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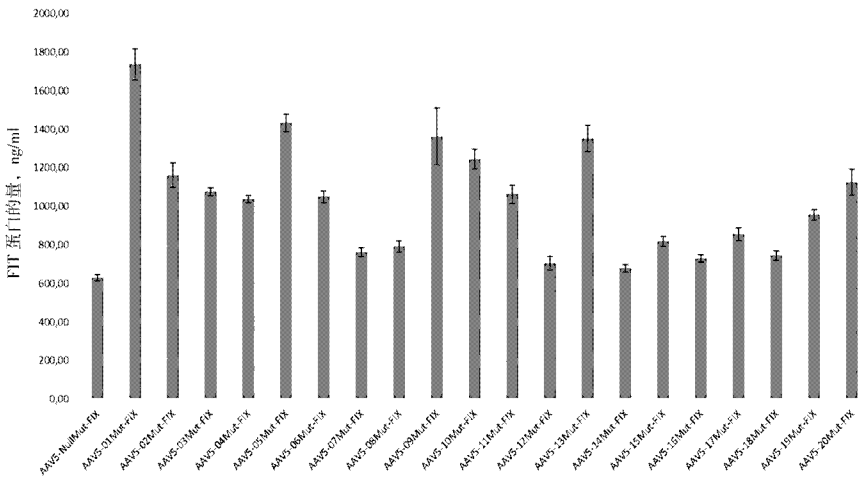
(54)名稱

獲得修飾的腺相關病毒衣殼的方法

(57)摘要

本申請涉及基因療法和分子生物學領域。更具體地，本發明涉及產生修飾的腺相關病毒(AAV)衣殼的方法和通過所述方法產生的修飾的 AAV 衣殼，以及編碼所述修飾的衣殼的分離的核酸和用於將異源核酸序列遞送至受試者的基於重組腺相關病毒的載體，所述載體包含所述修飾的衣殼。

指定代表圖：




【圖 12】

# 【發明摘要】

【中文發明名稱】

獲得修飾的腺相關病毒衣殼的方法

【英文發明名稱】

METHOD FOR OBTAINING A MODIFIED ADENO-ASSOCIATED VIRUS CAPSID

【中文】

本申請涉及基因療法和分子生物學領域。更具體地，本發明涉及產生修飾的腺相關病毒(AAV)衣殼的方法和通過所述方法產生的修飾的AAV衣殼，以及編碼所述修飾的衣殼的分離的核酸和用於將異源核酸序列遞送至受試者的基於重組腺相關病毒的載體，所述載體包含所述修飾的衣殼。

【指定代表圖】圖12

【代表圖之符號簡單說明】無

【特徵化學式】無

# 【發明說明書】

【中文發明名稱】

獲得修飾的腺相關病毒衣殼的方法

【英文發明名稱】

METHOD FOR OBTAINING A MODIFIED ADENO-ASSOCIATED VIRUS CAPSID

【技術領域】

本申請涉及基因療法和分子生物學領域。更具體地，本發明涉及產生修飾的腺相關病毒(AAV)衣殼的方法和通過所述方法產生的修飾的AAV衣殼，以及編碼所述修飾的衣殼的分離的核酸和用於將異源核酸序列遞送至受試者的基於重組腺相關病毒的載體，所述載體包含所述修飾的衣殼。

【先前技術】

腺相關病毒(AAV)是一種小型(25nm)、獨立的複製缺陷型無包膜病毒。已經在人和靈長類動物中描述了許多不同的AAV血清型。腺相關病毒基因組由(+或-)單鏈DNA(ssDNA)構成，長度約為4,700個核苷酸。在基因組DNA分子的末端，有末端反向重複序列(ITR)。基因組包含兩個開放閱讀框(ORF)：Rep和Cap，其含有編碼各種蛋白質產物的幾個可變閱讀框。rep產物對AAV複製至關重

要，而三種衣殼蛋白(VP1、VP2和VP3)以及其他可變產物由Cap基因編碼。VP1、VP2和VP3蛋白以1:1:10的比例存在以形成二十面體衣殼(Xie Q. et al. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. Proc Natl Acad Sci USA, 2002; 99:10405-10410)。在重組AAV(rAAV)載體產生過程中，將ITR兩側的表達盒包裝到AAV衣殼中。AAV複製所需的基因不包含在盒中。重組AAV被認為是用於體內基因轉移的最安全和最廣泛使用的病毒載體之一。載體可以感染多種組織類型的細胞，以提供強而持續的轉基因表達。它們也是非致病性的，並且具有低免疫原性特徵(High KA et al., "rAAV human trial experience" Methods Mol Biol. 2011; 807:429-57)。

在開發有效基因療法領域的研究的緊迫目的之一是優化載體以改善給定載體的某些特性。

各種AAV血清型的特徵在於對不同宿主細胞表面受體的親和力，它們對這些受體具有嗜性。因此，AAV2的主要已知受體是硫酸乙醯肝素蛋白聚糖，輔助受體是整聯蛋白異二聚體aVβ5、1型成纖維細胞生長因數受體和肝細胞生長因數受體c-Met。AAV12與硫酸乙醯肝素蛋白聚糖和唾液酸結合。AAV4和AAV5分別與N-和O-連接的唾液酸結合。AAV5啟動血小板衍生的生長因數受體。此外，已經在AAV衣殼蛋白的氨基酸序列與其組裝過程、基因組的包殼化、對宿主細胞表面上不同類型受體的親和力之間建立了關係(Govindasamy L. et. al. Structural insights into

adeno-associated virus serotype 5. J Virol. 2013 Oct;87 (20):11187-99)。

國際申請WO2012145601描述了具有變體衣殼蛋白的腺相關病毒(AAV)病毒體，其中與野生型AAV相比，當通過玻璃體內注射給藥時，AAV病毒體表現出對視網膜細胞更大的感染性。

國際申請WO2013158879描述了一種腺相關病毒(AAV)載體，用於向受試者遞送包含衣殼蛋白VP1的異源核酸序列，該衣殼蛋白VP1包含一個或多個賴氨酸置換，其中一個賴氨酸置換是K137R，其中所述賴氨酸置換有效抑制所述衣殼蛋白的泛素化，從而提高所述AVV載體在靶細胞中的轉導效率。

迄今為止，仍需要與野生型AAV相比具有改進性質的AAV，例如，其具有增強的轉導能力、更大的特異性轉導靶器官和組織細胞的能力、增加的衣殼容量、增加的AAV病毒基因組包裝效率，以及由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高的生成效率。

