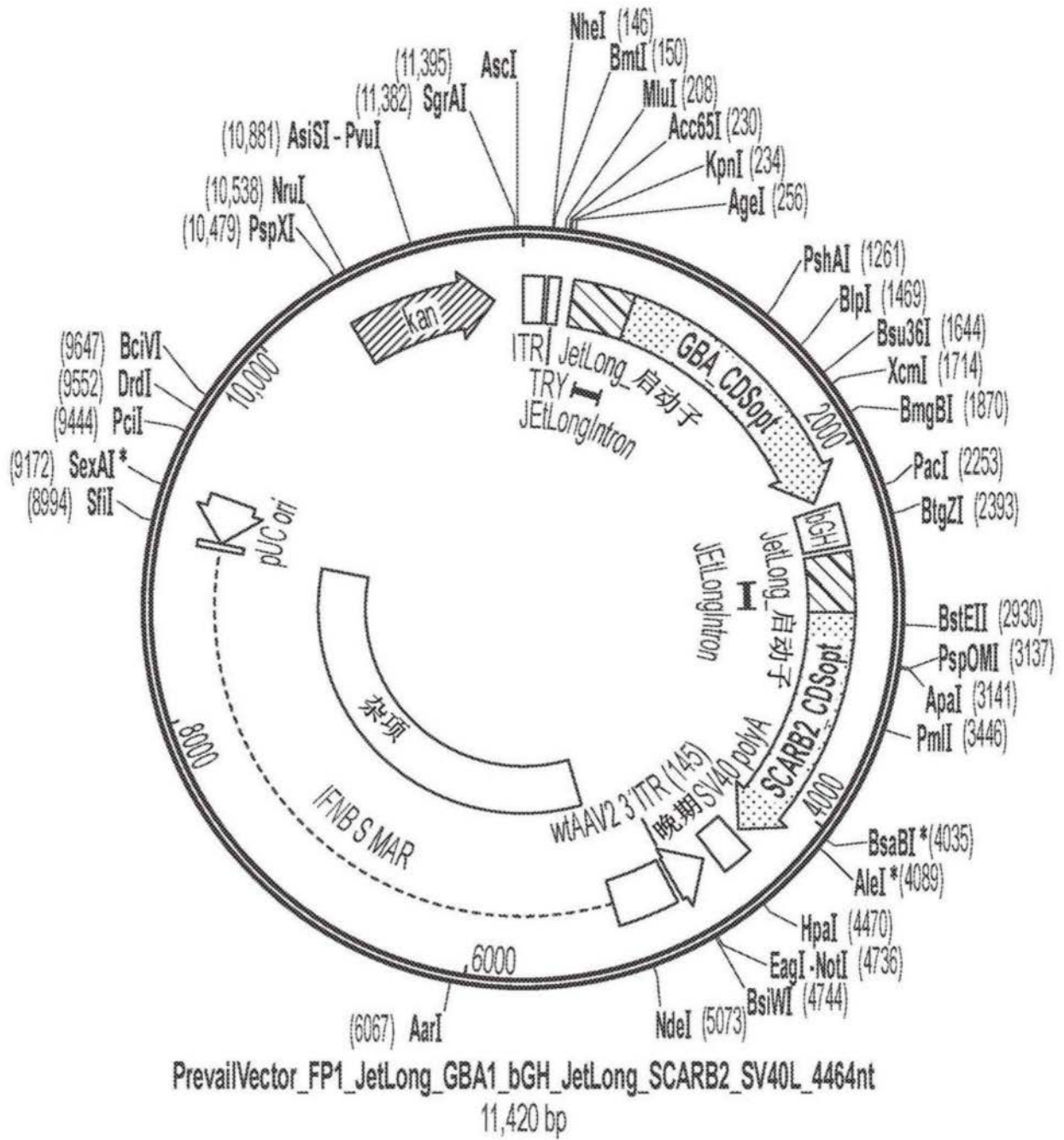


图3

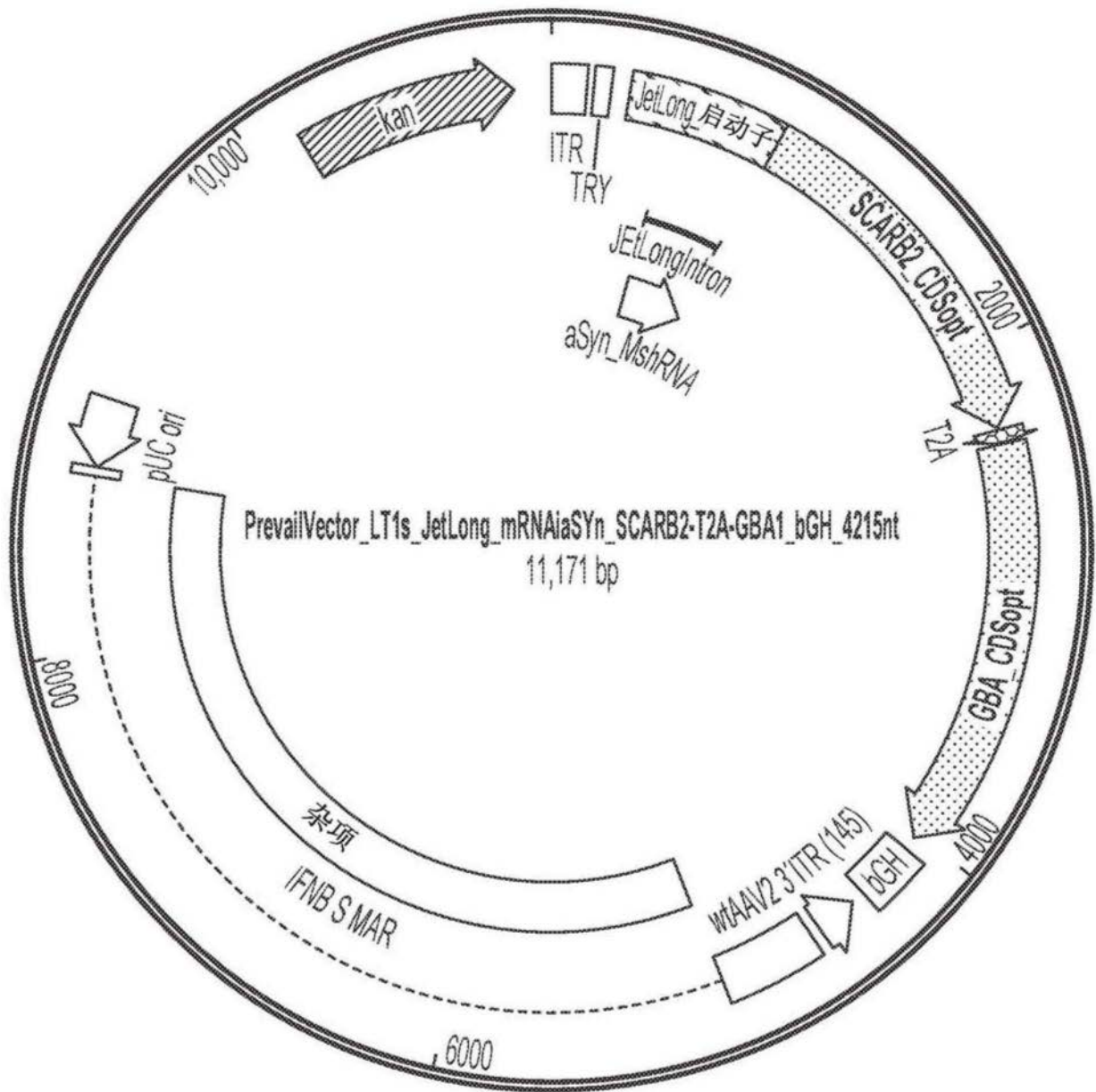


图4

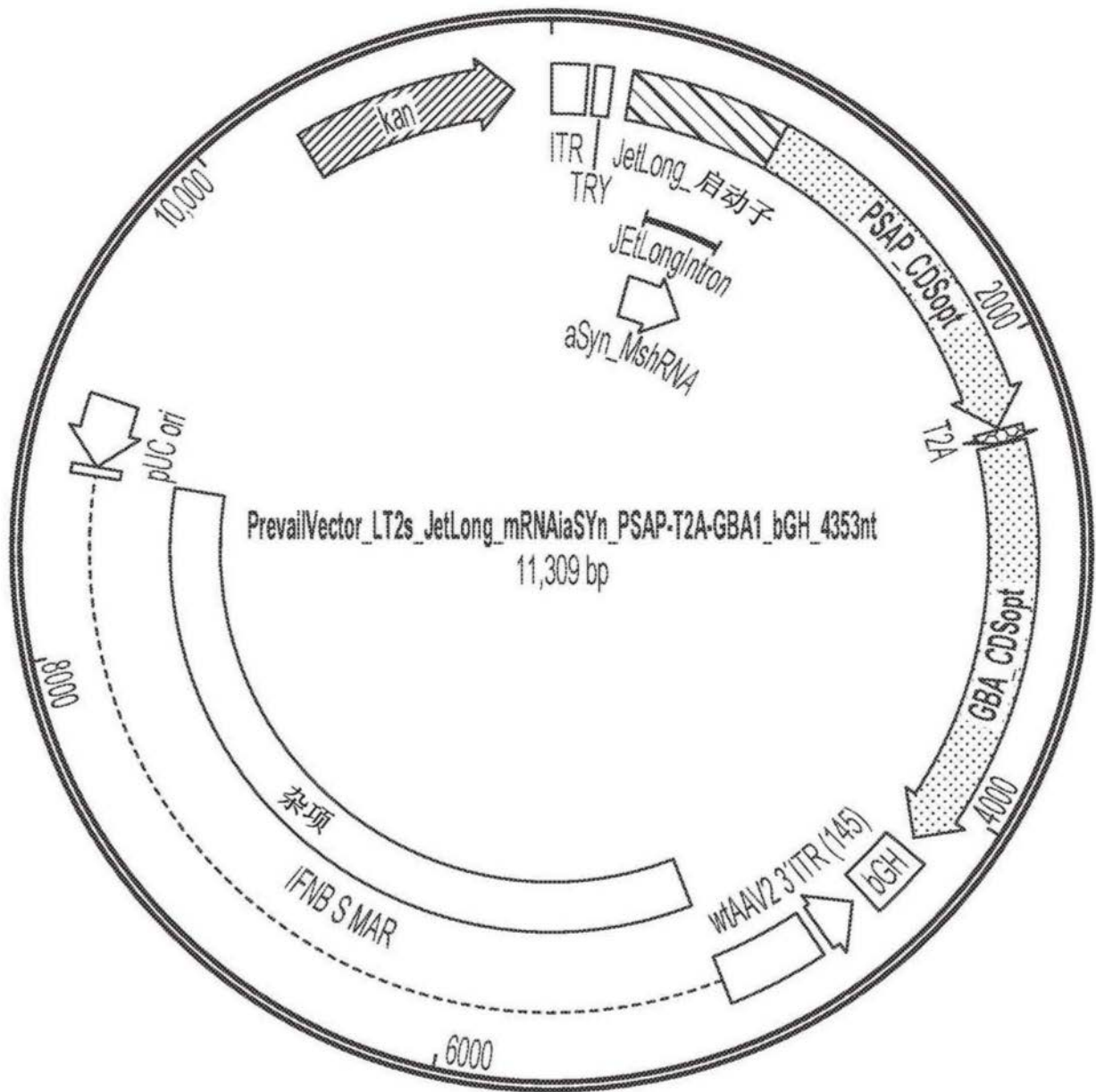


图5

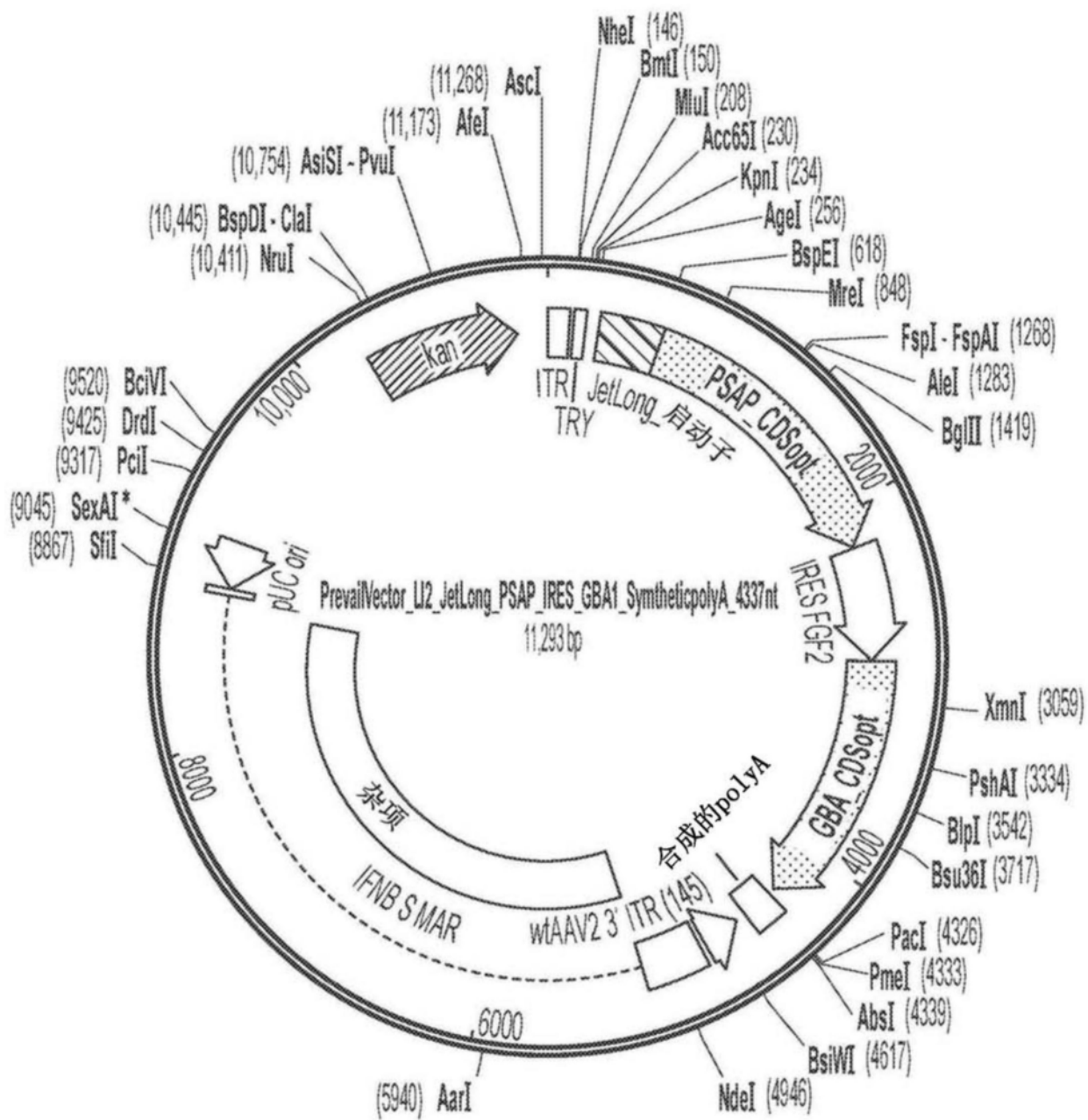


图6

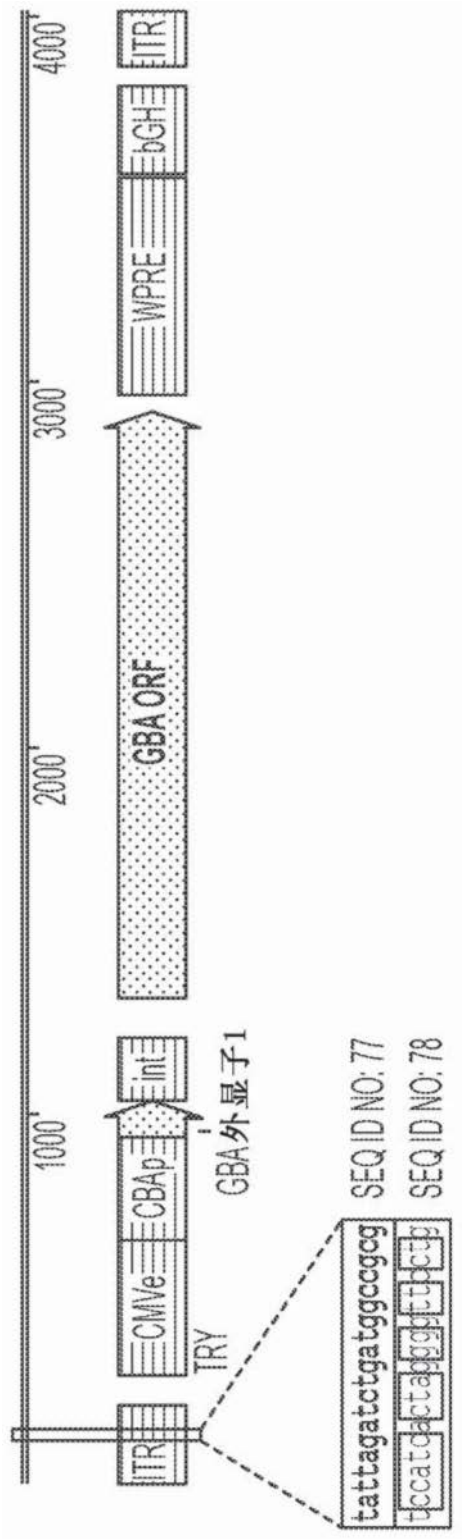


图7

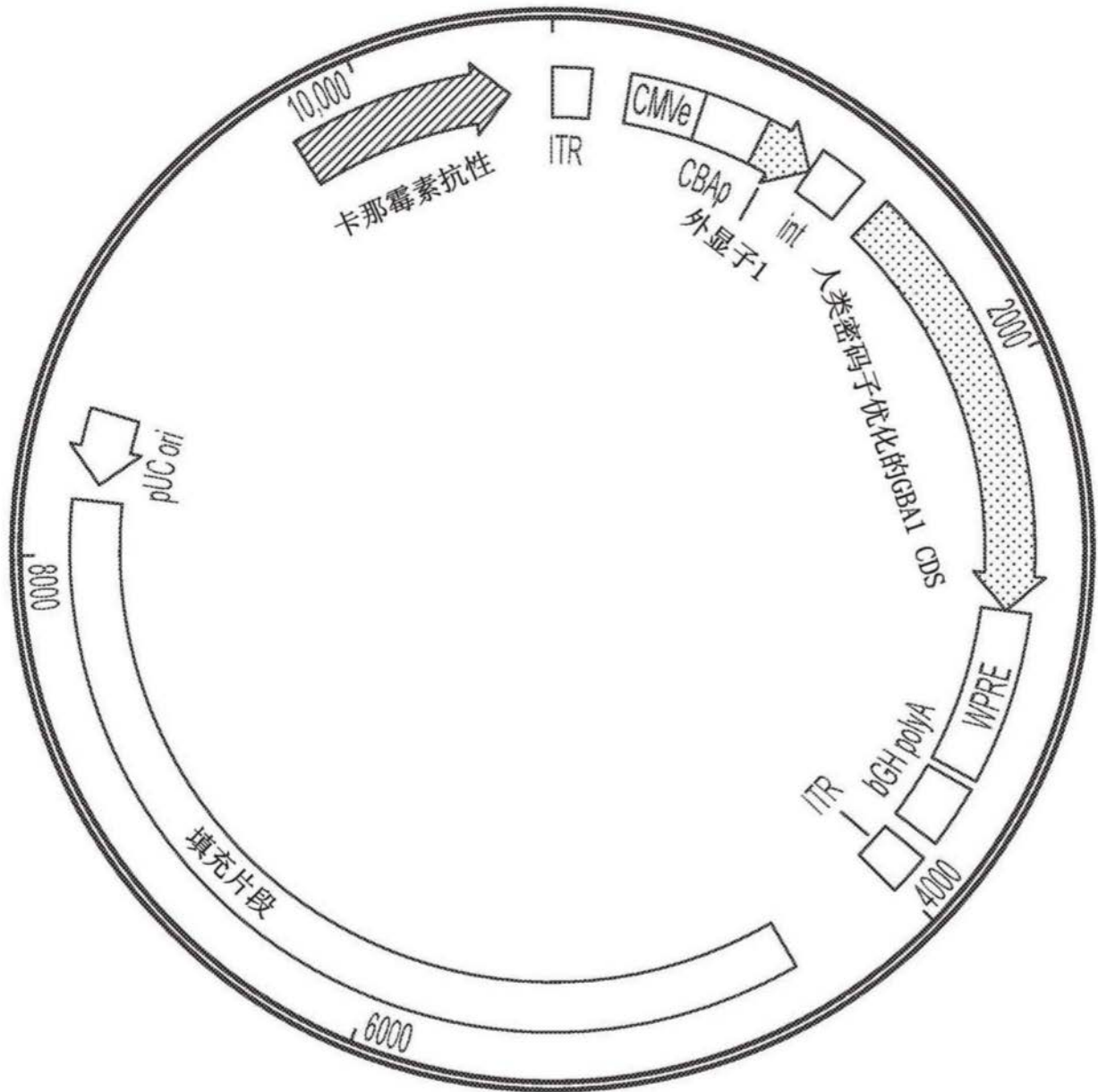


图8

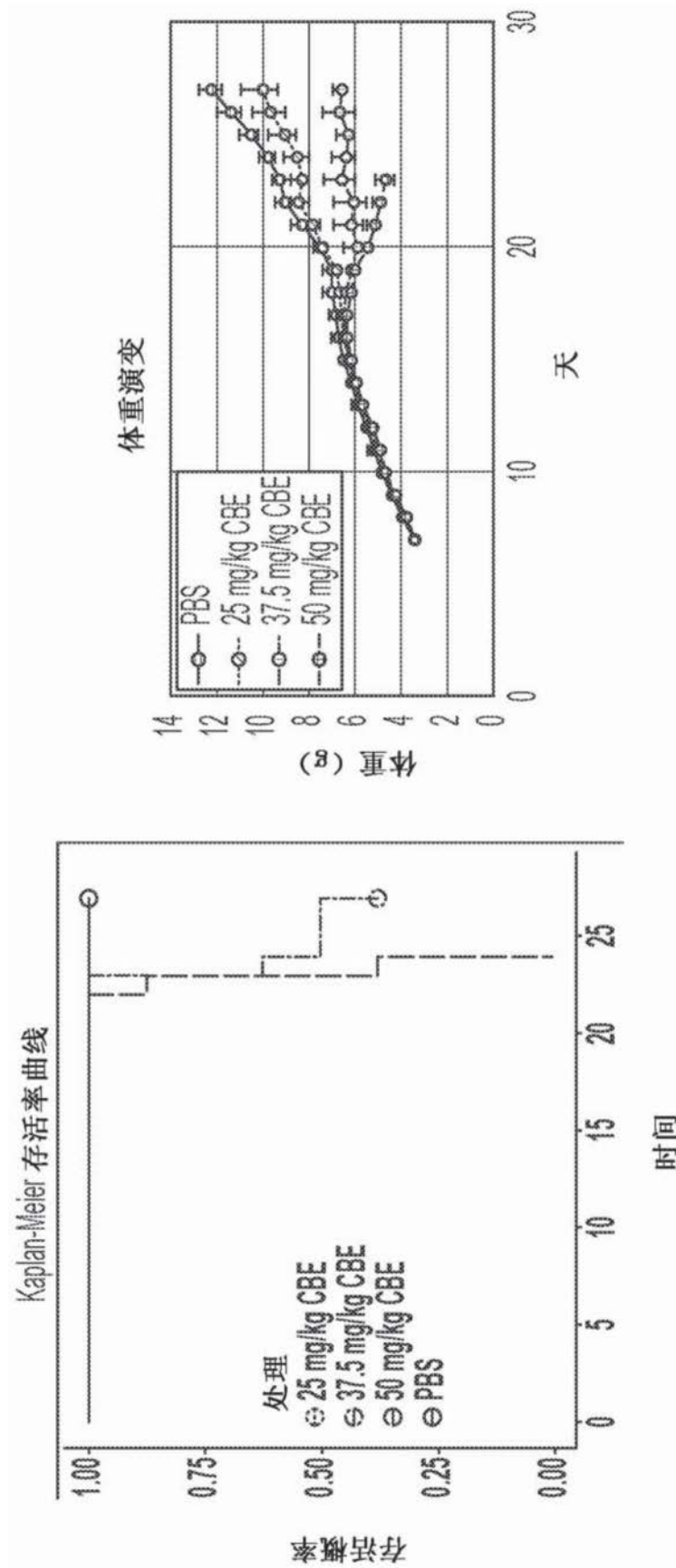


图9

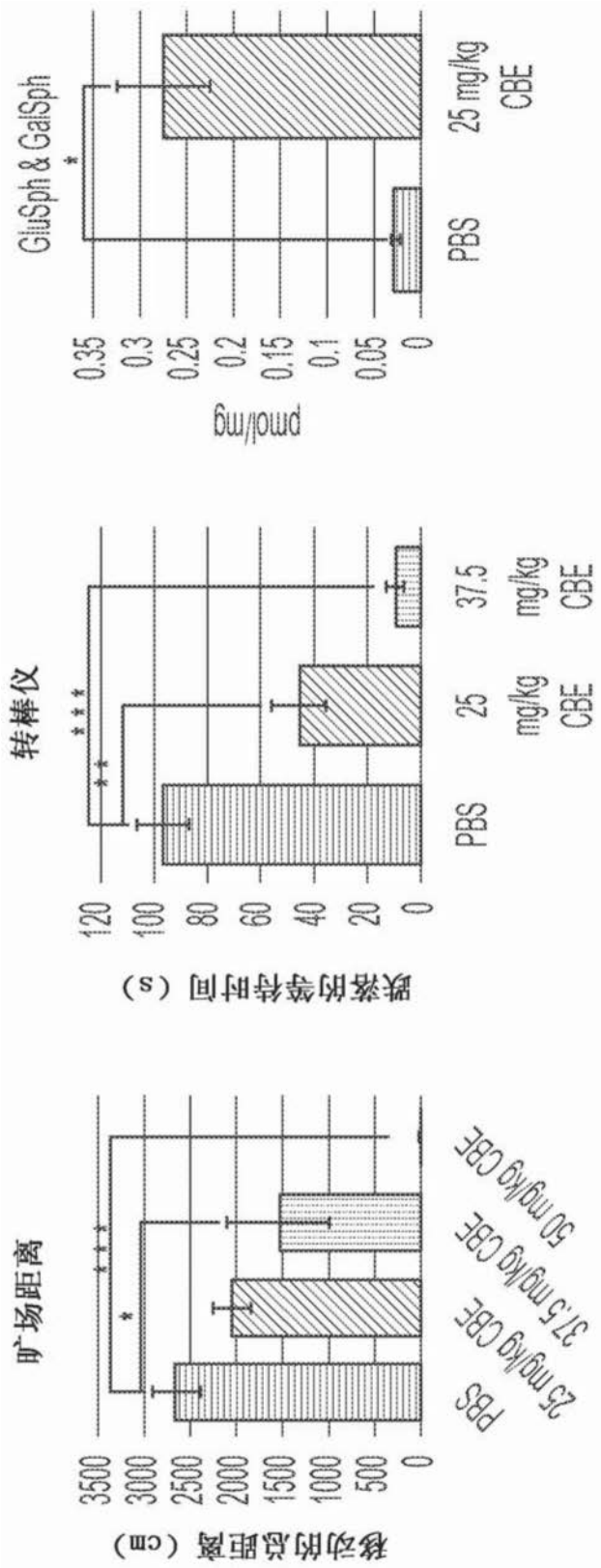


图9续

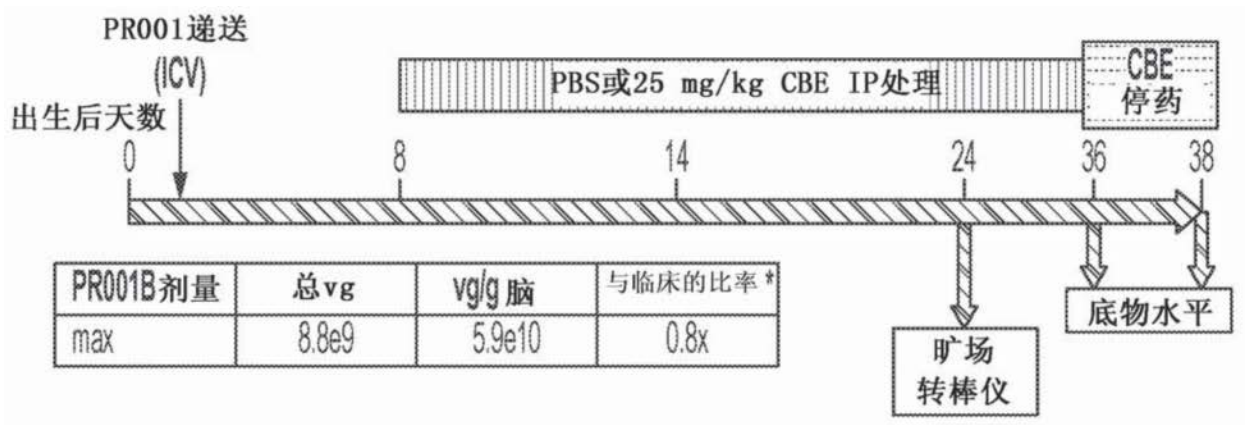


图10

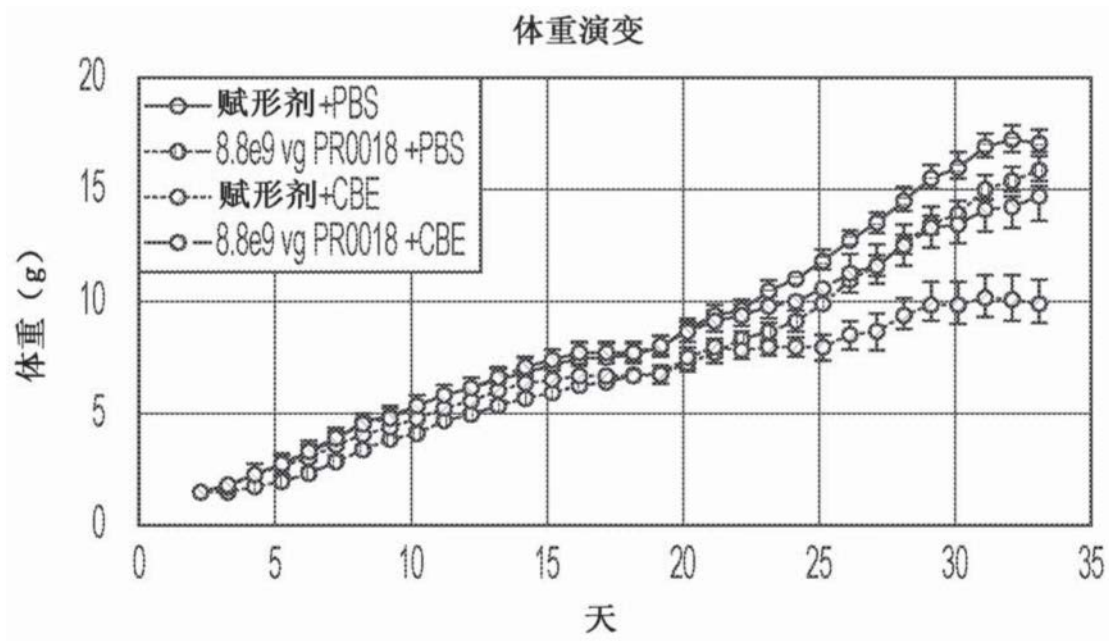


图11

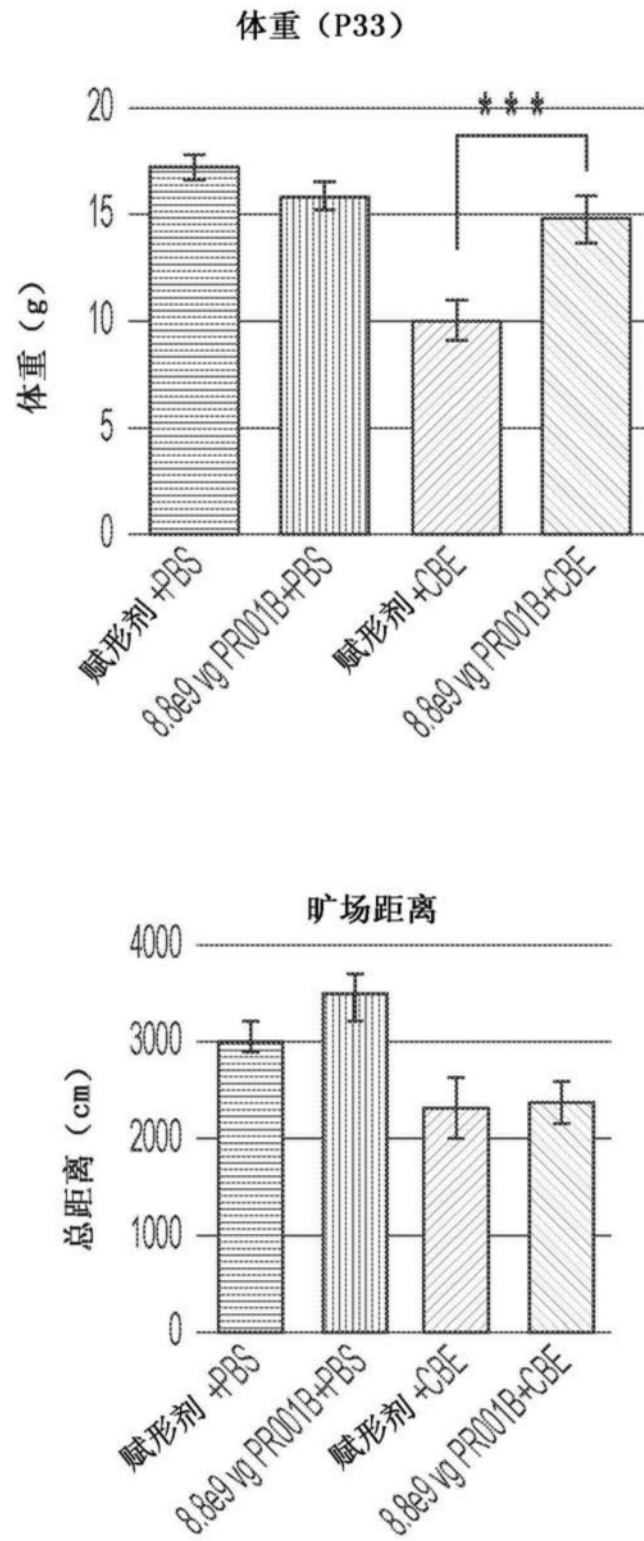


图11续

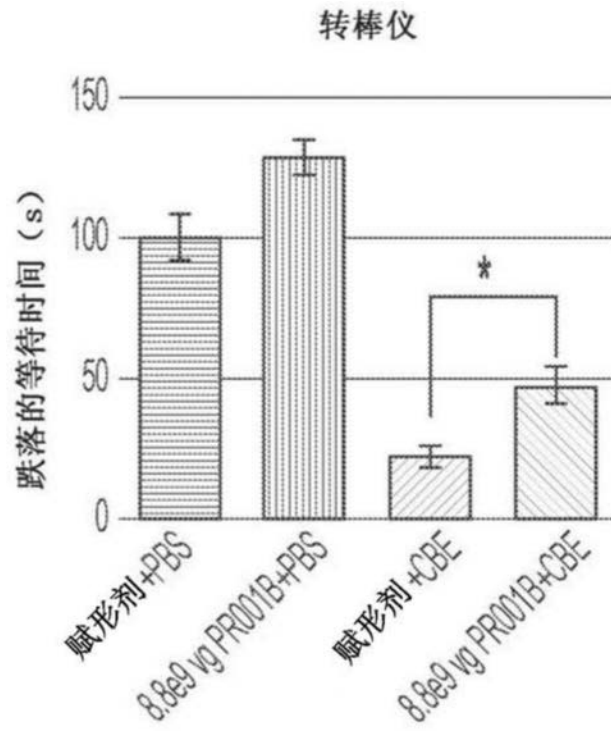


图11续