【發明內容】

本發明的作者已經發現，根據本發明的用於產生修飾的腺相關病毒(AAV)衣殼的方法意想不到地使得可以產生與不含這些修飾的AAV衣殼(野生型AAV衣殼)相比具有一種或多種改進性質的修飾的AAV衣殼，所述一種或多種改

進性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在一個方面，本發明涉及一種產生修飾的AAV衣殼的方法，包括：

a)確定修飾的AAV的殼粒(capsomere)蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的修飾的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在

修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換。

在本發明的一些實施方案中，產生修飾的AAV衣殼的方法進一步包括檢查步驟f)中產生的具有一個或多個氨基酸置換的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進性質，其中一種或多種改進性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在本發明的一些實施方案中，產生修飾的AAV衣殼的方法進一步包括檢查步驟f)中產生的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進性質，並選擇與不含這些修飾的AAV衣殼相比具有一種或多

種改進性質的修飾的AAV衣殼，其中所述一種或多種改進性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼選自以下AAV血清型：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15或AAV16。

在用於產生AAV衣殼的方法的一些實施方案中，所述結構相似的範本衣殼選自：細小病毒B19、人博卡病毒1(HBoV1)、牛細小病毒(BVP)。

在一個方面，本發明涉及一種用於產生基於重組腺相關病毒的病毒載體的修飾的AAV衣殼，所述修飾的AAV衣殼通過包括以下的方法產生：

a)確定修飾的AAV的殼粒蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間

的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換；

並且，如有必要，還包括

g)檢查步驟f)中產生的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進性質，並選擇

與不含這些修飾的AAV衣殼相比具有一種或多種改進性質的修飾的AAV衣殼，其中所述一種或多種改進性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼選自以下AAV血清型：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15或AAV16。

一方面，本發明涉及一種用於產生基於重組腺相關病毒的病毒載體的修飾的AAV衣殼，其包含具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368。

在本發明的一些實施方案中，修飾的AAV衣殼包含：

a)具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、

62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368；

b)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP2，所述修飾的AAV衣殼蛋白VP2具有選自下組的氨基酸序列：SEQ ID No: 16、22、28、34、40、46、52，58、64、70、76、82、88、94、100、106、112、118、124、130、136、142、148、154、160、166、172、178、184、190、196、202、208、214、220、226、232、238、244、250、256、262、268、274、280、286、292、298、304、310、316、322、328、334、340、346、352、358、364或370；

c)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP3，所述修飾的AAV衣殼蛋白VP3具有選自下組的氨基酸序列：SEQ ID No: 18、24、30、36、42、48、54，60、66、72、78、84、90、96、102、108、114、120、126、132、138、144、150、156、162、168、174、180、186、192、198、204、210、216、222、228、234、240、246、252、258、264、270、276、282、288、294、300、306、312、318、324、330、336、342、348、354、360、366或372。

一方面，本發明涉及編碼任何上述修飾衣殼的分離的核酸。

一方面，本發明涉及一種用於向受試者遞送異源核酸序列的基於重組腺相關病毒的載體，所述載體包含：

1)任何上述修飾的衣殼，和

2)異源核酸序列，其包含促進由異源核酸序列編碼的產物在靶細胞中表達的調節序列。

在一些實施方案中，基於重組腺相關病毒的載體具有異源核酸序列的表達產物，其為治療性多肽或報告多肽。

【圖式簡單說明】

[圖1]是旨在用於克隆AAV衣殼基因隨機變體文庫的質粒pAAV接頭的環狀示意圖。

GFP是編碼綠色螢光蛋白的序列，

PolyA是多聚腺苷酸化信號，

ITR是腺相關病毒的反向末端重複序列，

T2A是編碼來自病毒Thosea asigna的多肽鏈的自切割肽的序列，

HBG內含子是人β-珠蛋白內含子，

CMV啟動子是人巨細胞病毒啟動子，

AmpR是β-內醯胺酶基因的序列，提供大腸桿菌對氨苄青黴素的抗性，

pUC起點是高拷貝細菌複製起點，

EcoRI是EcoRI限制性內切酶識別位點，

AscI是AscI限制性內切酶識別位點。

[圖2]是旨在用於從隨機變體文庫中產生野生型AAV

的重組病毒產物的質粒pAAV-Rep的環狀示意圖。

AmpR是β-內醯胺酶基因的序列，提供大腸桿菌對氨苄青黴素的抗性，

pUC起點是高拷貝細菌複製起點，

AAV Rep基因是編碼病毒生命週期所需的Rep蛋白的序列，

SwaI是SwaI限制性內切酶識別位點，

NotI是NotI限制性內切酶識別位點。

[圖3]是旨在用於從隨機變體文庫中產生野生型AAV的重組病毒產物的質粒pHelper的環狀示意圖。

AmpR是β-內醯胺酶基因，提供對氨苄青黴素的抗性，

Ori是細菌的複製起點，

Adeno E2A是參與病毒DNA複製的輔助腺病毒基因序列，

Adeno E4是參與病毒DNA複製的輔助腺病毒基因序列，

Adeno VARNA是輔助腺病毒基因序列，負責翻譯早期和晚期病毒基因。

[圖4]是旨在用於產生重組AAV血清型5病毒產物的質粒pAAV-RC5的環狀圖。

AmpR是β-內醯胺酶基因的序列，提供大腸桿菌對氨苄青黴素的抗性，

pUC起點是高拷貝原核覆制起點。

AAV Rep基因是編碼腺相關病毒生命週期所需的Rep蛋白的序列，

AAV5 Cap基因是編碼腺相關病毒血清型5衣殼蛋白：VP1、VP2和VP3的重疊核苷酸序列的序列。

[圖5]是旨在用於產生重組AAV血清型9病毒產物的質粒pAAV-RC9的環狀圖。

AmpR是β-內醯胺酶基因的序列，提供大腸桿菌對氨苄青黴素的抗性，

pUC起點是高拷貝原核覆制起點。

AAV Rep基因是編碼腺相關病毒生命週期所需的Rep蛋白的序列，

AAV9 Cap基因是編碼腺相關病毒血清型9衣殼蛋白：VP1、VP2和VP3的重疊核苷酸序列的序列。

[圖6]是顯示基於修飾的腺相關病毒血清型5(rAAV5)的載體的生成效率的圖。

Vg/ml cf是指每毫升培養液的病毒基因組計數。

AAV5-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV5的載體。

AAV5-01Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-02Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、G226A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-03Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、D286E和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-04Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、L341Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-05Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有S2A、C387V和T711S突變的修飾的AAV5衣殼蛋白VP1。