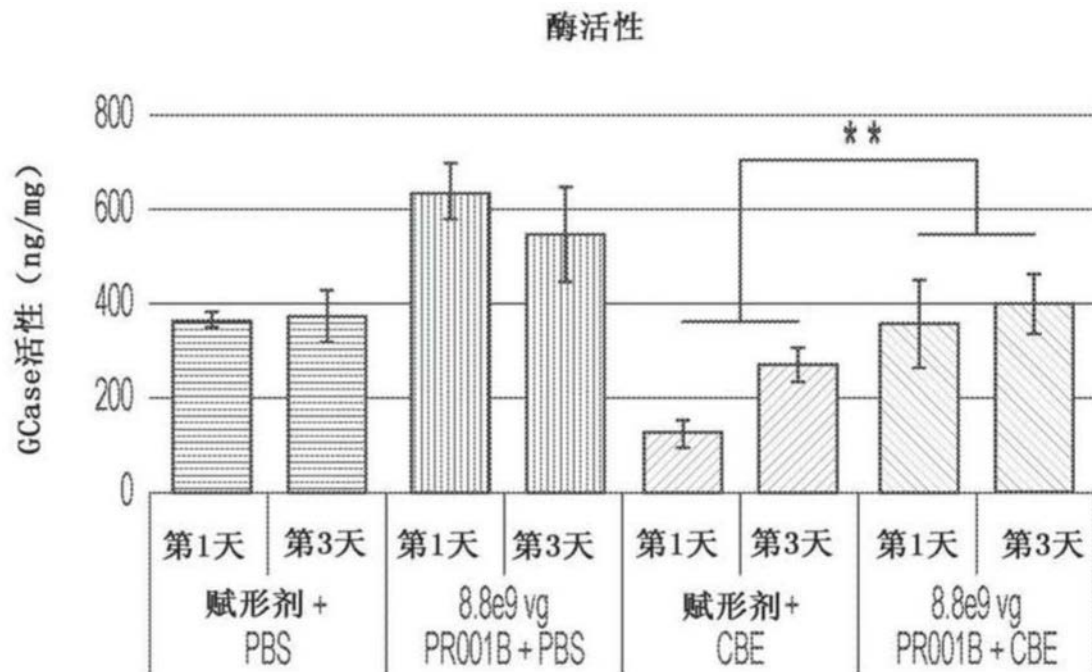


图12

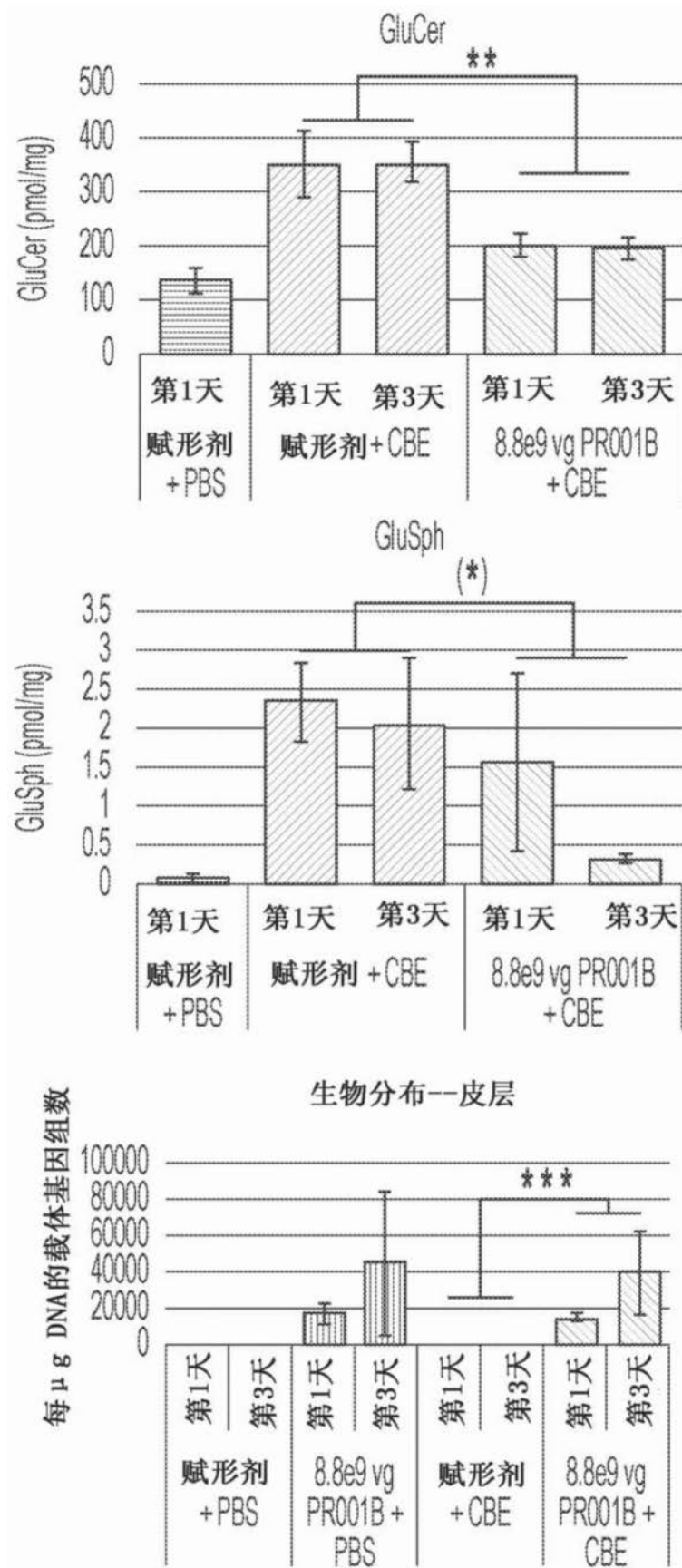


图12续

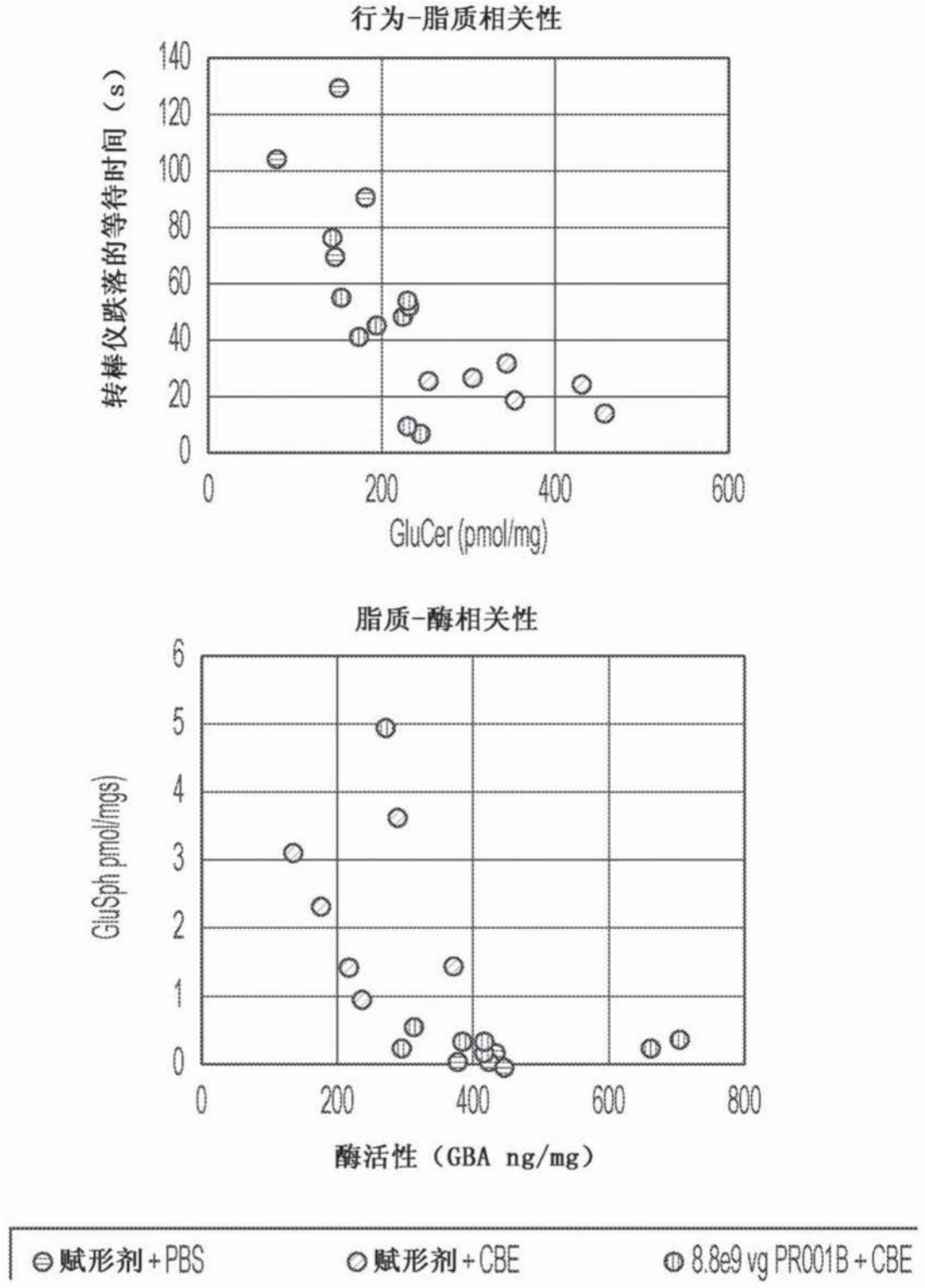


图13

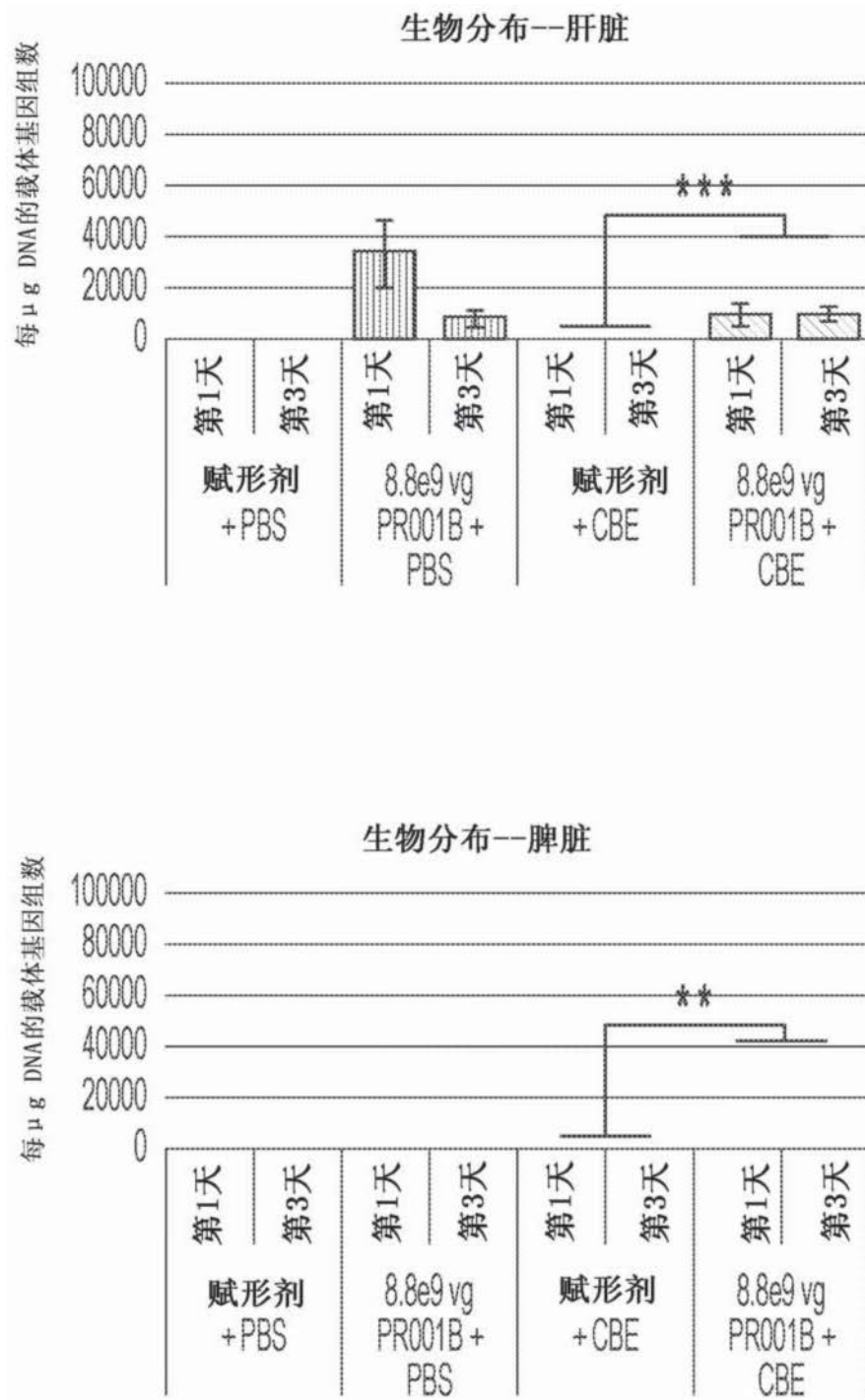


图14

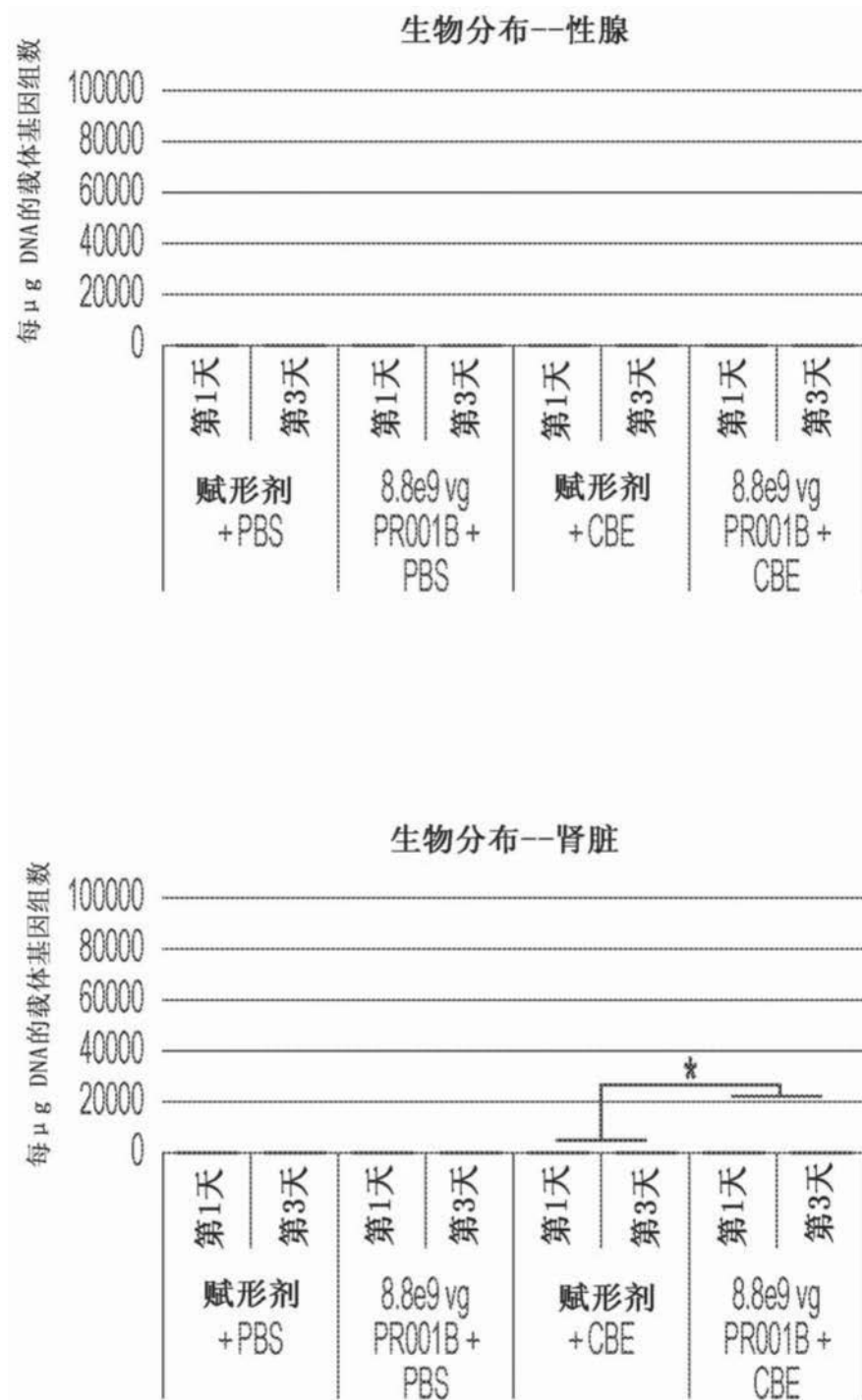


图14续

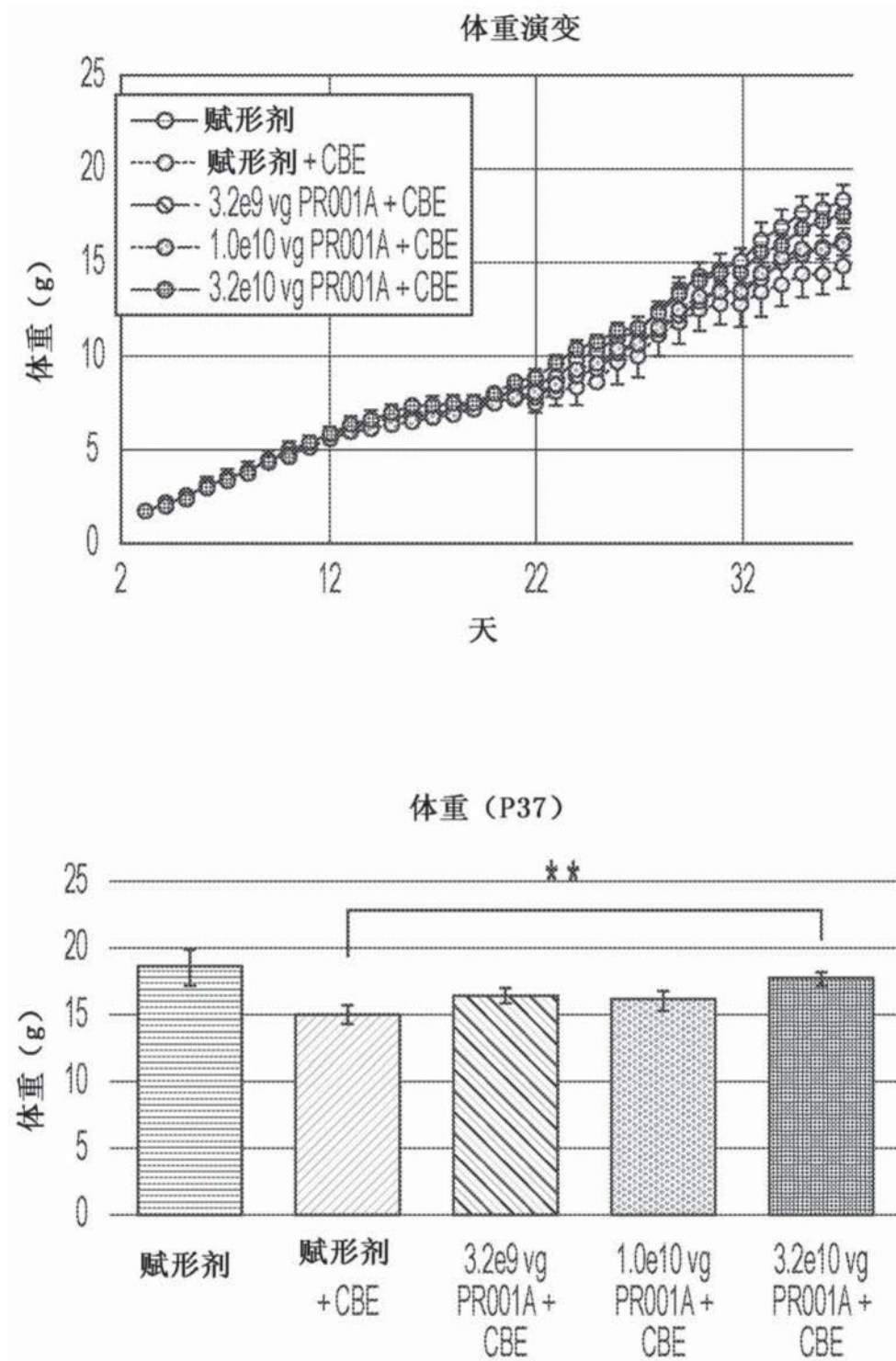


图15

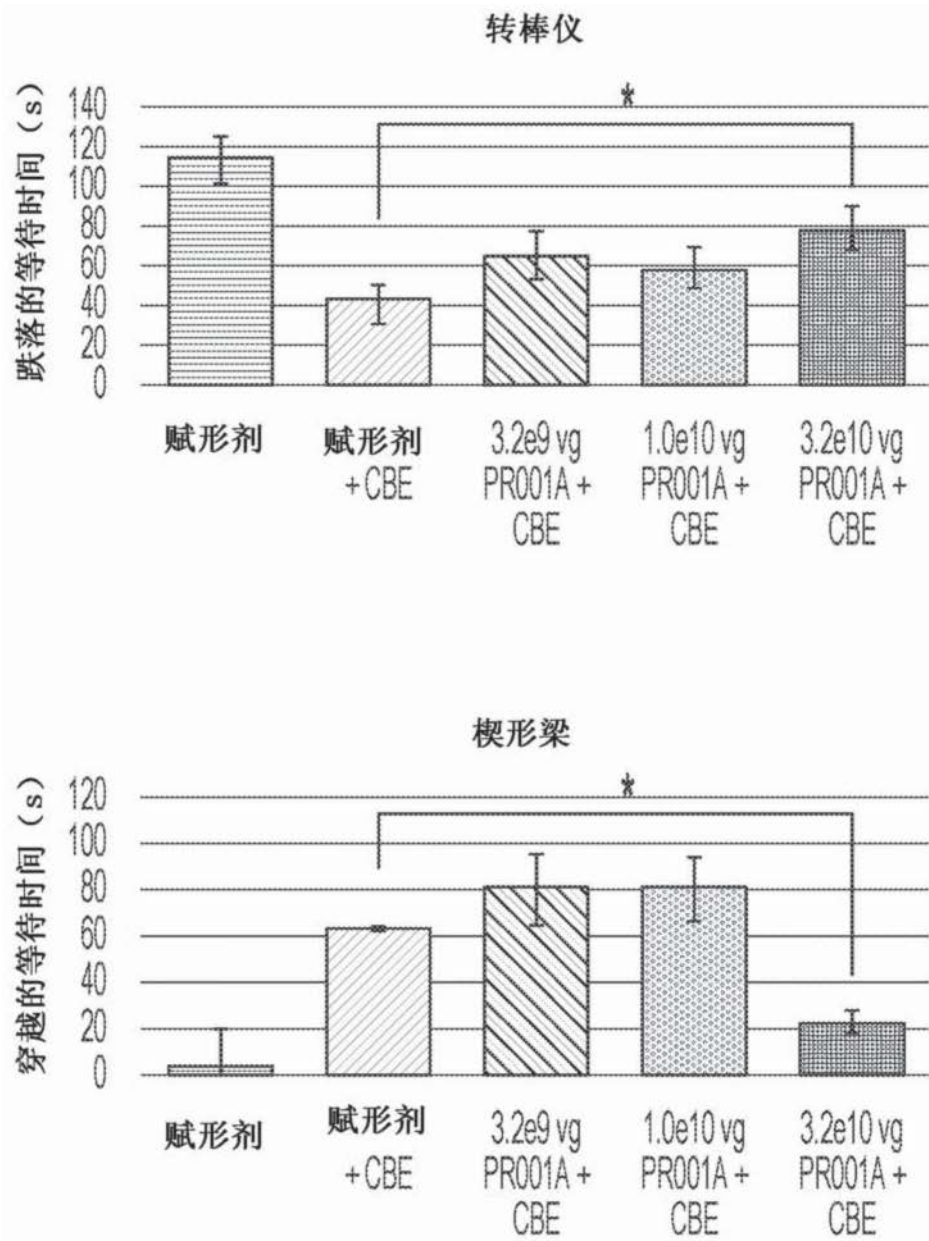