AAV5-06Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、Q421H和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-07Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P466T和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-08Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S594Q和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-09Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T614L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-10Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、N679K和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-11Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P723V和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-12Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、V431Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-13Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、A616D和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-14Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、W683R和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-15Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S222A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-16Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、R285L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-17Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有S2A、Q340A和T711S突變的修飾的AAV5衣殼蛋白VP1。

AAV5-18Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S420F和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-19Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S680A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-20Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T721V和T711S的修飾的AAV5衣殼蛋白VP1。

[圖7]是顯示基於修飾的腺相關病毒血清型5(rAAV5)的載體的生成效率的圖。

Vg/ml cf是指每毫升培養液的病毒基因組計數。

AAV5-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV5載體。

AAV5-01Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-21Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變G226A的修飾的AAV5衣殼蛋白VP1。

AAV5-22Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變D286E的修飾的AAV5衣殼蛋白VP1。

AAV5-23Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變L341Y的修飾的AAV5衣殼蛋白VP1。

AAV5-24Mut-GFP是指基於修飾的腺相關病毒血清型

5(rAAV5)的載體，所述rAAV5包含具有突變C387V的修飾的AAV5衣殼蛋白VP1。

AAV5-25Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q421H的修飾的AAV5衣殼蛋白VP1。

AAV5-26Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P466T的修飾的AAV5衣殼蛋白VP1。

AAV5-27Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S594Q的修飾的AAV5衣殼蛋白VP1。

AAV5-28Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T614L的修飾的AAV5衣殼蛋白VP1。

AAV5-29Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變N679K的修飾的AAV5衣殼蛋白VP1。

AAV5-30Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P723V的修飾的AAV5衣殼蛋白VP1。

AAV5-31Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變V431Y的修飾的AAV5衣殼蛋白VP1。

AAV5-32Mut-GFP是指基於修飾的腺相關病毒血清型

5(rAAV5)的載體，所述rAAV5包含具有突變A616D的修飾的AAV5衣殼蛋白VP1。

AAV5-33Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變W683R的修飾的AAV5衣殼蛋白VP1。

AAV5-34Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S222A的修飾的AAV5衣殼蛋白VP1。

AAV5-35Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變R285L的修飾的AAV5衣殼蛋白VP1。

AAV5-36Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q340A的修飾的AAV5衣殼蛋白VP1。

AAV5-37Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S420F的修飾的AAV5衣殼蛋白VP1。

AAV5-38Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S680A的修飾的AAV5衣殼蛋白VP1。

AAV5-39Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T721V的修飾的AAV5衣殼蛋白VP1。

[圖8]是顯示基於修飾的腺相關病毒血清型9(rAAV9)

的載體的生成效率的圖。

Vg/ml cf是指每毫升培養液的病毒基因組計數。

AAV9-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV9的載體。

AAV9-01Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變S232T的修飾的AAV9衣殼蛋白VP1。

AAV9-02Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D297E的修飾的AAV9衣殼蛋白VP1。

AAV9-03Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351K的修飾的AAV9衣殼蛋白VP1。

AAV9-04Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351R的修飾的AAV9衣殼蛋白VP1。

AAV9-05Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變C396V的修飾的AAV9衣殼蛋白VP1。

AAV9-06Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D433Y的修飾的AAV9衣殼蛋白VP1。

AAV9-07Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變L444R的修飾

的AAV9衣殼蛋白VP1。

AAV9-08Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y478F的修飾的AAV9衣殼蛋白VP1。

AAV9-09Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G604N的修飾的AAV9衣殼蛋白VP1。

AAV9-10Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G627K的修飾的AAV9衣殼蛋白VP1。

AAV9-11Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625L的修飾的AAV9衣殼蛋白VP1

AAV9-12Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625V的修飾的AAV9衣殼蛋白VP1。

AAV9-13Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變S692T的修飾的AAV9衣殼蛋白VP1。

AAV9-14Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T733V的修飾的AAV9衣殼蛋白VP1。

AAV9-15Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變A427R的修飾

的AAV9衣殼蛋白VP1。

AAV9-16Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變M635D的修飾的AAV9衣殼蛋白VP1。

AAV9-17Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變W695R的修飾的AAV9衣殼蛋白VP1。

AAV9-18Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變R296L的修飾的AAV9衣殼蛋白VP1。

AAV9-19Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351A的修飾的AAV9衣殼蛋白VP1。

AAV9-20Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y395F的修飾的AAV9衣殼蛋白VP1。

AAV9-21Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變R434L的修飾的AAV9衣殼蛋白VP1。

AAV9-22Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變N691A的修飾的AAV9衣殼蛋白VP1。

[圖9]是顯示在野生型rAAV5衣殼蛋白VP1中或在已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中包含點突變

的基於rAAV5的產物的細胞轉導效率的圖。

AAV5-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV5的載體。

AAV5-01Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-02Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、G226A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-03Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、D286E和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-04Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、L341Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-05Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有S2A、C387V和T711S突變的修飾的AAV5衣殼蛋白VP1。

AAV5-06Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、Q421H和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-07Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P466T和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-08Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S594Q和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-09Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T614L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-10Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、N679K和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-11Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P723V和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-12Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、V431Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-13Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、A616D和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-14Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、W683R和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-15Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S222A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-16Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、R285L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-17Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有S2A、Q340A和T711S突變的修飾的AAV5衣殼蛋白VP1。

AAV5-18Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S420F和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-19Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S680A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-20Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T721V和T711S的修飾的AAV5衣殼蛋白VP1。

[圖10]是顯示在野生型rAAV5衣殼蛋白VP1中包含點突變的基於rAAV5的產物的細胞轉導效率的圖。

AAV5-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV5的載體。

AAV5-01Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-21Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變G226A的修飾

的AAV5衣殼蛋白VP1。

AAV5-22Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變D286E的修飾的AAV5衣殼蛋白VP1。

AAV5-23Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變L341Y的修飾的AAV5衣殼蛋白VP1。

AAV5-24Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變C387V的修飾的AAV5衣殼蛋白VP1。

AAV5-25Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q421H的修飾的AAV5衣殼蛋白VP1。

AAV5-26Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P466T的修飾的AAV5衣殼蛋白VP1。

AAV5-27Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S594Q的修飾的AAV5衣殼蛋白VP1。

AAV5-28Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T614L的修飾的AAV5衣殼蛋白VP1。

AAV5-29Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變N679K的修飾

的AAV5衣殼蛋白VP1。

AAV5-30Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P723V的修飾的AAV5衣殼蛋白VP1。

AAV5-31Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含帶有V431Y突變的修飾的AAV5衣殼蛋白VP1。

AAV5-32Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變A616D的修飾的AAV5衣殼蛋白VP1。

AAV5-33Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變W683R的修飾的AAV5衣殼蛋白VP1。

AAV5-34Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S222A的修飾的AAV5衣殼蛋白VP1。

AAV5-35Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變R285L的修飾的AAV5衣殼蛋白VP1。

AAV5-36Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q340A的修飾的AAV5衣殼蛋白VP1。

AAV5-37Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S420F的修飾

的AAV5衣殼蛋白VP1。

AAV5-38Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S680A的修飾的AAV5衣殼蛋白VP1。

AAV5-39Mut-GFP是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T721V的修飾的AAV5衣殼蛋白VP1。

[圖11]是顯示在野生型rAAV9衣殼蛋白VP1中包含點突變的基於rAAV9的產物的細胞轉導效率的圖。

AAV9-NullMut-GFP是指基於具有野生型衣殼蛋白VP1的rAAV9的載體。

AAV9-01Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變S232T的修飾的AAV9衣殼蛋白VP1。

AAV9-02Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D297E的修飾的AAV9衣殼蛋白VP1。

AAV9-03Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351K的修飾的AAV9衣殼蛋白VP1。

AAV9-04Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351R的修飾的AAV9衣殼蛋白VP1。

AAV9-05Mut-GFP是指基於修飾的腺相關病毒血清型

9(rAAV9)的載體，所述rAAV9包含具有突變C396V的修飾的AAV9衣殼蛋白VP1。

AAV9-06Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D433Y的修飾的AAV9衣殼蛋白VP1。

AAV9-07Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變L444R的修飾的AAV9衣殼蛋白VP1。

AAV9-08Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y478F的修飾的AAV9衣殼蛋白VP1。

AAV9-09Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G604N的修飾的AAV9衣殼蛋白VP1。

AAV9-10Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G627K的修飾的AAV9衣殼蛋白VP1。

AAV9-11Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625L的修飾的AAV9衣殼蛋白VP1。

AAV9-12Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625V的修飾的AAV9衣殼蛋白VP1。

AAV9-13Mut-GFP是指基於修飾的腺相關病毒血清型

9(rAAV9)的載體，所述rAAV9包含具有突變S692T的修飾的AAV9衣殼蛋白VP1。

AAV9-14Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T733V的修飾的AAV9衣殼蛋白VP1。

AAV9-15Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變A427R的修飾的AAV9衣殼蛋白VP1。

AAV9-16Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變M635D的修飾的AAV9衣殼蛋白VP1。

AAV9-17Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變W695R的修飾的AAV9衣殼蛋白VP1。

AAV9-18Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變R296L的修飾的AAV9衣殼蛋白VP1。

AAV9-19Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351A的修飾的AAV9衣殼蛋白VP1。

AAV9-20Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y395F的修飾的AAV9衣殼蛋白VP1。

AAV9-21Mut-GFP是指基於修飾的腺相關病毒血清型

9(rAAV9)的載體，所述rAAV9包含具有突變R434L的修飾的AAV9衣殼蛋白VP1。

AAV9-22Mut-GFP是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變N691A的修飾的AAV9衣殼蛋白VP1。

[圖12]是顯示用在野生型rAAV5衣殼蛋白VP1或已經含有突變S2A和T711S的rAAV5衣殼蛋白VP1中包含單突變的基於rAAV5的產物進行細胞轉導後由轉基因編碼的目標蛋白的生產效率的圖。

AAV5-NullMut-FIX是指基於具有野生型衣殼蛋白VP1的rAAV5的載體。

AAV5-01Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-02Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、G226A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-03Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、D286E和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-04Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、L341Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-05Mut-FIX是指基於修飾的腺相關病毒血清型

5(rAAV5)的載體，所述rAAV5包含具有突變S2A、C387V和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-06Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、Q421H和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-07Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P466T和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-08Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S594Q和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-09Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T614L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-10Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、N679K和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-11Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、P723V和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-12Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、V431Y和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-13Mut-FIX是指基於修飾的腺相關病毒血清型

5(rAAV5)的載體，所述rAAV5包含具有突變S2A、A616D和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-14Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、W683R和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-15Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S222A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-16Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、R285L和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-17Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、Q340A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-18Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S420F和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-19Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、S680A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-20Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A、T721V和T711S的修飾的AAV5衣殼蛋白VP1。

[圖13]是顯示用在野生型rAAV5衣殼蛋白VP1中包含

單突變的基於rAAV5的產物進行細胞轉導後由轉基因編碼的目標蛋白的生產效率的圖。

AAV5-NullMut-FIX是指基於具有野生型衣殼蛋白VP1的rAAV5的載體。

AAV5-01Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S2A和T711S的修飾的AAV5衣殼蛋白VP1。

AAV5-21Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變G226A的修飾的AAV5衣殼蛋白VP1。

AAV5-22Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變D286E的修飾的AAV5衣殼蛋白VP1。

AAV5-23Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變L341Y的修飾的AAV5衣殼蛋白VP1。

AAV5-24Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變C387V的修飾的AAV5衣殼蛋白VP1。

AAV5-25Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q421H的修飾的AAV5衣殼蛋白VP1。

AAV5-26Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P466T的修飾

的AAV5衣殼蛋白VP1。

AAV5-27Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S594Q的修飾的AAV5衣殼蛋白VP1。

AAV5-28Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T614L的修飾的AAV5衣殼蛋白VP1。

AAV5-29Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變N679K的修飾的AAV5衣殼蛋白VP1。

AAV5-30Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變P723V的修飾的AAV5衣殼蛋白VP1。

AAV5-31Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變V431Y的修飾的AAV5衣殼蛋白VP1。

AAV5-32Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變A616D的修飾的AAV5衣殼蛋白VP1。

AAV5-33Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變W683R的修飾的AAV5衣殼蛋白VP1。

AAV5-34Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S222A的修飾

的AAV5衣殼蛋白VP1。

AAV5-35Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變R285L的修飾的AAV5衣殼蛋白VP1。

AAV5-36Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變Q340A的修飾的AAV5衣殼蛋白VP1。

AAV5-37Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S420F的修飾的AAV5衣殼蛋白VP1。

AAV5-38Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變S680A的修飾的AAV5衣殼蛋白VP1。

AAV5-39Mut-FIX是指基於修飾的腺相關病毒血清型5(rAAV5)的載體，所述rAAV5包含具有突變T721V的修飾的AAV5衣殼蛋白VP1。

[圖14]是顯示在用包含野生型rAAV9衣殼蛋白VP1突變的基於rAAV9的產物進行細胞轉導後由轉基因編碼的目標蛋白的生產效率的圖。

AAV9-NullMut-FIX是指基於具有野生型衣殼蛋白VP1的rAAV9的載體。

AAV9-01Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變S232T的修飾的AAV9衣殼蛋白VP1。

AAV9-02Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D297E的修飾的AAV9衣殼蛋白VP1。