图15续

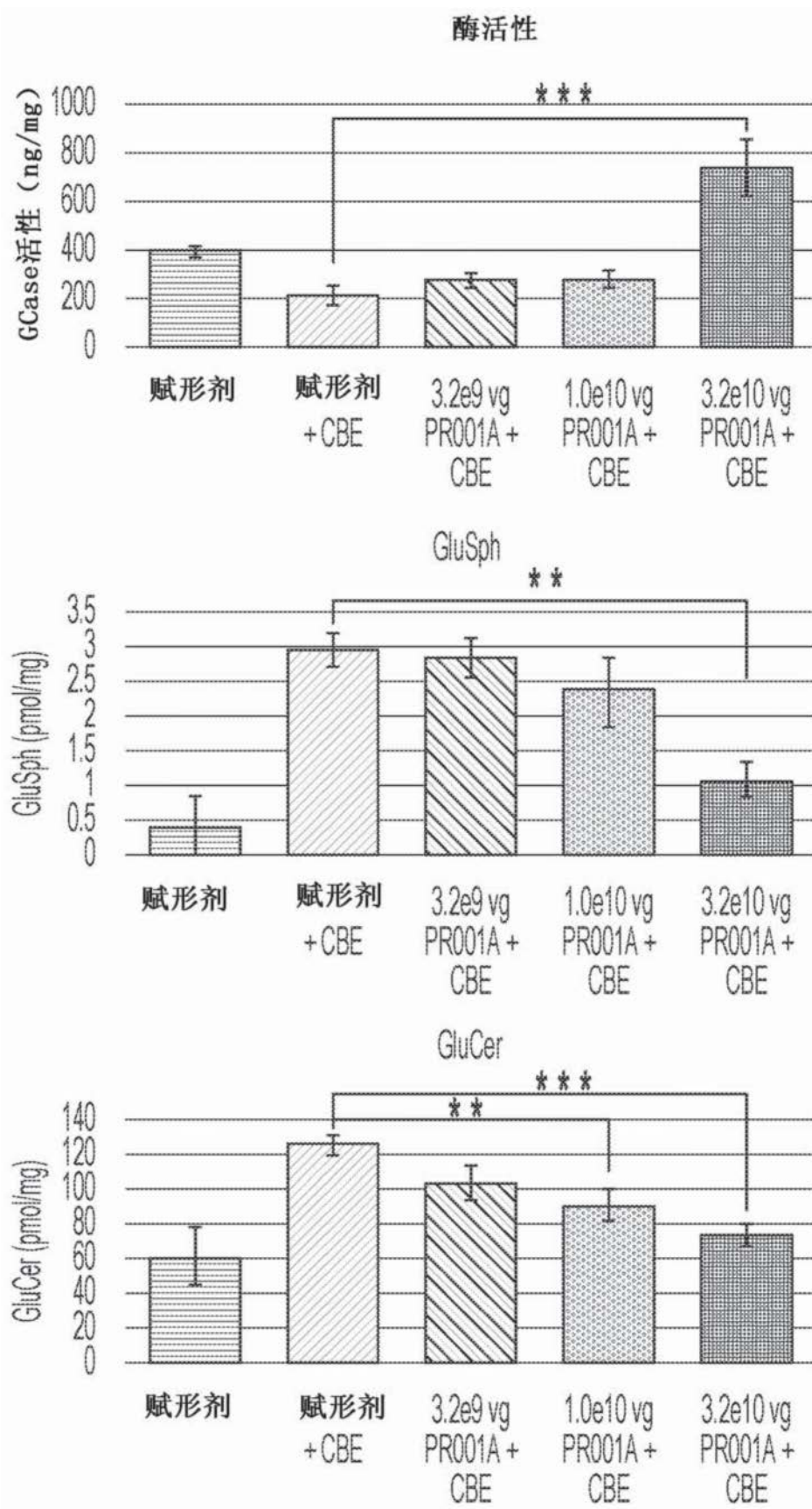


图16

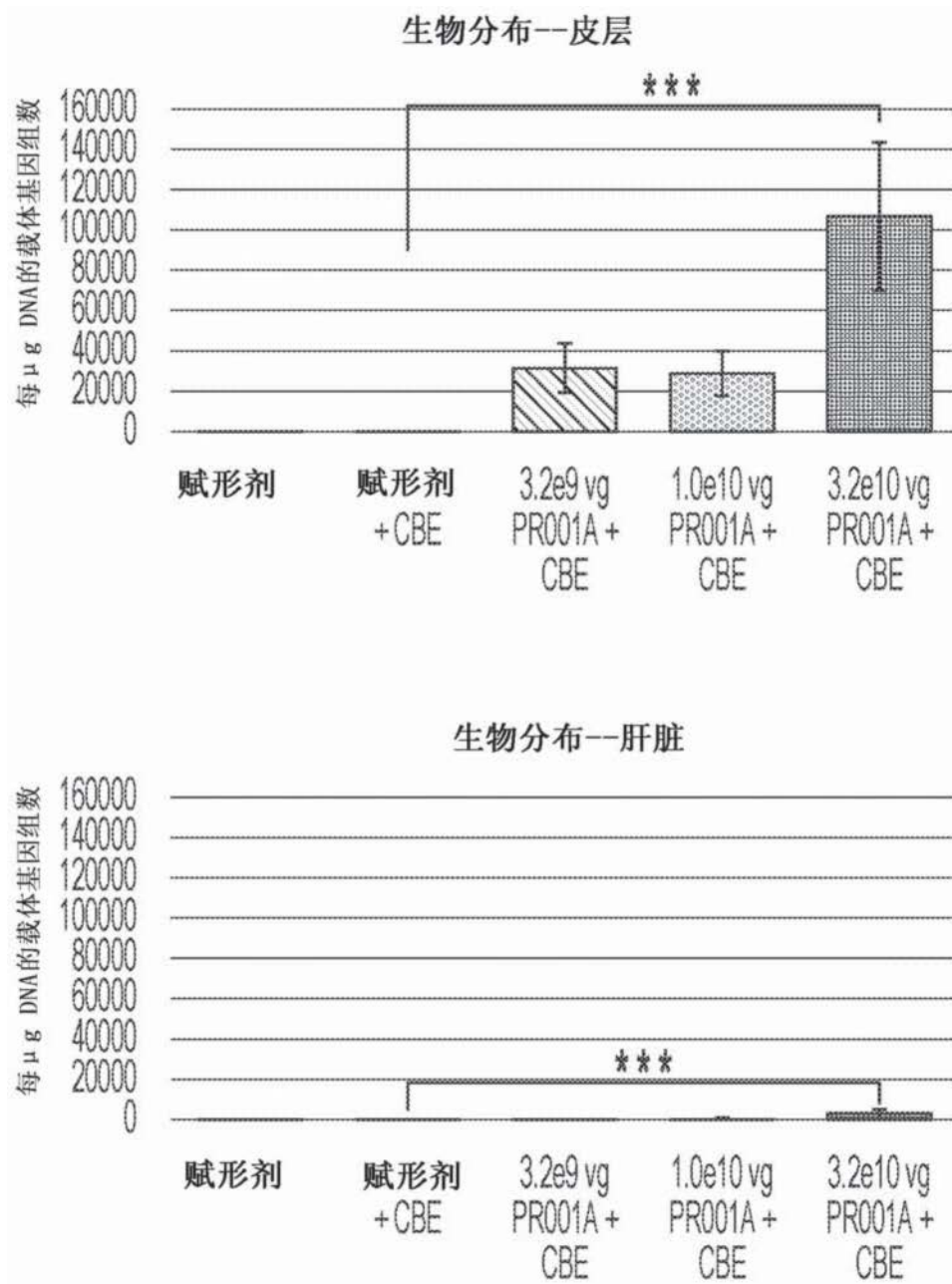


图16续

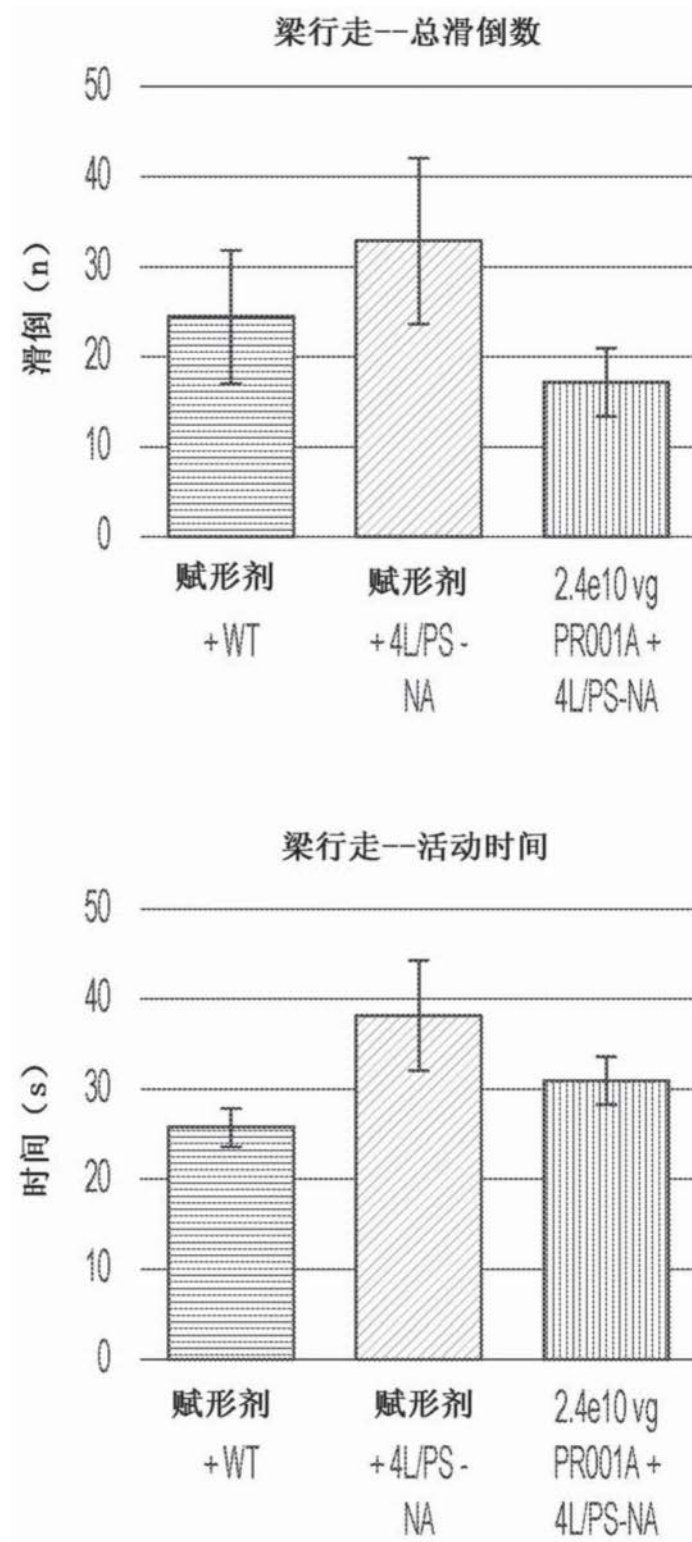


图17

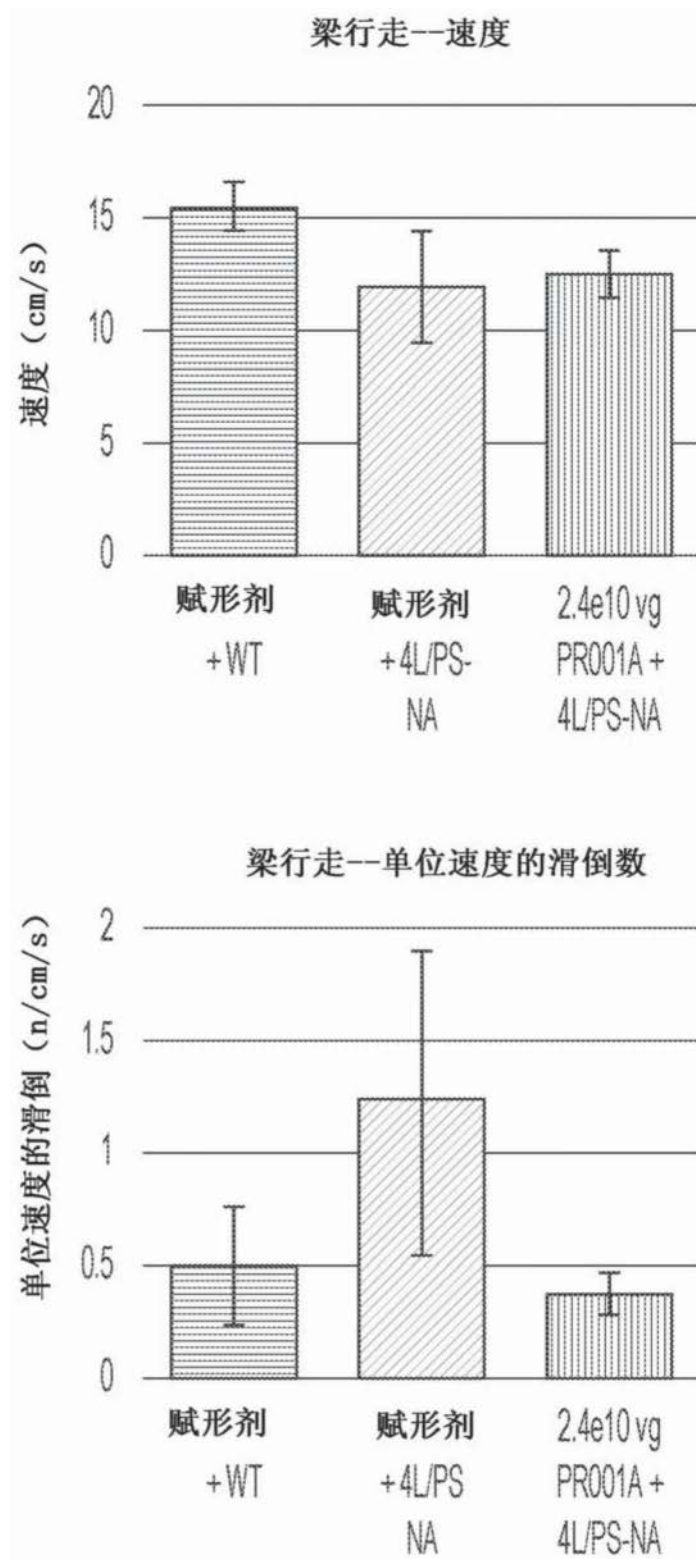


图17续

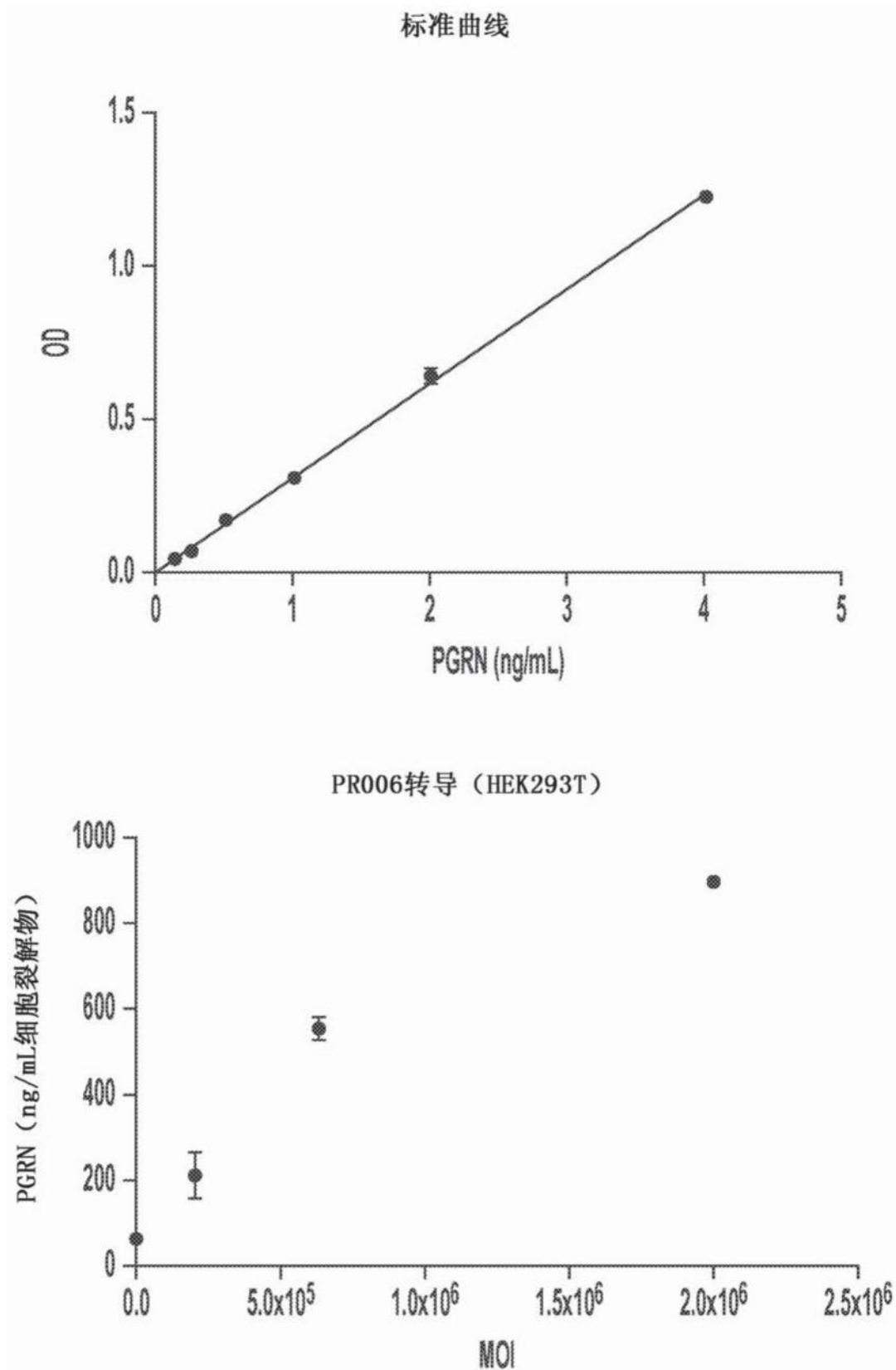


图18

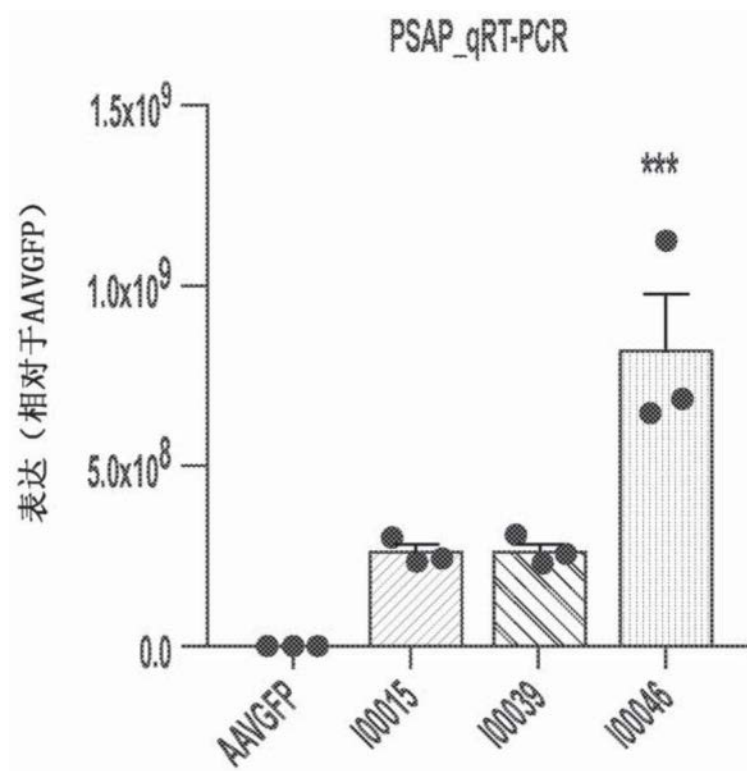


图19

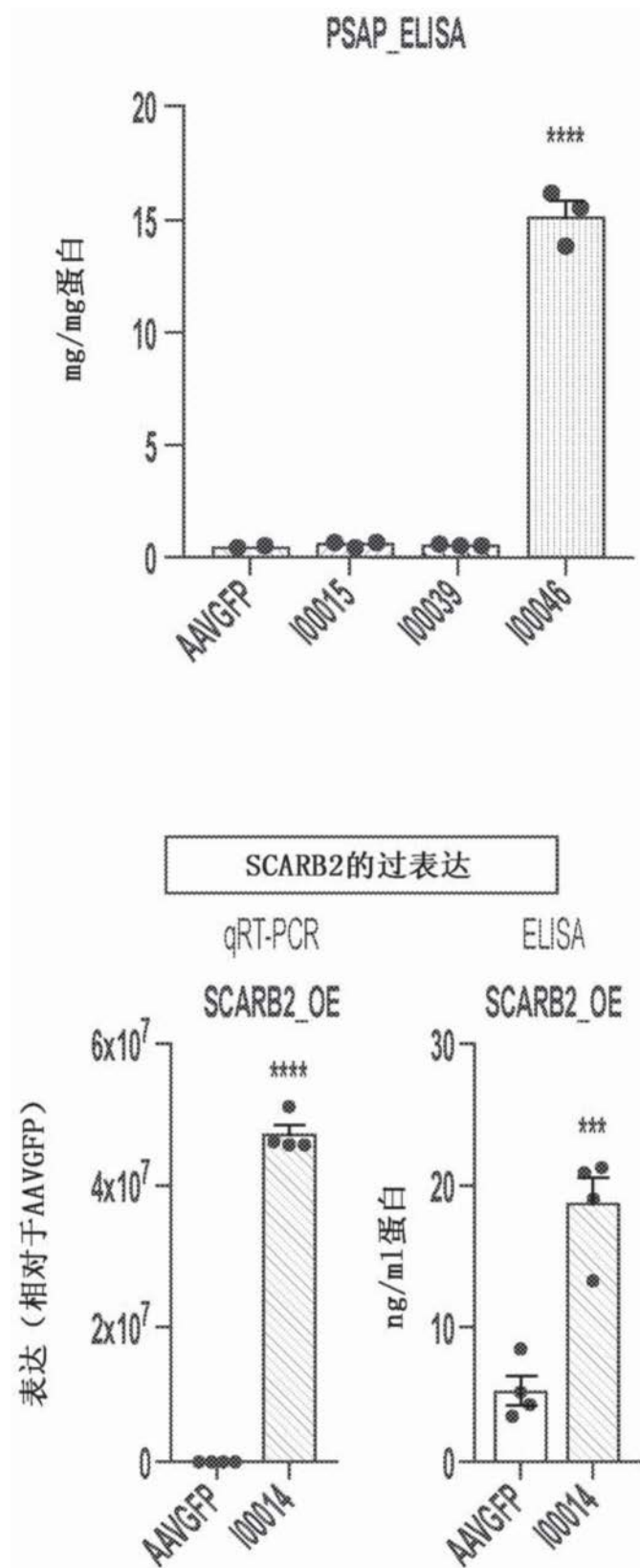


图19续

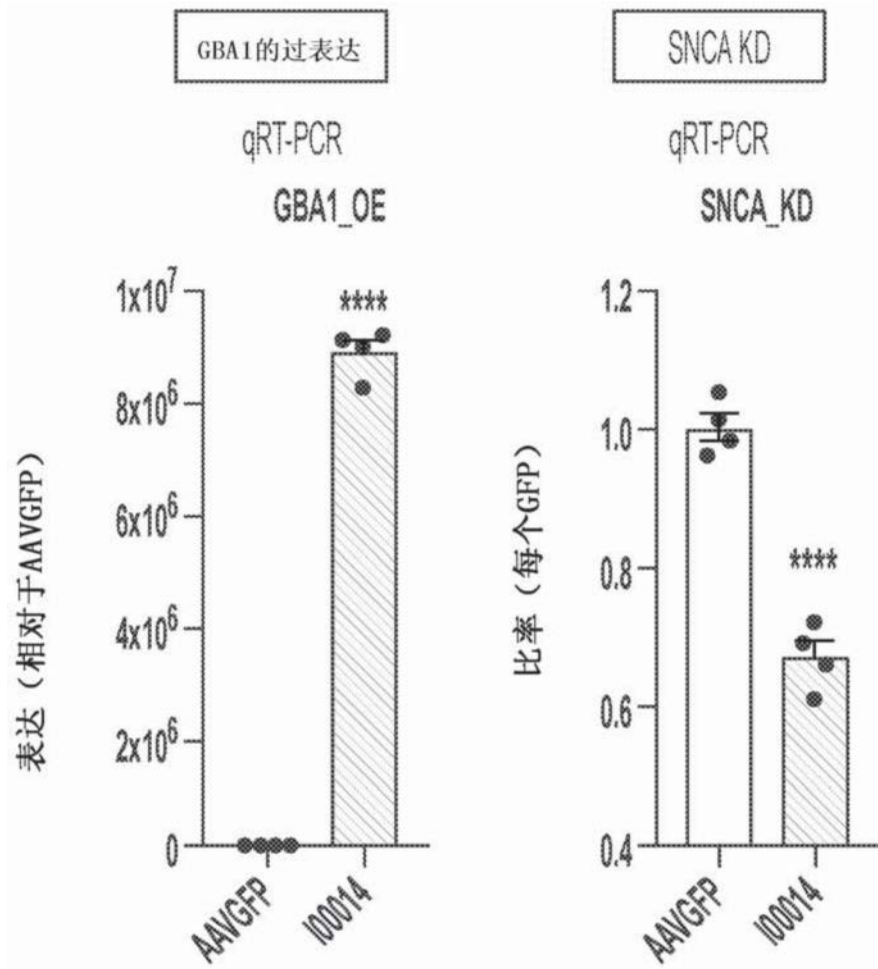


图19续

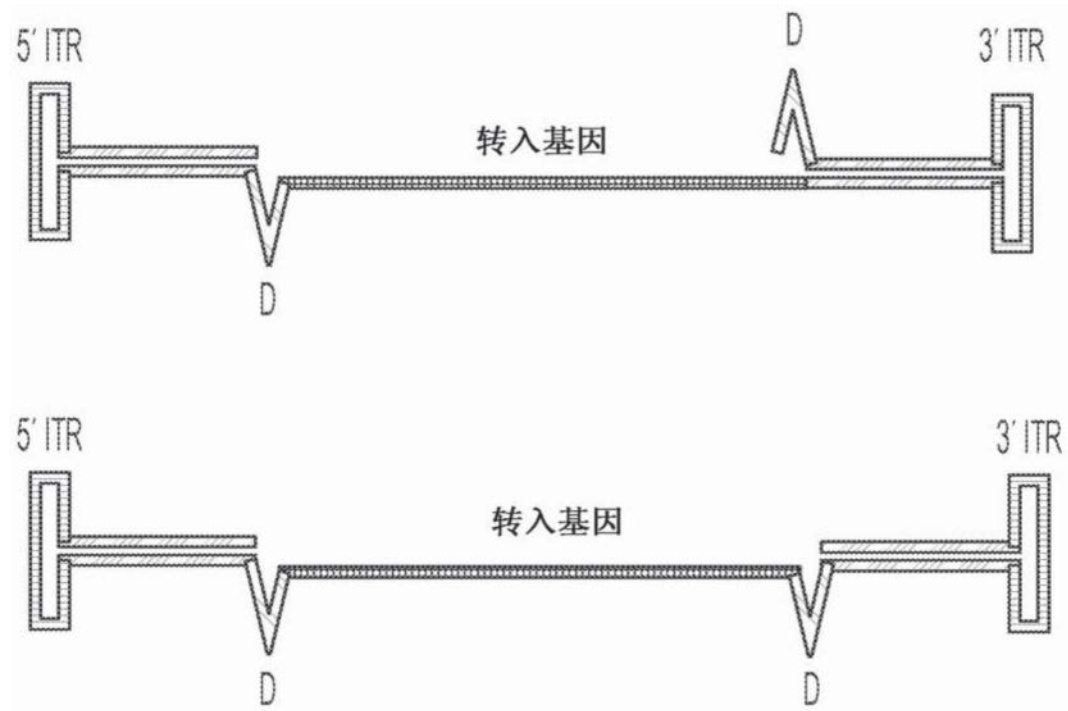


图20

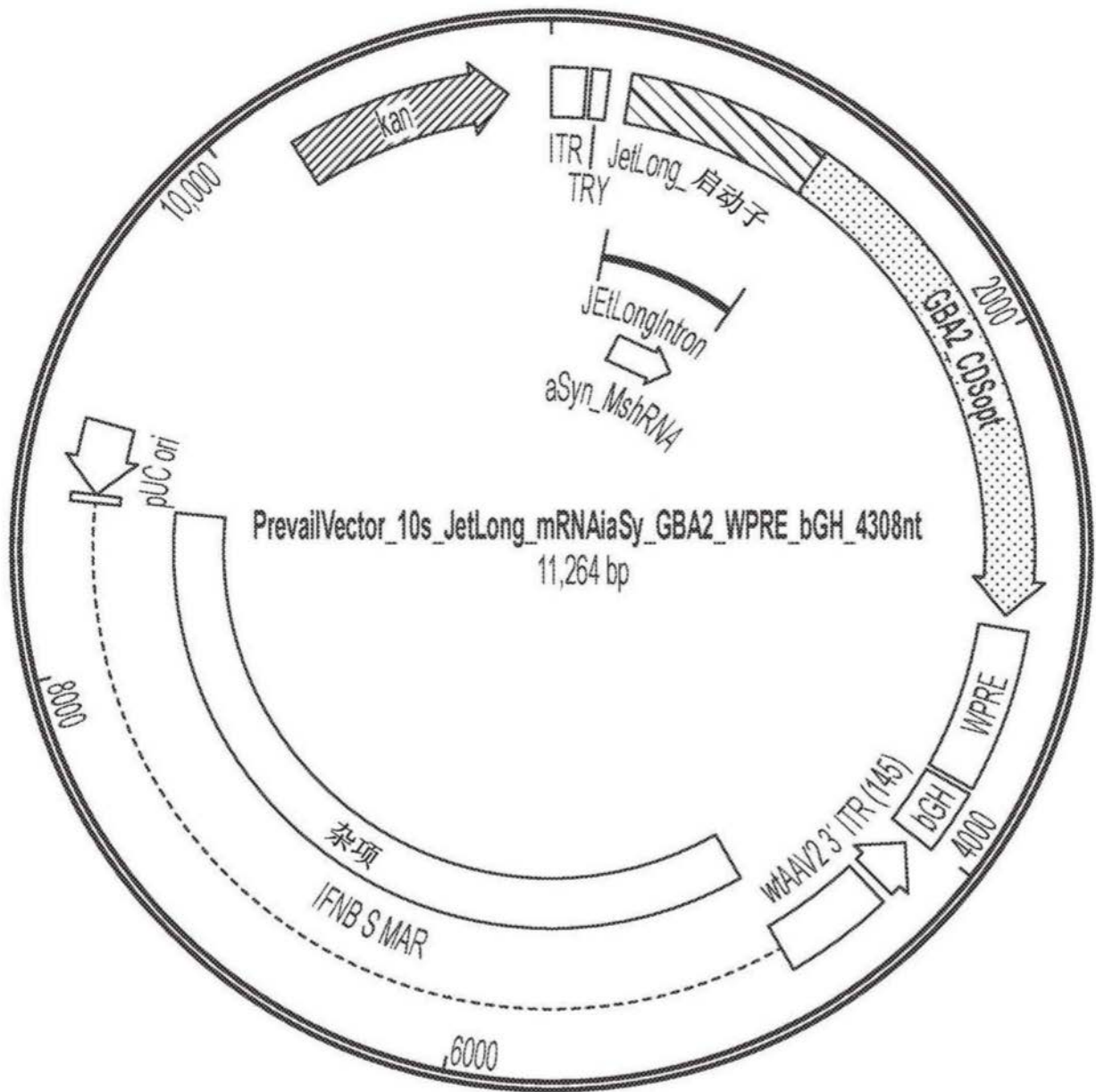


图21

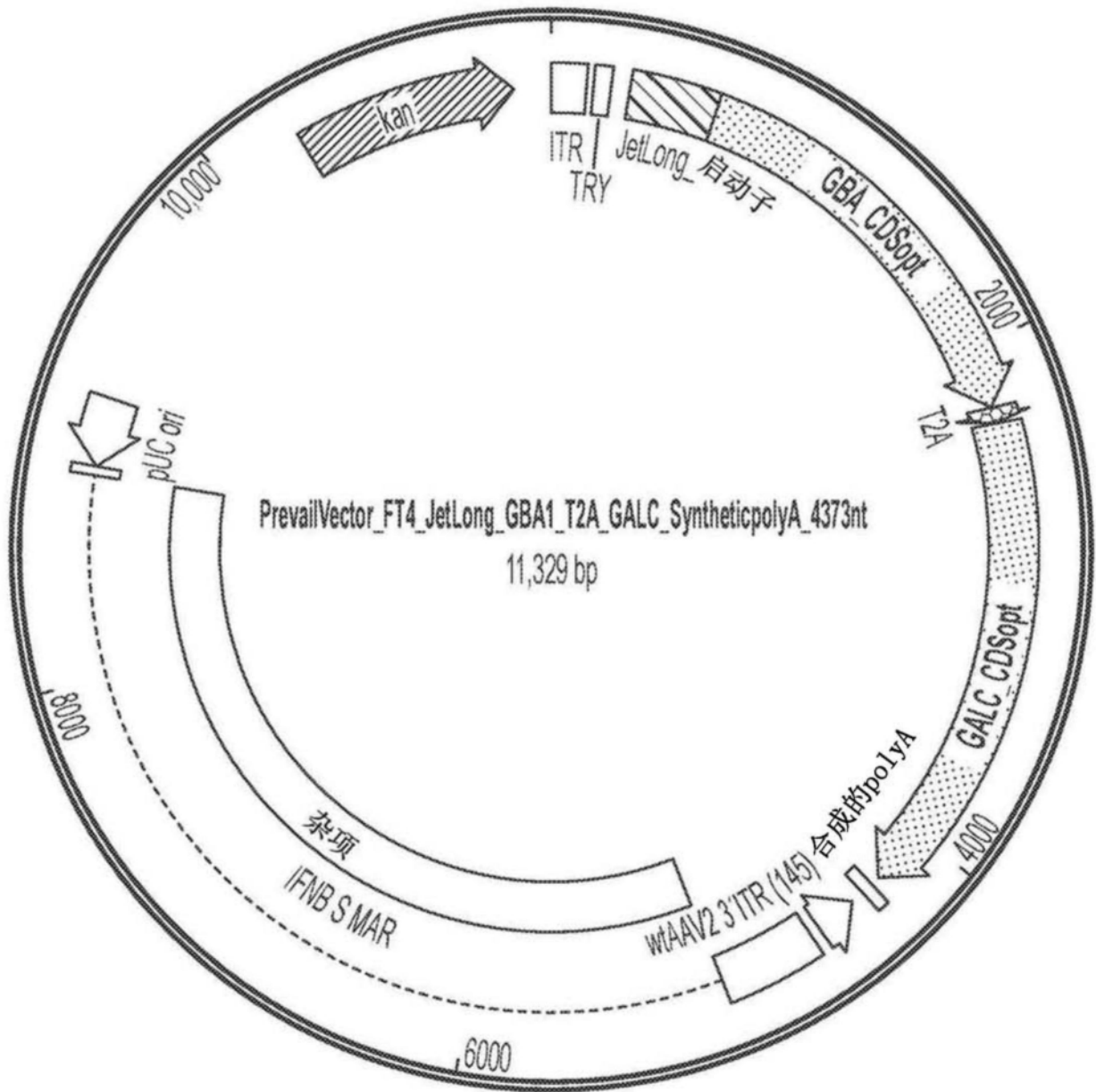


图22

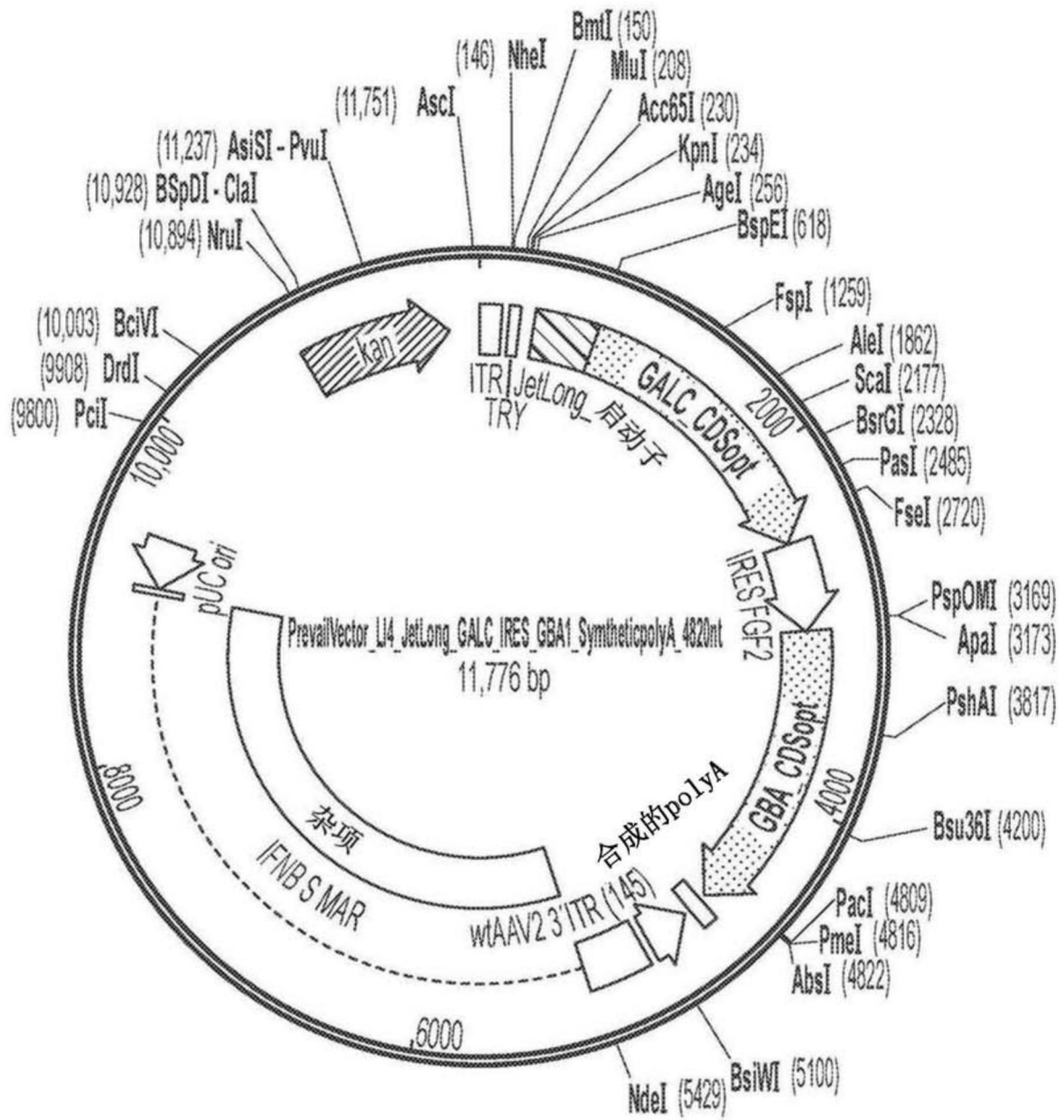