AAV9-03Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351K的修飾的AAV9衣殼蛋白VP1。

AAV9-04Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351R的修飾的AAV9衣殼蛋白VP1。

AAV9-05Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變C396V的修飾的AAV9衣殼蛋白VP1。

AAV9-06Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變D433Y的修飾的AAV9衣殼蛋白VP1。

AAV9-07Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變L444R的修飾的AAV9衣殼蛋白VP1。

AAV9-08Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y478F的修飾的AAV9衣殼蛋白VP1。

AAV9-09Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G604N的修飾的AAV9衣殼蛋白VP1。

AAV9-10Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變G627K的修飾的AAV9衣殼蛋白VP1。

AAV9-11Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625L的修飾的AAV9衣殼蛋白VP1。

AAV9-12Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T625V的修飾的AAV9衣殼蛋白VP1。

AAV9-13Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變S692T的修飾的AAV9衣殼蛋白VP1。

AAV9-14Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變T733V的修飾的AAV9衣殼蛋白VP1。

AAV9-15Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含帶有突變A427R的修飾的AAV9衣殼蛋白VP1。

AAV9-16Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變M635D的修飾的AAV9衣殼蛋白VP1。

AAV9-17Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變W695R的修飾的AAV9衣殼蛋白VP1。

AAV9-18Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變R296L的修飾的AAV9衣殼蛋白VP1。

AAV9-19Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Q351A的修飾的AAV9衣殼蛋白VP1。

AAV9-20Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變Y395F的修飾的AAV9衣殼蛋白VP1。

AAV9-21Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變R434L的修飾的AAV9衣殼蛋白VP1。

AAV9-22Mut-FIX是指基於修飾的腺相關病毒血清型9(rAAV9)的載體，所述rAAV9包含具有突變N691A的修飾的AAV9衣殼蛋白VP1。

*定義和一般方法*

除非本文另有定義，否則與本發明有關的所有技術和科學術語將具有與本領域技術人員通常理解的相同的含義。

此外，除非上下文另有要求，否則單數術語應包括複數術語，並且複數術語應包括單數術語。通常，本文所述的細胞培養、分子生物學、免疫學、微生物學、遺傳學、分析化學、有機合成化學、醫學和藥物化學以及蛋白質和

核酸的雜交和化學的本分類和方法是本領域技術人員眾所周知的並廣泛應用於本領域。酶反應和純化方法根據製造商的指南進行，如本領域中常見的，或如本文所述。

術語“天然存在的”、“天然的”或“野生型”用於描述可以在自然界中找到的與人工產生不同的物件。例如，存在於生物體(包括病毒)中的蛋白質或核苷酸序列，可以從自然界的來源中分離出來，並且未經實驗室人員有意修改，是天然存在的。

如在本說明書和隨附申請專利範圍中使用的，除非上下文另有說明，否則詞語“包括”和“包含”或其變體例如“包括”、“包括”、“包含”或“含有”，將被理解為暗示包含指定的整體或整體組，但不排除任何其他整體或整體組。

*腺相關病毒(AAV)*

細小病毒科(*Parvoviridae*)病毒是含有DNA的小型動物病毒。細小病毒科可分為兩個亞科：其成員感染脊椎動物的細小病毒亞科(*Parvovirinae*)和其成員感染昆蟲的濃核病毒亞科(*Densovirinae*)。到2006年，已經描述了11種腺相關病毒血清型(Mori, S. ET AL., 2004, "Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein", Virology, T. 330 (2): 375-83)。2008年描述了腺相關病毒的血清型12(Michael Schmidt ET AL., Adeno-associated virus type 12 (AAV12): a novel AAV serotype with sialic acid- and heparan sulfate

proteoglycan-independent transduction activity, J Virol. 2008 Feb;82(3):1399-406. doi: 10.1128/JVI.02012-07)。所有已知的血清型都可以感染多種組織類型的細胞。組織特異性由衣殼蛋白血清型決定；因此，基於腺相關病毒的載體是通過指定所需的血清型來構建的。有關細小病毒和細小病毒科其他成員的更多資訊已在文獻中進行了描述(Kenneth I. Berns, «Parvoviridae: The Viruses and Their Replication», Chapter 69 in Fields Virology (3d Ed. 1996))。

所有已知AAV血清型的基因組組構非常相似。AAV的基因組是長度小於約5000個核苷酸(nt)的線性單鏈DNA分子。反向末端重複序列(ITR)位於對病毒生命週期至關重要的蛋白質(Rep)的獨特編碼核苷酸序列以及重疊衣殼蛋白(Cap)的序列的兩側。Cap基因編碼形成衣殼的VP蛋白(VP1、VP2和VP3)，以及AAP(蛋白啟動腺相關病毒(AAV)組裝)(Sonntag F, Köther K, Schmidt K, et al. The assembly-activating protein promotes capsid assembly of different adeno-associated virus serotypes. J Virol. 2011;85(23): 12686-12697. doi:10.1128/JVI.05359-11)和MAAP(膜相關輔助蛋白)(Ogden PJ, Kelsic ED, Sinai S, Church GM. Comprehensive AAV capsid fitness landscape reveals a viral gene and enables machine-guided design. Science. 2019;366(6469):1139-1143. doi:10.1126/science.aaw2900)。145個核苷酸長的AAV基因組側翼序列是自我互補的，並

且被組構成可以形成形成T形髮夾的能量穩定的分子內雙鏈體。這種髮夾結構作為病毒DNA複製的起點其作用，用作細胞DNA聚合酶複合物的引物。在哺乳動物細胞中感染野生型AAV(wtAAV)後，Rep基因(如Rep78和Rep52)分別使用P5啟動子和P19啟動子表達，並且兩種Rep蛋白在病毒基因組的複製中都具有一定的功能。Rep開放閱讀框(Rep ORF)中的剪接事件導致實際上四種Rep蛋白(例如Rep78、Rep68、Rep52和Rep40)的表達。然而，已經表明編碼Rep78和Rep52蛋白的未剪接mRNA足以在哺乳動物細胞中產生AAV載體。

*基於重組腺相關病毒(rAAV)的載體*

如本文所用，術語“載體”是指能夠轉運與其連接的另一種核酸的核酸分子。此外，本文中的術語“載體”是指能夠轉運核酸的病毒顆粒。

如在本說明書中使用的，術語“表達”被定義為由其啟動子驅動的特定核苷酸序列的轉錄和/或翻譯。

*用途*

“向受試者遞送異源核酸序列”是將基因插入受試者的細胞和/或組織中。

術語“受試者”、“患者”、“個體”等在本說明書中可互換使用，並且它們指的是適用於本說明書中描述的方法的任何動物。在某些非限制性實施方案中，受試者、患者或

個體是人。所述受試者可以是任何年齡的男性或女性。

【實施方式】

*產生修飾的腺相關病毒衣殼的方法*

在一個方面，本發明涉及一種產生修飾的AAV衣殼的方法，包括：

a)確定修飾的AAV的殼粒蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的修飾的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於

誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換。

相互作用介面(或表面)是指一組與相鄰五聚體亞單位的氨基酸相互作用的殼粒蛋白多肽鏈片段，其中五聚體亞單位是指具有正五邊形形狀且由位於中心孔周圍的5種殼粒蛋白組成的衣殼結構元件(L. M. Drouin and M. Agbandje-McKenna. Adeno-associated virus structural biology as a tool in vector development. Future Virol., vol. 8, no. 12, pp. 1183-1199, 2013)。

給定亞單位的相互作用介面旨在指所有氨基酸，其至少一個原子與相鄰五聚體亞單位的任何原子的距離小於5Å。

對於AAV5，計算基於衣殼結構PDB ID: 6JCT。

根據計算，AAV5的相鄰五聚體亞單位之間的相互作用介面包括以下範圍的AAV5衣殼蛋白VP1殘基：220-226、283-293、339-342、385-392、412-435、462-466、590-598、611-626、677-685、721-724。

對於AAV9，基於結構PDB ID: 3UX1進行了類似的計算。

根據計算，AAV9的相鄰五聚體亞單位的相互作用介面包括以下範圍的AAV9衣殼蛋白VP1殘基：230-236、294-304、350-353、394-401、421-444、476-480、601-609、622-637、689-697、733-736。

“一個或多個氨基酸置換”是指1、2、3、4、5、6、7、8、9或10個氨基酸置換。例如，1至10個氨基酸置換、1至9個氨基酸置換、1至8個氨基酸置換、1至7個氨基酸置換、1至6個氨基酸置換、1至5個氨基酸置換，1至4個氨基酸置換，1至3個氨基酸置換或1至2個氨基酸置換。

在本發明的一些實施方案中，產生修飾的AAV衣殼的方法進一步包括檢查步驟f)中產生的具有一個或多個氨基酸置換的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，其中一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在本發明的一些實施方案中，產生修飾的AAV衣殼的方法進一步包括檢查步驟f)中產生的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，並選擇與不含這些修飾的AAV衣殼相比具有一種或多

種改進的性質的修飾的AAV衣殼，其中所述一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼選自以下AAV血清型：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15、AAV16、rAAV.rh8、rAAV.rhlO、rAAV.rh20、rAAV.rh39、rAAV.Rh74、rAAV.RHM4-1、AAV.hu37、rAAV.Anc80、rAAV.Anc80L65、rAAV.7m8、rAAV.PHP.B、rAAV2.5、rAAV2tYF、rAAV3B、rAAV.LK03、AAV.HSC1、AAV.HSC2、AAV.HSC3、AAV.HSC4、AAV.HSC5、AAV.HSC6、AAV.HSC7、AAV.HSC8、AAV.HSC9、AAV.HSC10、AAV.HSC11、AAV.HSC12、AAV.HSC13、AAV.HSC14、AAV.HSC15或AAV.HSC16。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼可以是野生型AAV衣殼或在蛋白質VP1、VP2和/或VP3中包含一個或多個氨基酸置換的AAV衣殼。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼可以是野生型AAV5衣殼或在蛋白質VP1、VP2和/或VP3中包含一個或多個氨基酸置換的AAV5衣殼。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼可以是在蛋白質VP1中包含氨基酸置換S2A和T711S的AAV5衣殼。

在用於產生AAV衣殼的方法的一些實施方案中，修飾的AAV衣殼可以是野生型AAV9衣殼或在蛋白質VP1、VP2和/或VP3中包含一個或多個氨基酸置換的AAV9衣殼。

在用於產生AAV衣殼的方法的一些實施方案中，結構相似的範本衣殼選自：細小病毒B19、人博卡病毒1(HBoV1)、牛細小病毒(BVP)。

*根據本發明的修飾的AAV衣殼*

在一個方面，本發明涉及一種用於產生基於重組腺相關病毒的病毒載體的修飾的AAV衣殼，所述修飾的AAV衣殼通過包括以下的方法產生：

a)確定修飾的AAV的殼粒蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換；

並且，如有必要，還包括

g)檢查步驟f)中產生的修飾的AAV衣殼與沒有這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，並選擇與不含這些修飾的AAV衣殼相比具有一種或多種改進的性質的修飾的AAV衣殼，其中所述一種或多種改進的性質

選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼選自以下AAV血清型：AAV: AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15、AAV16、rAAV.rh8、rAAV.rhlO、rAAV.rh20、rAAV.rh39、rAAV.Rh74、rAAV.RHM4-1、AAV.hu37、rAAV.Anc80、rAAV.Anc80L65、rAAV.7m8、rAAV.PHP.B、rAAV2.5、rAAV2tYF、rAAV3B、rAAV.LK03、AAV.HSC1、AAV.HSC2、AAV.HSC3、AAV.HSC4、AAV.HSC5、AAV.HSC6、AAV.HSC7、AAV.HSC8、AAV.HSC9、AAV.HSC10、AAV.HSC11、AAV.HSC12、AAV.HSC13、AAV.HSC14、AAV.HSC15或AAV.HSC16。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼可以是野生型AAV衣殼或在蛋白質VP1、VP2和/或VP3中包含一個或多個氨基酸置換的AAV衣殼。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼可以是野生型AAV5衣殼或在蛋白質VP1、VP2和/或VP3

中包含一個或多個氨基酸置換的AAV5衣殼。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼可以是在蛋白質VP1中包含氨基酸置換S2A和T711S的AAV5衣殼。

在修飾的AAV衣殼的一些實施方案中，修飾的AAV衣殼可以是野生型AAV9衣殼或在蛋白質VP1、VP2和/或VP3中包含一個或多個氨基酸置換的AAV9衣殼。

腺相關病毒基因組DNA(+)-鏈的“右側”包含編碼三種衣殼蛋白的重疊序列：VP1、VP2和VP3。這些基因的轉錄從單個啟動子p40開始。相應蛋白質的分子量分別為87、72和62 kDa。所有這三種蛋白質都是從單個mRNA翻譯而來的。轉錄後，pre-mRNA可以以兩種不同的方式剪接，其中較長或較短的內含子被切除以形成2300或2600個核苷酸長的mRNA。