图23

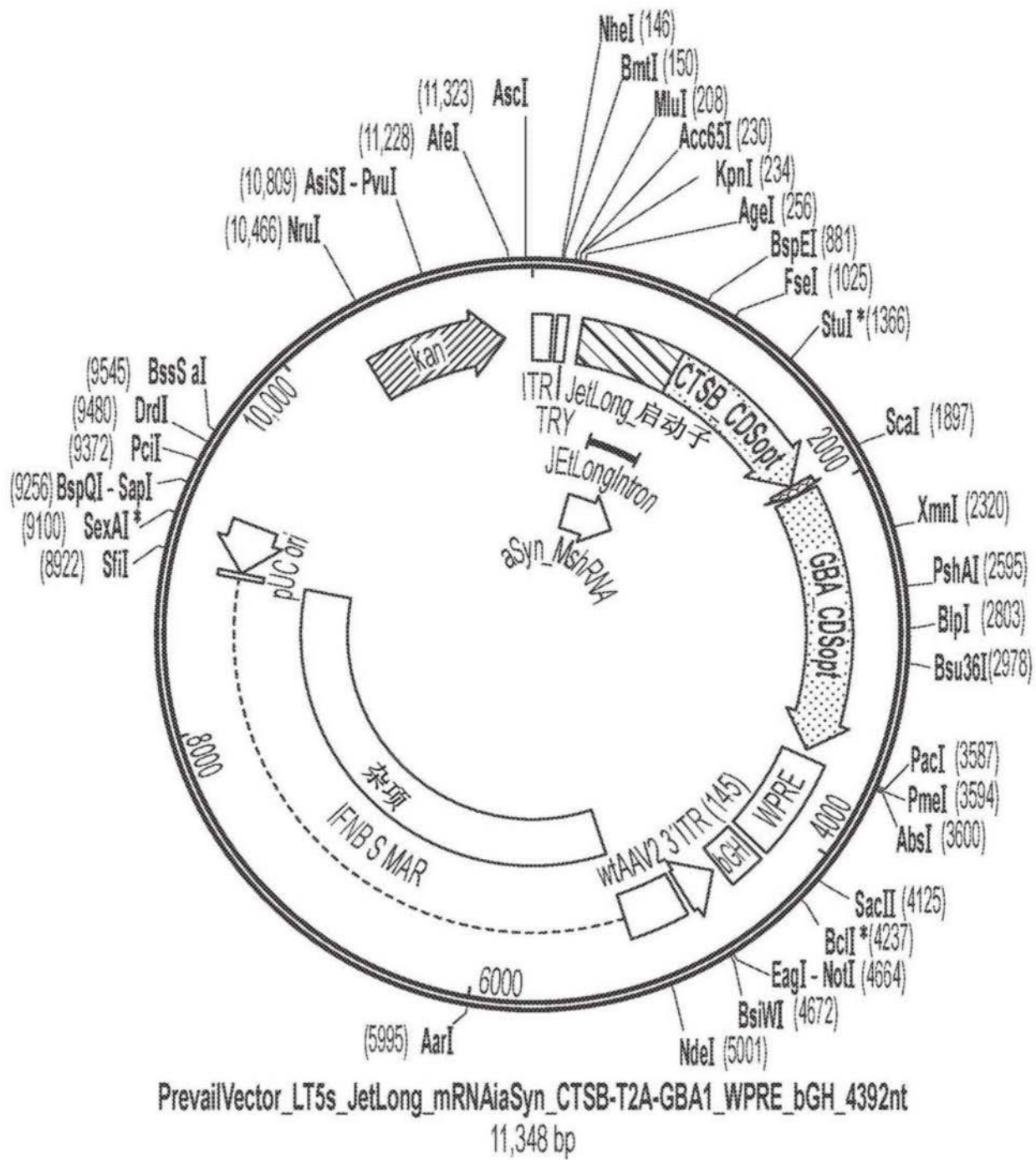


图24

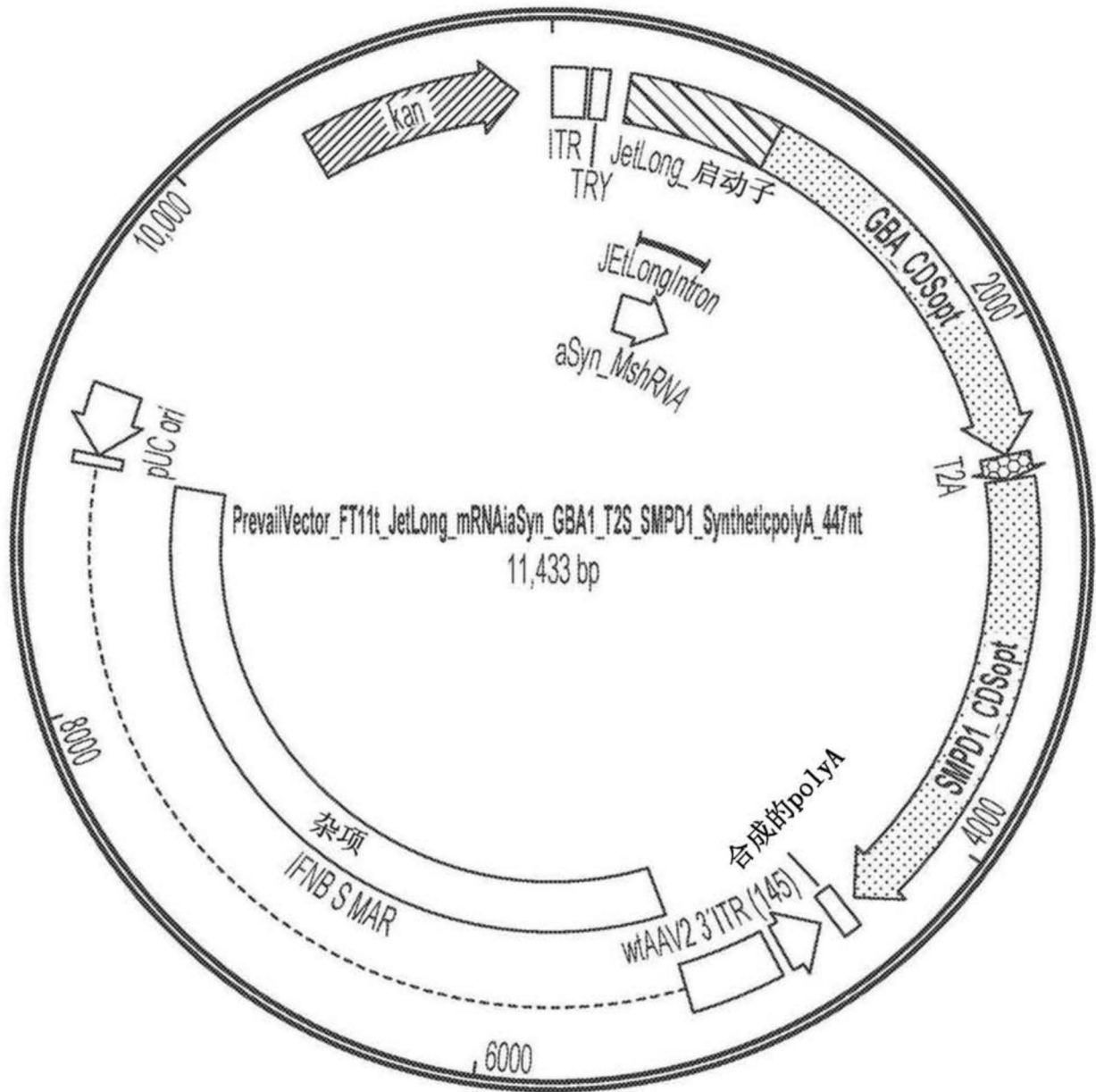


图25

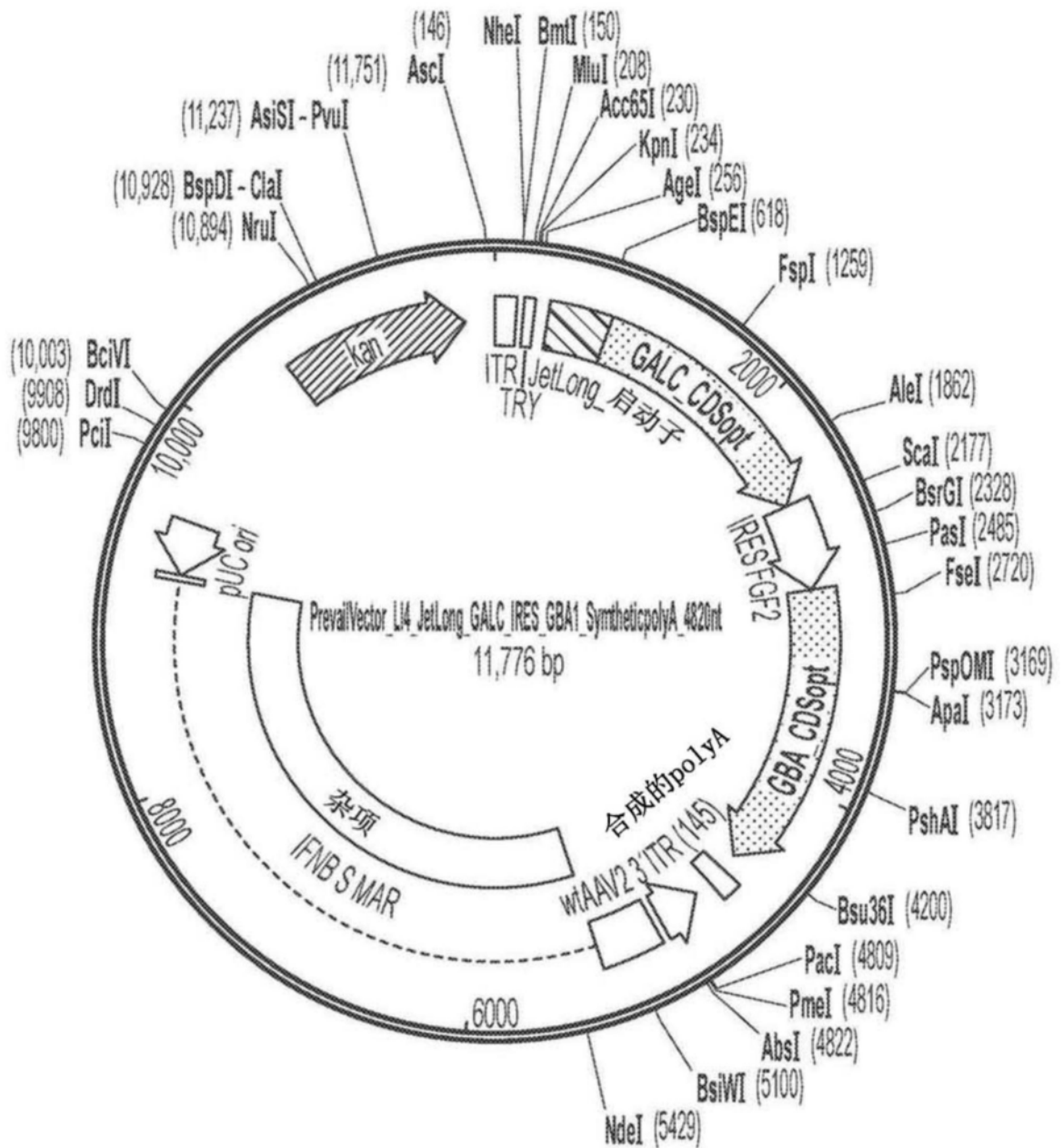


图26

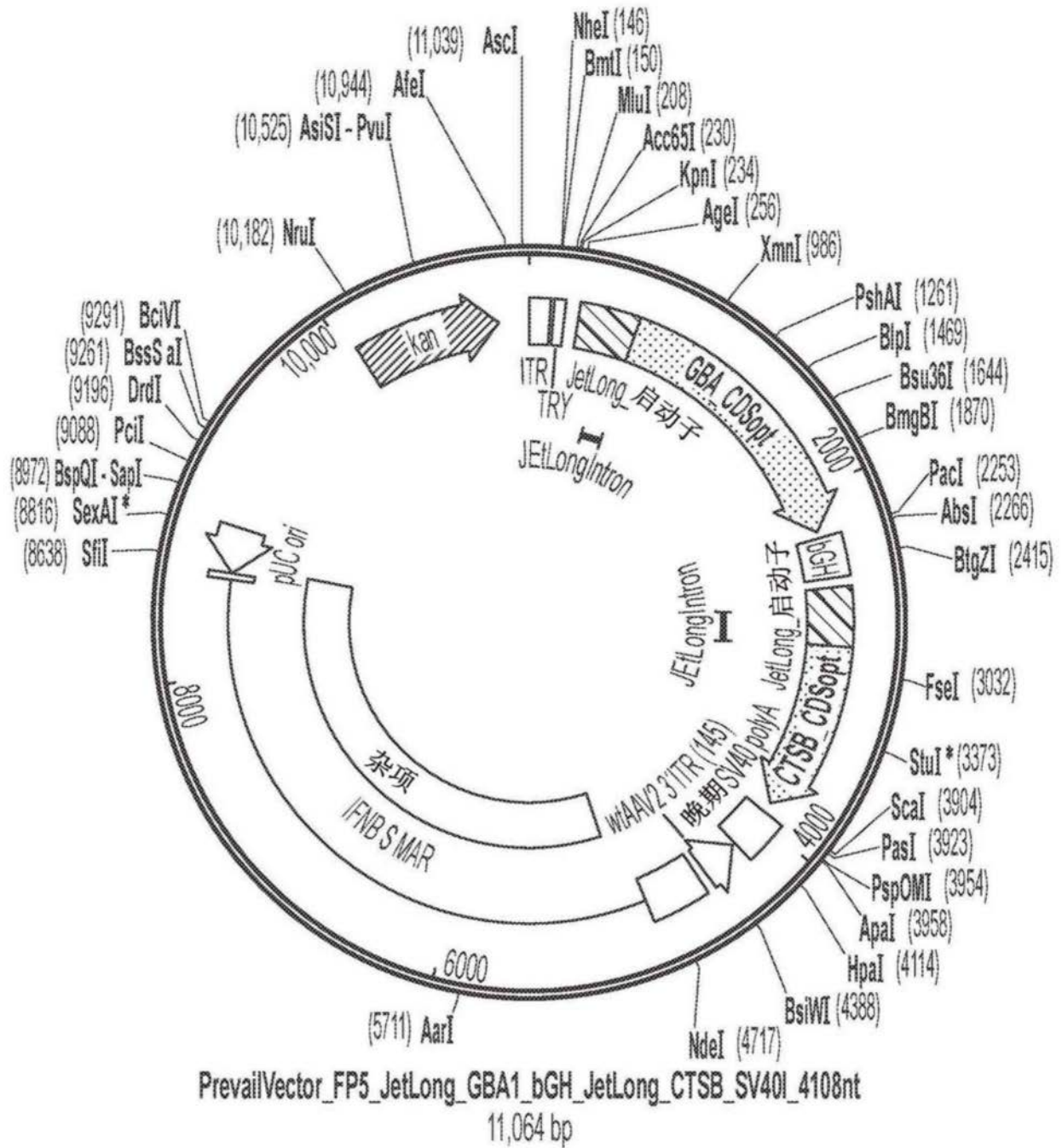


图27

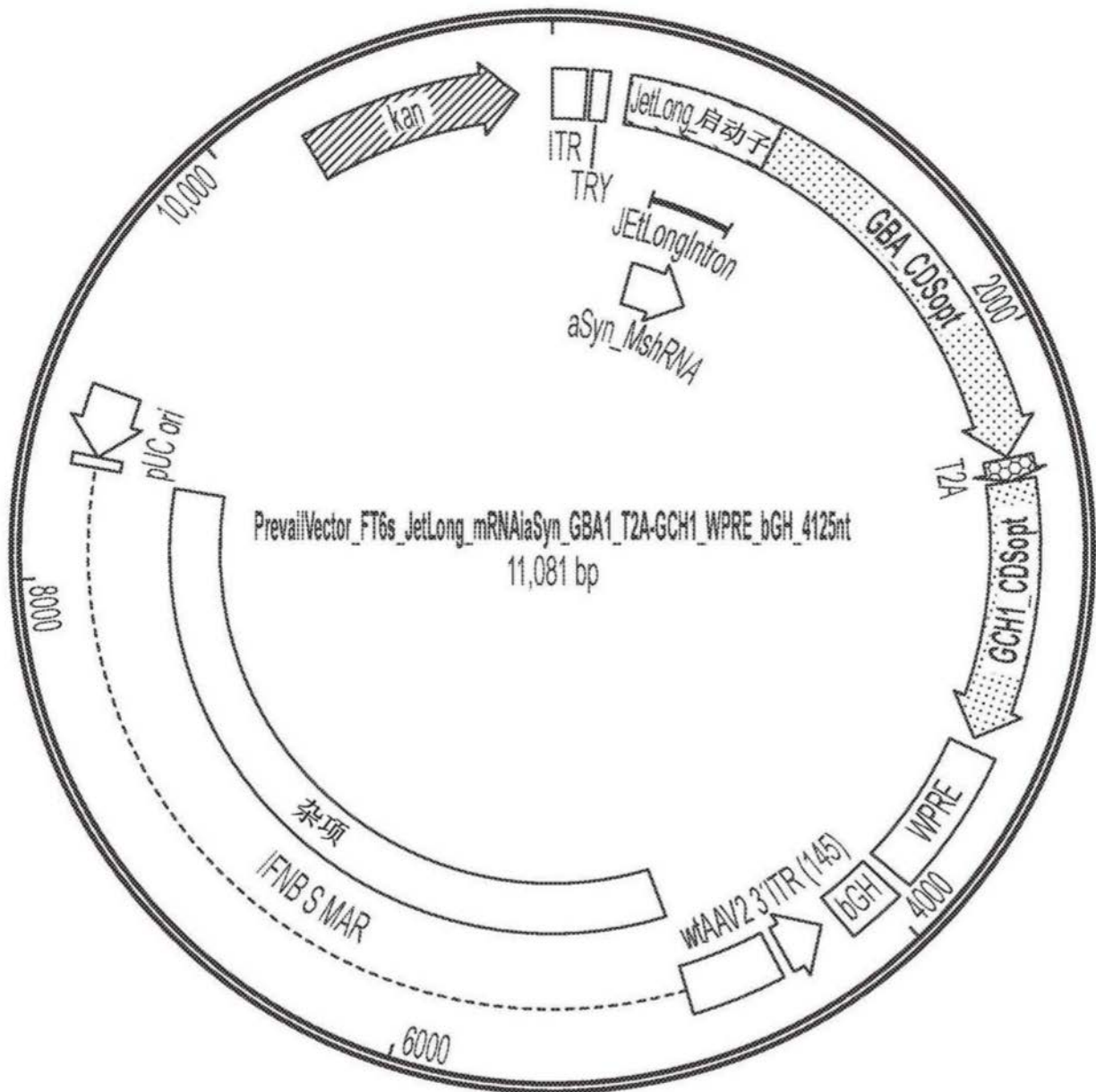


图28

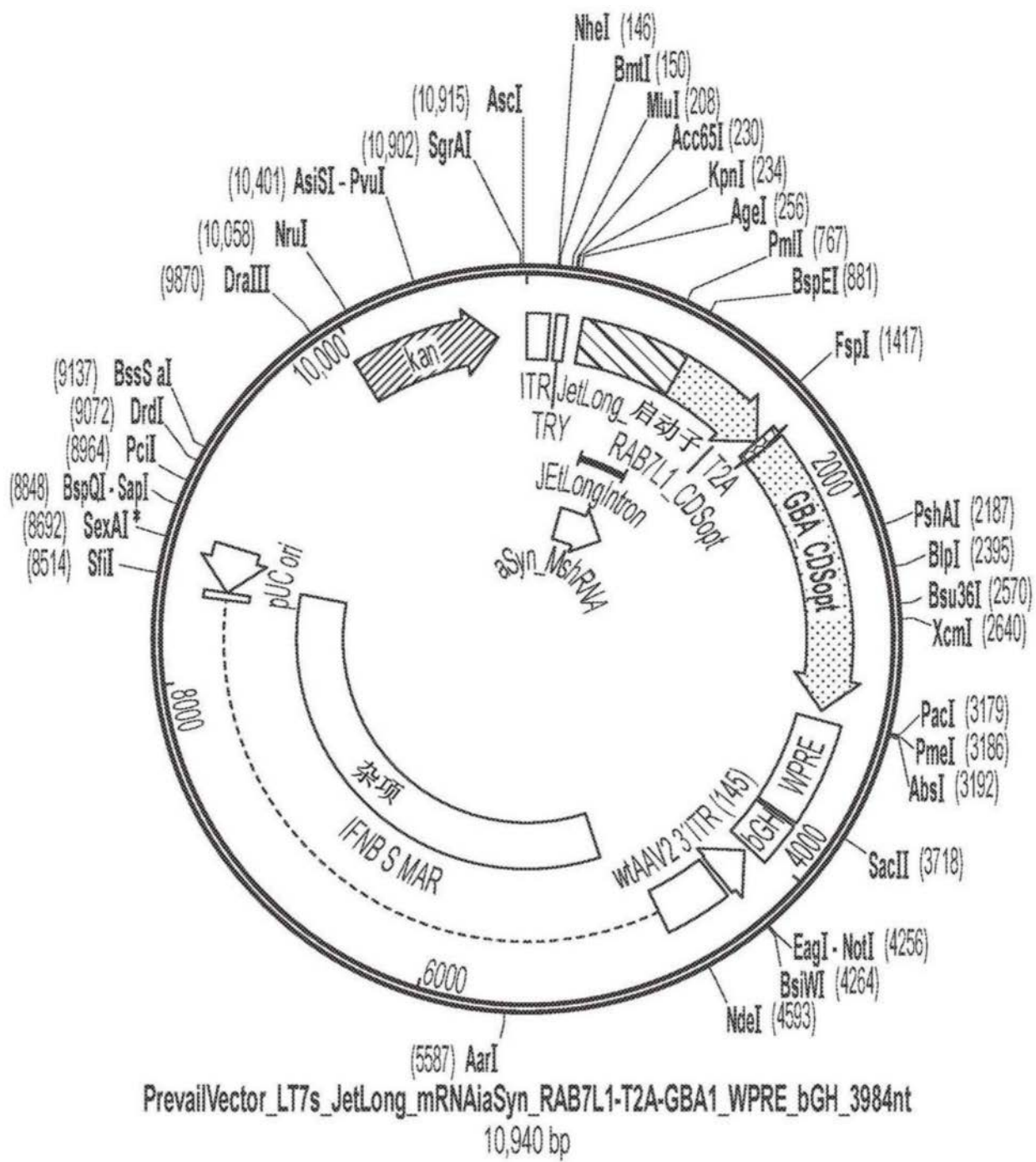