因此，將突變引入*Cap*基因不僅會影響AAV衣殼蛋白VP1，還會影響AAV VP2和VP3衣殼蛋白。

下面是Cap AAV5基因序列中AAV5衣殼結構蛋白的位置：

1-725 aa-VP1、

137-725 aa-VP2、

193-725 aa-VP3。

下面是Cap AAV9基因序列中AAV9衣殼結構蛋白的位置：

1-737 aa-VP1、

138-737 aa-VP2、

203-737 aa-VP3。

在本發明的一些實施方案中，修飾的AAV衣殼包含具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368。

在本發明的一些實施方案中，修飾的AAV衣殼包含具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP2：SEQ ID No: 16、22、28、34、40、46、52、58、64、70、76、82、88、94、100、106、112、118、124、130、136、142、148、154、160、166、172、178、184、190、196、202、208、214、220、226、232、238、244、250、256、262、268、274、280、286、292、298、304、310、316、322、328、334、340、346、352、358、364或370。

在本發明的一些實施方案中，修飾的AAV衣殼包含具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP3：SEQ ID No: 18、24、30、36、42、48、54、60、66、72、78、84、90、96、102、108、114、120、126、132、138、144、150、156、162、168、174、180、186、192、198、204、210、216、222、228、234、240、246、252、

258、264、270、276、282、288、294、300、306、312、318、324、330、336、342、348、354、360、366或372。

在本發明的一些實施方案中，修飾的AAV衣殼包含：

a)具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368；

b)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP2，所述修飾的AAV衣殼蛋白VP2具有選自下組的氨基酸序列：SEQ ID No: 16、22、28、34、40、46、52、58、64、70、76、82、88、94、100、106、112、118、124、130、136、142、148、154、160、166、172、178、184、190、196、202、208、214、220、226、232、238、244、250、256、262、268、274、280、286、292、298、304、310、316、322、328、334、340、346、352、358、364或370；

c)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP3，所述修飾的AAV衣殼蛋白VP3具有選自下組的氨基酸序列：SEQ ID No: 18、24、30、36、42、48、54、60、66、72、78、84、90、96、102、108、114、120、126、132、138、144、150、156、162、168、174、180、186、192、

198、204、210、216、222、228、234、240、246、252、258、264、270、276、282、288、294、300、306、312、318、324、330、336、342、348、354、360、366或372。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換G226A(AAV5衣殼VP1 G226A)並且具有SEQ ID No: 14的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換G90A(AAV5衣殼VP2 G90A)並且具有SEQ ID No: 16的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換G34A(AAV5衣殼VP3 G34A)並且具有SEQ ID No: 18的氨基酸序列

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A、G226A和T711S(AAV5衣殼VP1 S2A、G226A和T711S)並且具有SEQ ID No: 20的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換G90A和T575S(AAV5衣殼VP2 G90A和T575S)並且具有SEQ ID No: 22的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換G34A T519S(AAV5衣殼VP3 G34A T519S)並且具有SEQ ID No: 24的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換D286E(AAV5衣殼VP1 D286E)並且具有SEQ ID No: 26的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換D150E(AAV5衣殼VP2 D150E)並且具有SEQ ID No: 28的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換D94E(AAV5衣殼VP3 D94E)並且具有SEQ ID No: 30的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A、D286E和T711S(AAV5衣殼VP1 S2A、D286E和T711S)並且具有SEQ ID No: 32的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換D150E和T575S(AAV5衣殼VP2 D150E和T575S)並且具有SEQ ID No: 34的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換D94E和T519S(AAV5衣殼VP3 D94E和T519S)並且具有SEQ ID No: 36的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換L341Y(AAV5衣殼VP1 L341Y)並且具有SEQ ID No: 38的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換L205Y(AAV5衣殼VP2 L205Y)並且具有SEQ ID No: 40的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換L149Y(AAV5衣殼VP3 L149Y)並且具有SEQ ID No: 42的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A、L341Y、T711S(AAV5衣殼VP1 S2A、L341Y、T711S)並且具有SEQ ID No: 44的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換L205Y、T575S(AAV5衣殼VP2 L205Y、T575S)並且具有SEQ ID No: 46的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換L149Y、T519S(AAV5衣殼VP3 L149Y、T519S)並且具有SEQ ID No: 48的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換C387V(AAV5衣殼VP1 C387V)並且具有SEQ ID No: 50的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換C251V(AAV5衣殼VP2 C251V)並且具有SEQ ID No: 52的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換C195V(AAV5衣殼VP3 C195V)並且具有SEQ ID No: 54的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A、C387V、T711S(AAV5衣殼VP1 S2A、C387V、T711S)並且具有SEQ ID No: 56的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換C251V、T575S(AAV5衣殼VP2 C251V、T575S)並且具有SEQ ID No: 58的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換C195V、T519S(AAV5衣殼VP3 C195V、T519S)並且具有SEQ ID No: 60的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換Q421H(AAV5衣殼VP1 Q421H)並且具有SEQ ID No: 62的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換Q285H(AAV5衣殼VP2 Q285H)並且具有SEQ ID No: 64的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換Q229H(AAV5衣殼VP3 Q229H)並且具有SEQ ID No: 66的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A Q421H T711S(AAV5衣殼VP1 S2A Q421H T711S)並且具有SEQ ID No: 68的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換Q285H T575S(AAV5衣殼VP2 Q285H T575S)並且具有SEQ ID No: 70的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換Q229H T519S(AAV5衣殼VP3 Q229H T519S)並且具有SEQ ID No: 72的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換P466T(AAV5衣殼VP1 P466T)並且具有SEQ ID No: 74的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換P330T(AAV5衣殼VP2 P330T)並且具有SEQ ID No: 76的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換P274T(AAV5衣殼VP3 P274T)並且具有SEQ ID No: 78的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A P466T T711S(AAV5衣殼VP1 S2A P466T T711S)並且具有SEQ ID

No: 80的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換P330T T575S(AAV5衣殼VP2 P330T T575S)並且具有SEQ ID No: 82的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換P274T T519S(AAV5衣殼VP3 P274T T519S)並且具有SEQ ID No: 84的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S594Q(AAV5衣殼VP1 S594Q)並且具有SEQ ID No: 86的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S458Q(AAV5衣殼VP2 S458Q)並且具有SEQ ID No: 88的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S402Q(AAV5衣殼VP3 S402Q)並且具有SEQ ID No: 90的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A S594Q T711S(AAV5衣殼VP1 S2A S594Q T711S)並且具有SEQ ID No: 92的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S458Q T575S(AAV5