图29

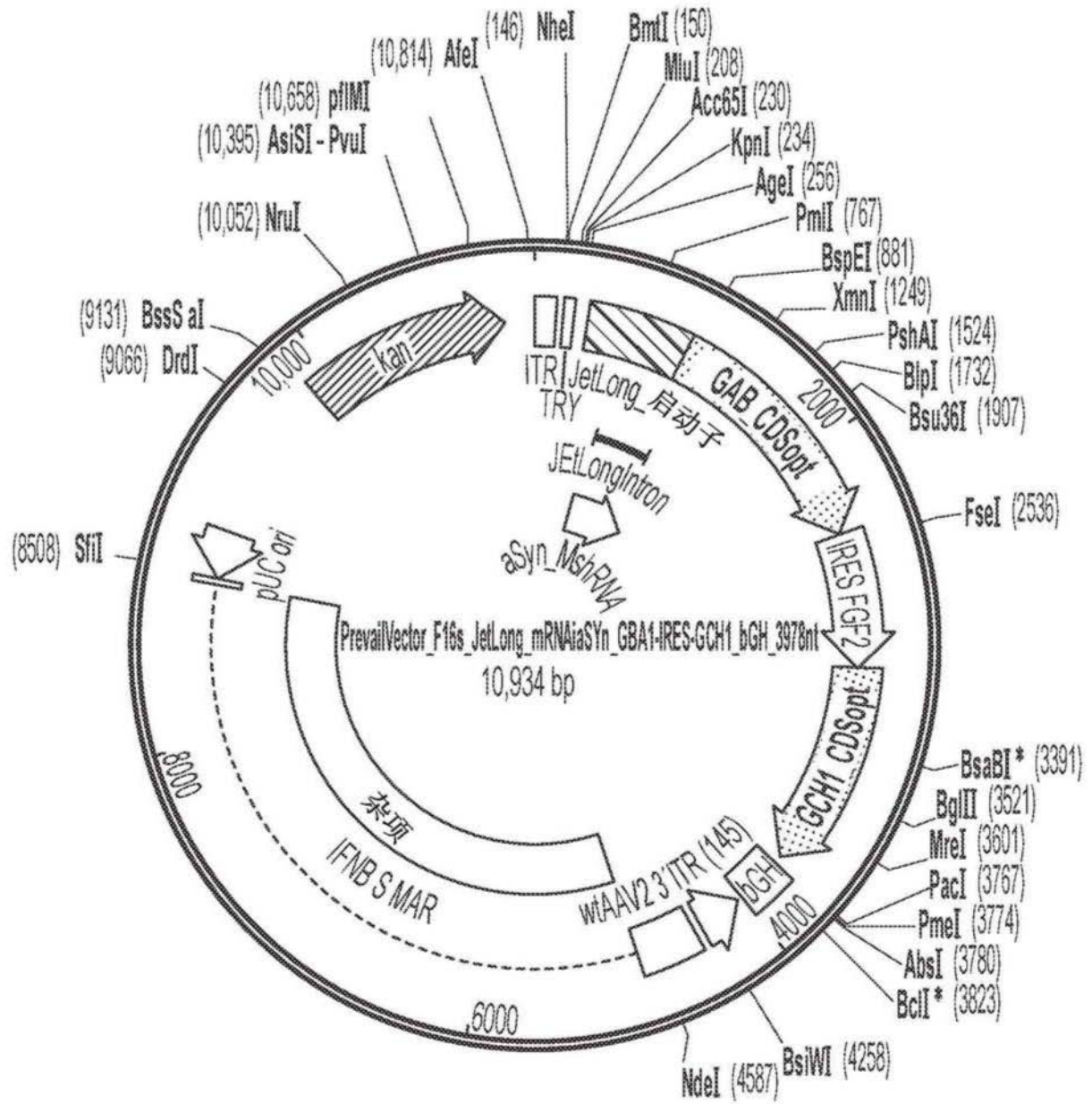


图30

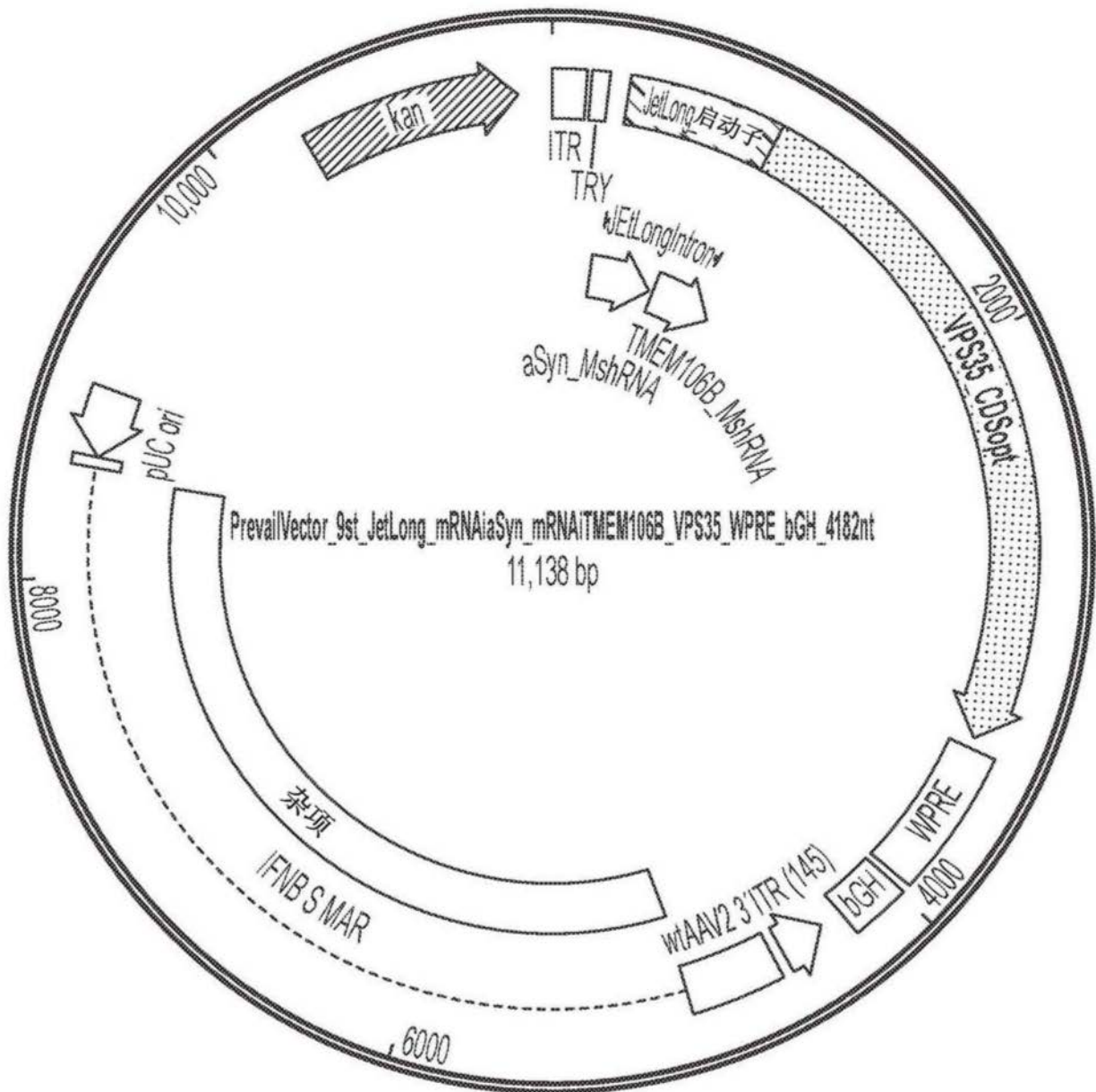


图31

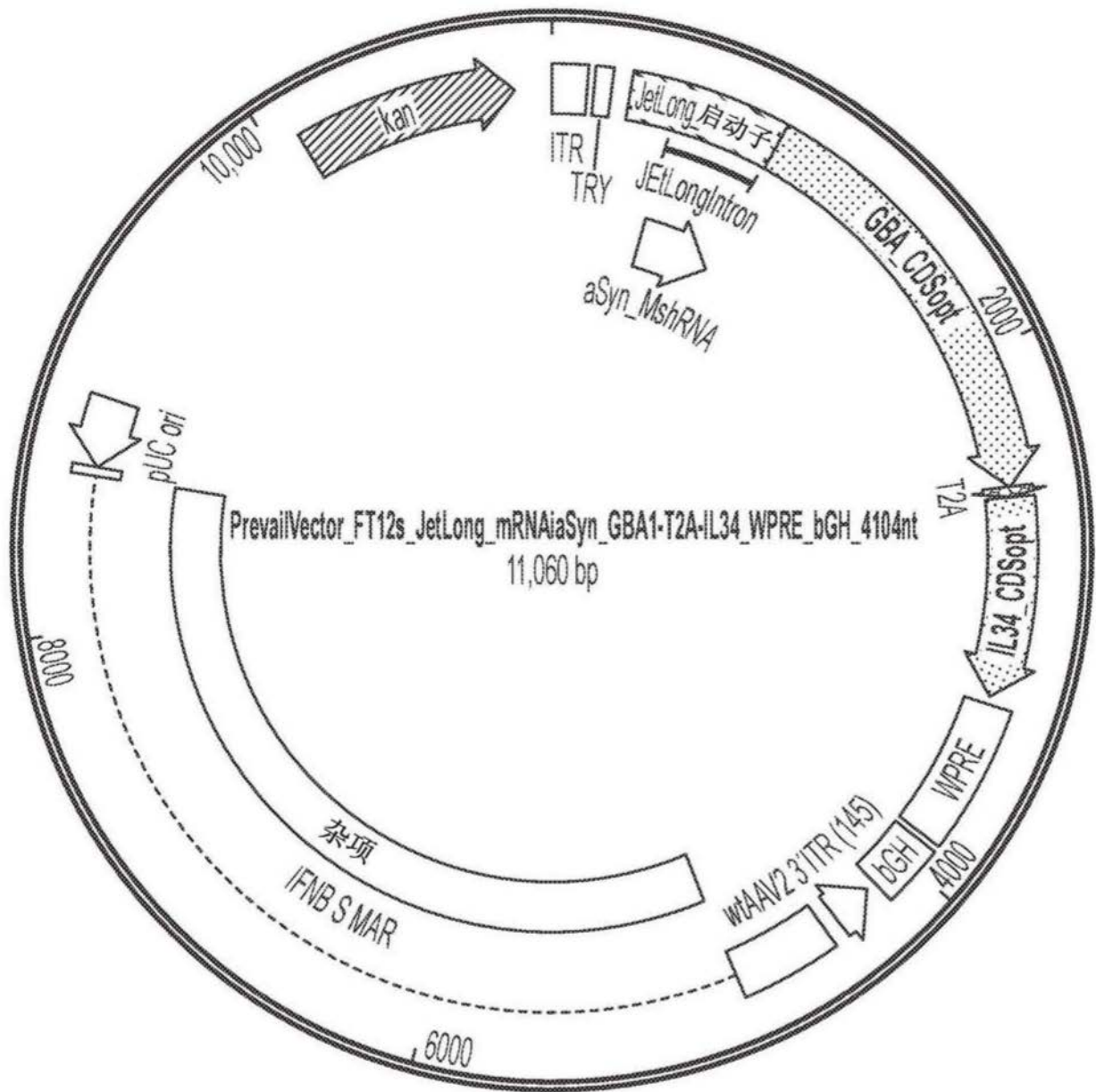


图32

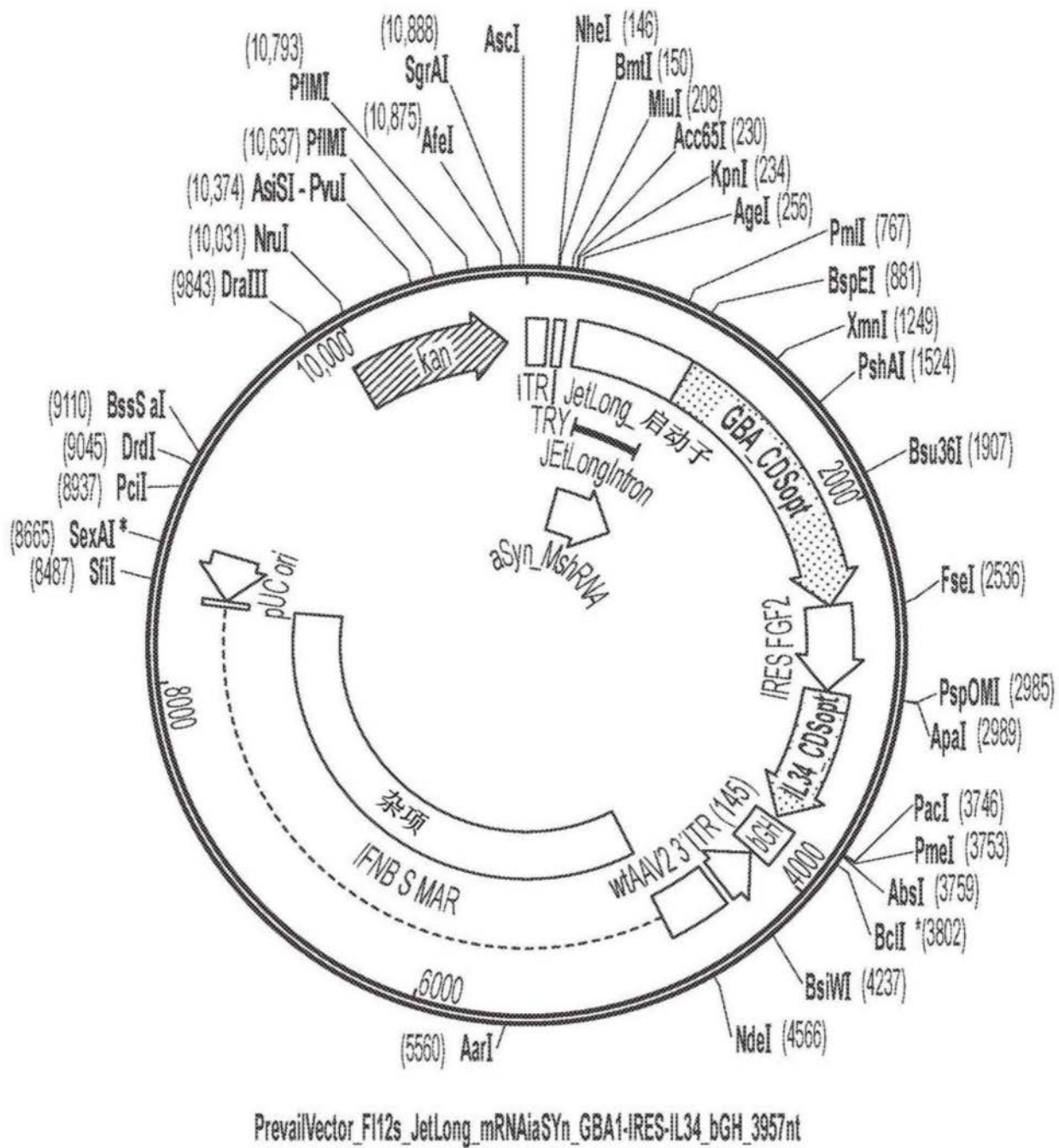


图33

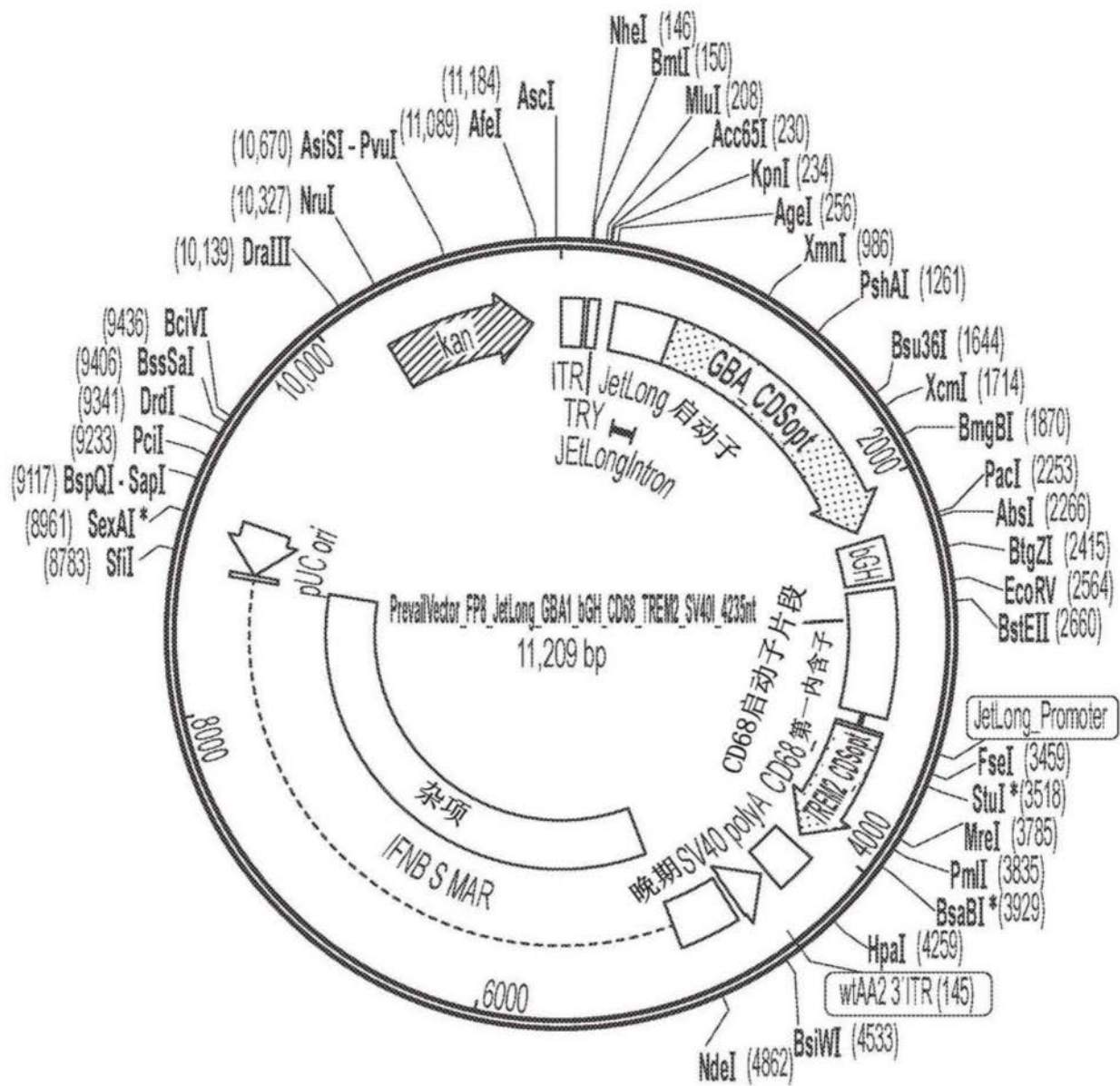


图34

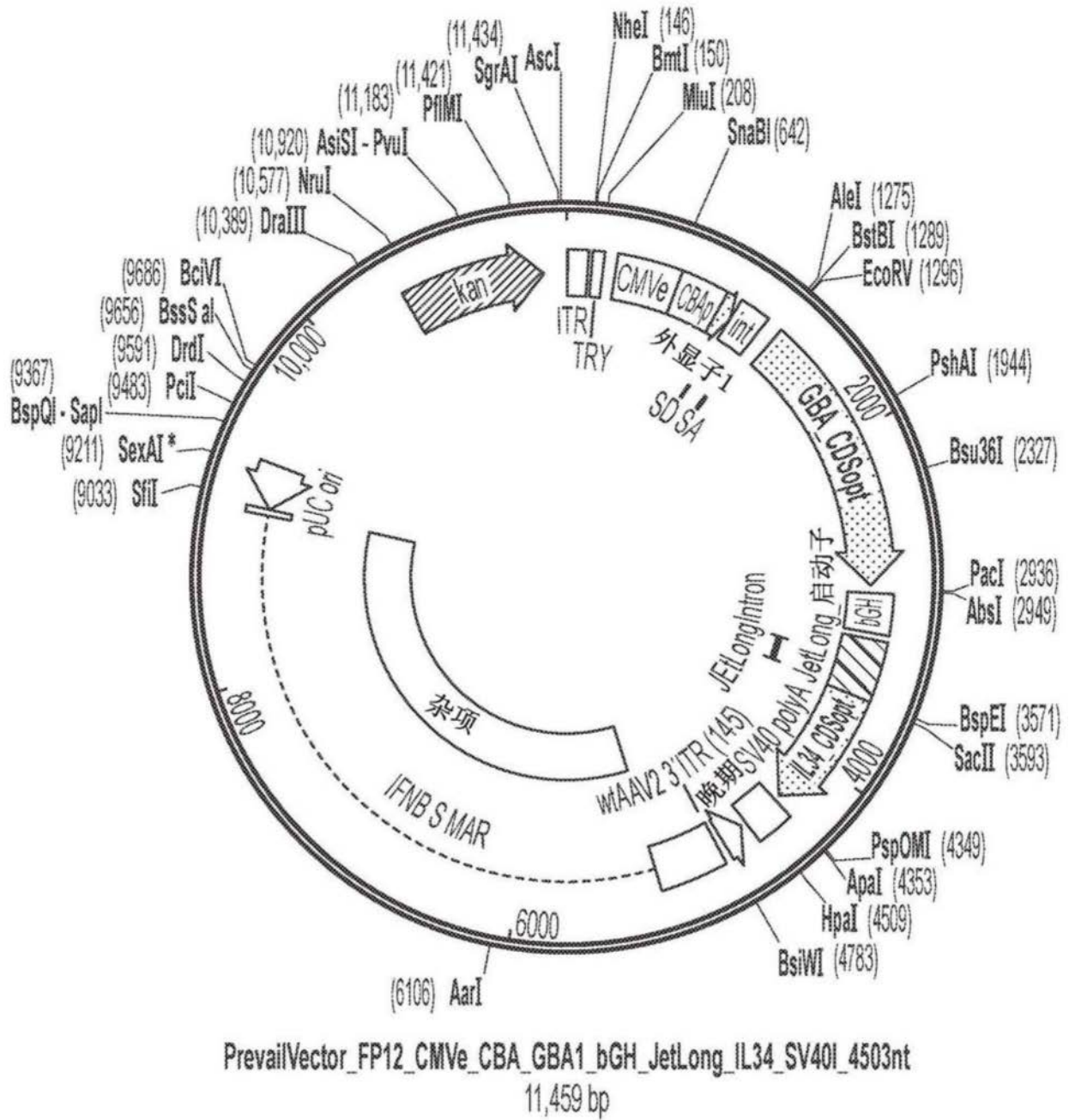


图35

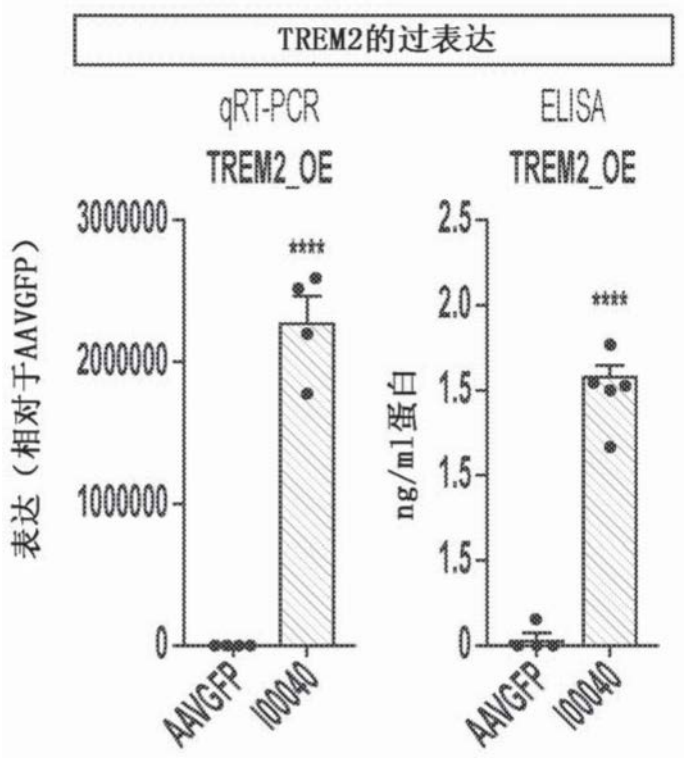


图36A

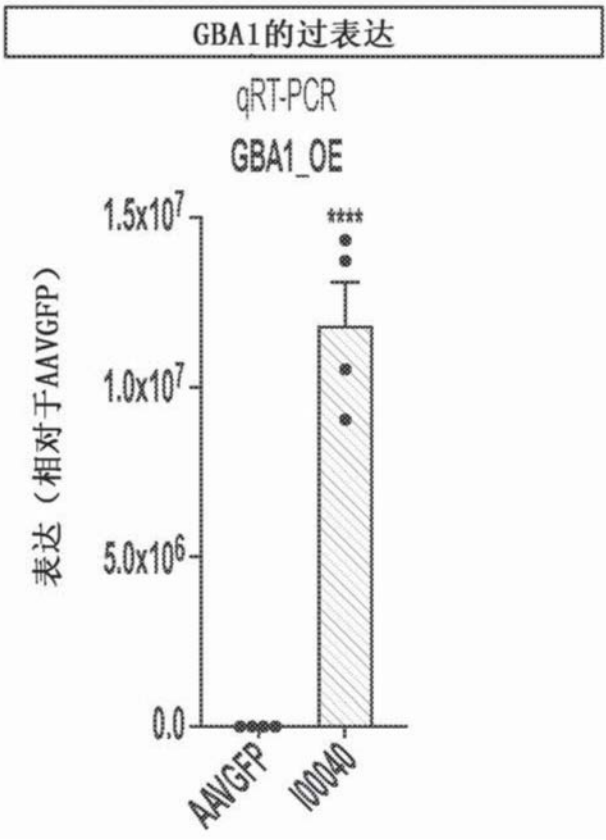


图36B

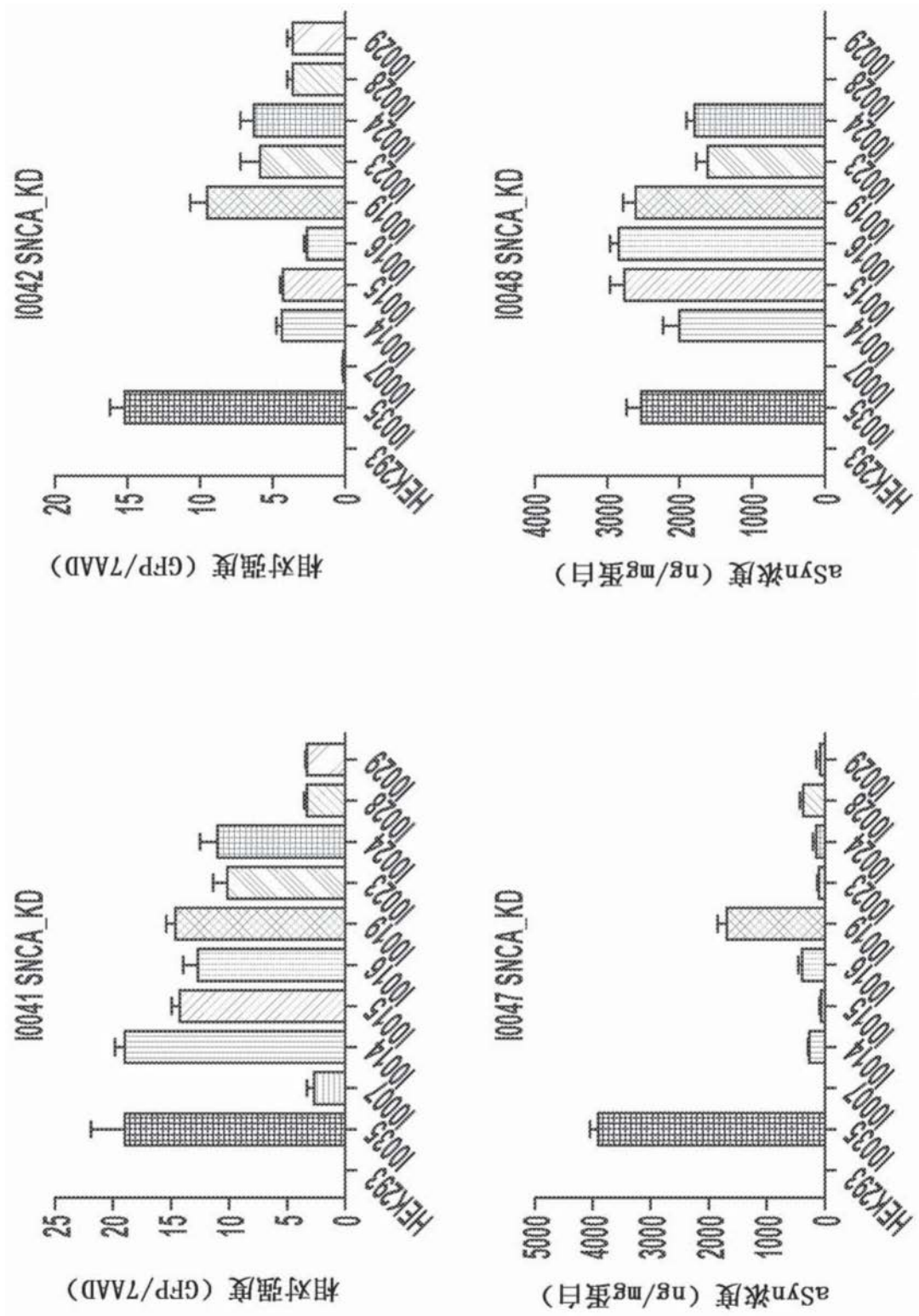


图37

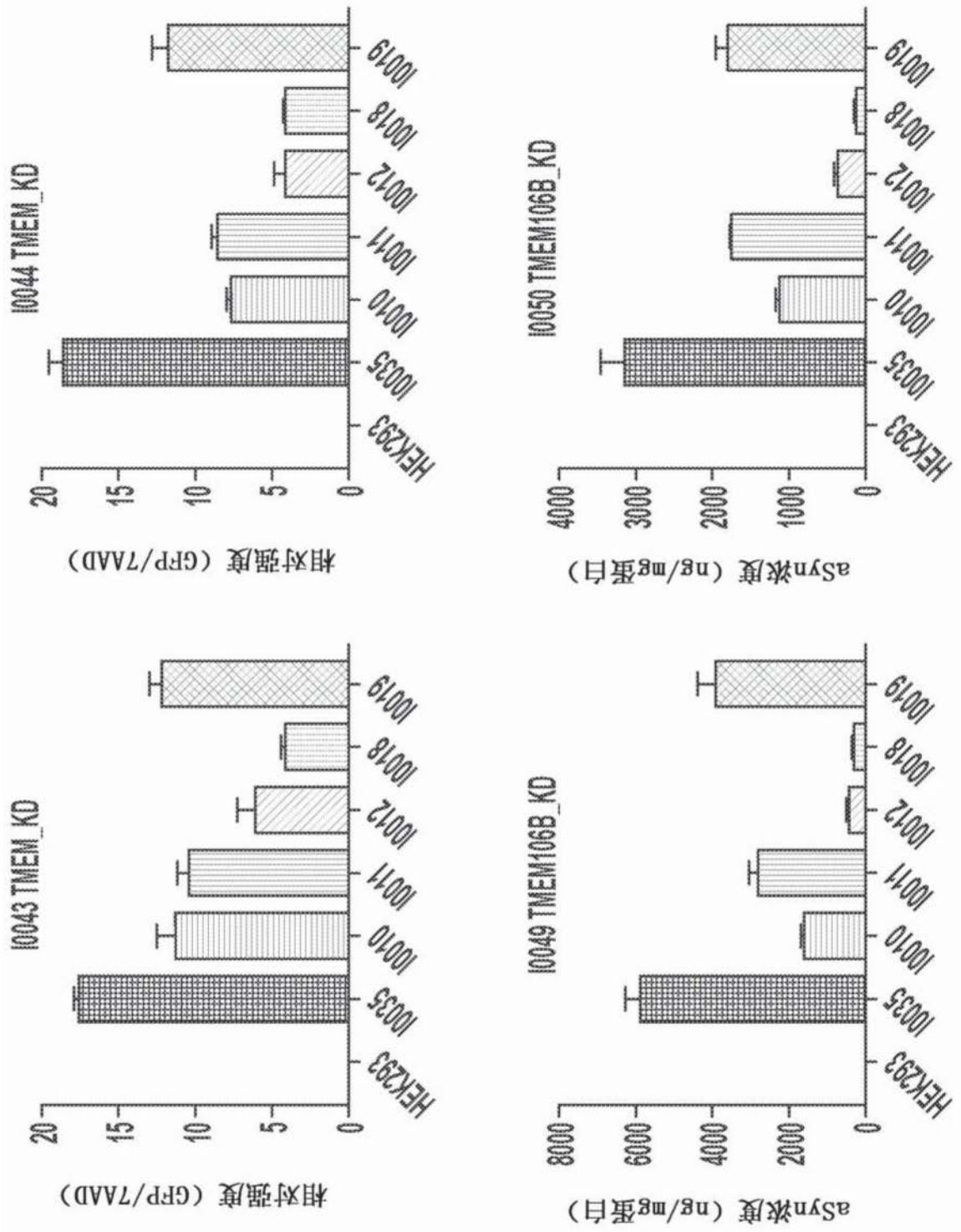


图38

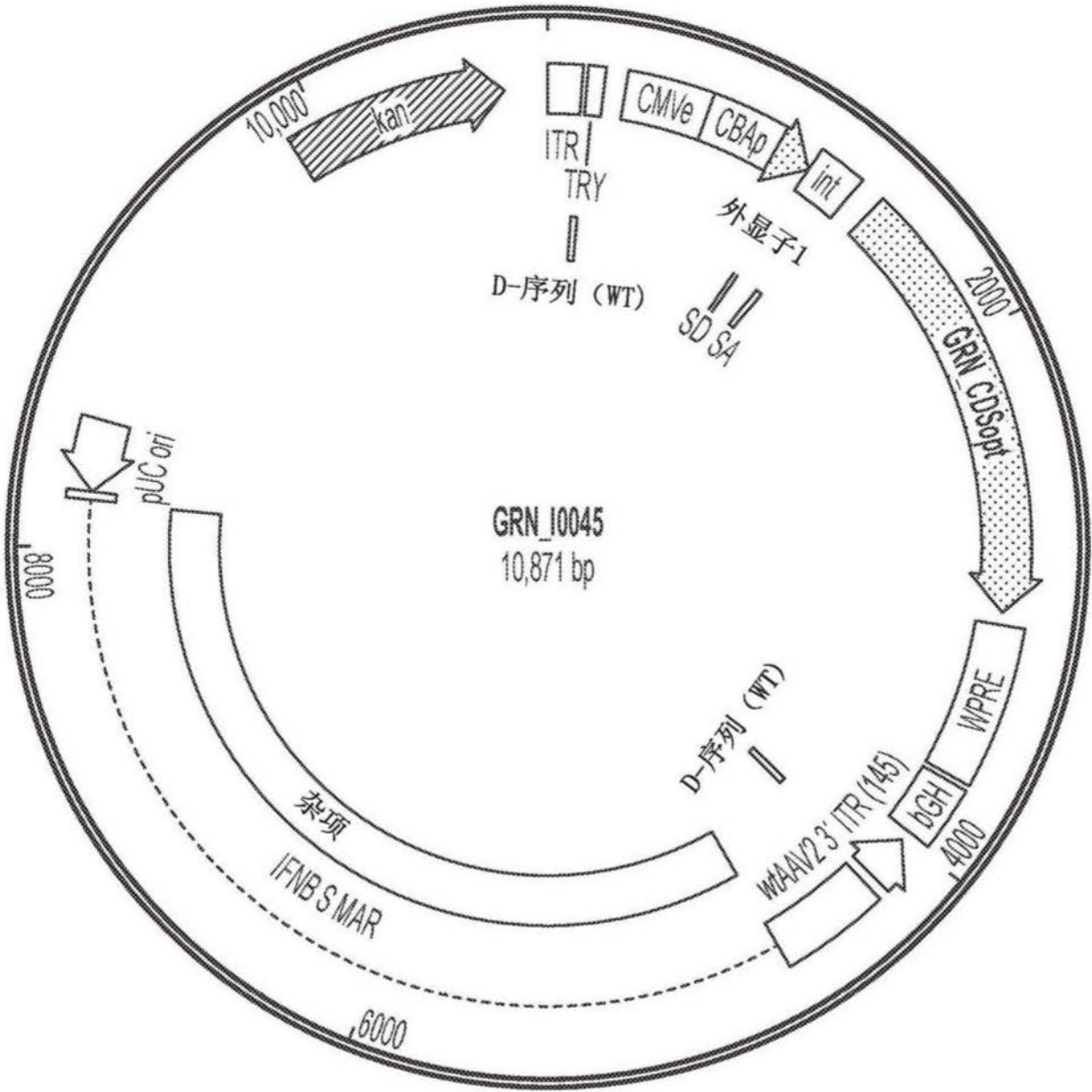


图39

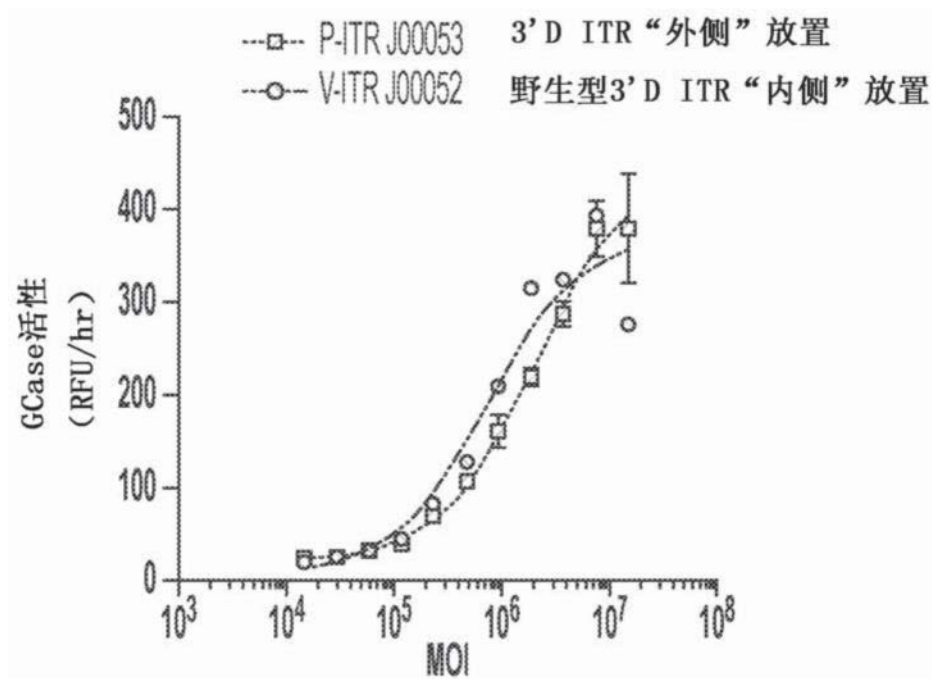


图40