衣殼VP2 S458Q T575S)並且具有SEQ ID No: 94的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S402Q T519S(AAV5衣殼VP3 S402Q T519S)並且具有SEQ ID No: 96的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換T614L(AAV5衣殼VP1 T614L)並且具有SEQ ID No: 98的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換T478L(AAV5衣殼VP2 T478L)並且具有SEQ ID No: 100的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換T422L(AAV5衣殼VP3 T422L)並且具有SEQ ID No: 102的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A T614L T711S(AAV5衣殼VP1 S2A T614L T711S)並且具有SEQ ID No: 104的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換T478L T575S(AAV5衣殼VP2 T478L T575S)並且具有SEQ ID No: 106的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP3，其包含置換(AAV5衣殼VP3 T422L T519S)並且具有SEQ ID No: 108的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換N679K(AAV5衣殼VP1 N679K)並且具有SEQ ID No: 110的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換N543K(AAV5衣殼VP2 N543K)並且具有SEQ ID No: 112的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換N487K(AAV5衣殼VP3 N487K)並且具有SEQ ID No: 114的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A N679K T711S(AAV5衣殼VP1 S2A N679K T711S)並且具有SEQ ID No: 116的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換N543K T575S(AAV5衣殼VP2 N543K T575S)並且具有SEQ ID No: 118的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換N487K T519S(AAV5衣殼VP3 N487K T519S)並且具有SEQ ID No: 120的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP1，其包含置換P723V(AAV5衣殼VP1 P723V)並且具有SEQ ID No: 122的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換P587V(AAV5衣殼VP2 P587V)並且具有SEQ ID No: 124的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換P531V(AAV5衣殼VP3 P531V)並且具有SEQ ID No: 126的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換P723V T711S(AAV5衣殼VP1 S2A P723V T711S)並且具有SEQ ID No: 128的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換P587V T575S(AAV5衣殼VP2 P587V T575S)並且具有SEQ ID No: 130的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換P531V T519S(AAV5衣殼VP3 P531V T519S)並且具有SEQ ID No: 132的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換V431Y(AAV5衣殼VP1 V431Y)並且具有SEQ ID No: 134的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP2，其包含置換V295Y(AAV5衣殼VP2 V295Y)並且具有SEQ ID No: 136的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換V239Y(AAV5衣殼VP3 V239Y)並且具有SEQ ID No: 138的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A V431Y T711S(AAV5衣殼VP1 S2A V431Y T711S)並且具有SEQ ID No: 140的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換V295Y T575S(AAV5衣殼VP2 V295Y T575S)並且具有SEQ ID No: 142的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換V239Y T519S(AAV5衣殼VP3 V239Y T519S)並且具有SEQ ID No: 144的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換A616D(AAV5衣殼VP1 A616D)並且具有SEQ ID No: 146的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換A480D(AAV5衣殼VP2 A480D)並且具有SEQ ID No: 148的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP3，其包含置換A424D(AAV5衣殼VP3 A424D)並且具有SEQ ID No: 150的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A A616D T711S(AAV5衣殼VP1 S2A A616D T711S)並且具有SEQ ID No: 152的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換A480D T575S(AAV5衣殼VP2 A480D T575S)並且具有SEQ ID No: 154的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換A424D T519S(AAV5衣殼VP3 A424D T519S)並且具有SEQ ID No: 156的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換W683R(AAV5衣殼VP1 W683R)並且具有SEQ ID No: 158的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換W547R(AAV5衣殼VP2 W547R)並且具有SEQ ID No: 160的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換W491R(AAV5衣殼VP3 W491R)並且具有SEQ ID No: 162的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP1，其包含置換S2A W683R T711S(AAV5衣殼VP1 S2A W683R T711S)並且具有SEQ ID No: 164的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換W547R T575S(AAV5衣殼VP2 W547R T575S)並且具有SEQ ID No: 166的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換W491R T519S(AAV5衣殼VP3 W491R T519S)並且具有SEQ ID No: 168的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S222A(AAV5衣殼VP1 S222A)並且具有SEQ ID No: 170的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S86A(AAV5衣殼VP2 S86A)並且具有SEQ ID No: 172的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S30A(AAV5衣殼VP3 S30A)並且具有SEQ ID No: 174的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A S222A T711S(AAV5衣殼VP1 S2A S222A T711S)並且具有SEQ ID No: 176的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S86A T575S(AAV5衣殼VP2 S86A T575S)並且具有SEQ ID No: 178的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S30A T519S(AAV5衣殼VP3 S30A T519S)並且具有SEQ ID No: 180的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換R285L(AAV5衣殼VP1 R285L)並且具有SEQ ID No: 182的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換R149L(AAV5衣殼VP2 R149L)並且具有SEQ ID No: 184的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換R93L(AAV5衣殼VP3 R93L)並且具有SEQ ID No: 186的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A R285L T711S(AAV5衣殼VP1 S2A R285L T711S)並且具有SEQ ID No: 188的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換R149L T575S(AAV5衣殼VP2 R149L T575S)並且具有SEQ ID No: 190的氨基酸

序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換R93L T519S(AAV5衣殼VP3 R93L T519S)並且具有SEQ ID No: 192的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換Q340A(AAV5衣殼VP1 Q340A)並且具有SEQ ID No: 194的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換(AAV5衣殼VP2 Q204A)並且具有SEQ ID No: 196的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換Q148A(AAV5衣殼VP3 Q148A)並且具有SEQ ID No: 198的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A Q340A T711S(AAV5衣殼VP1 S2A Q340A T711S)並且具有SEQ ID No: 200的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換Q204A T575S(AAV5衣殼VP2 Q204A T575S)並且具有SEQ ID No: 202的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換Q148A T519S(AAV5

衣殼VP3 Q148A T519S)並且具有SEQ ID No: 204的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S420F(AAV5衣殼VP1 S420F)並且具有SEQ ID No: 206的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S284F(AAV5衣殼VP2 S284F)並且具有SEQ ID No: 208的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S228F(AAV5衣殼VP3 S228F)並且具有SEQ ID No: 210的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A S420F T711S(AAV5衣殼VP1 S2A S420F T711S)並且具有SEQ ID No: 212的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S284F T575S(AAV5衣殼VP2 S284F T575S)並且具有SEQ ID No: 214的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S228F T519S(AAV5衣殼VP3 S228F T519S)並且具有SEQ ID No: 216的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP1，其包含置換S680A(AAV5衣殼VP1 S680A)並且具有SEQ ID No: 218的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S544A(AAV5衣殼VP2 S544A)並且具有SEQ ID No: 220的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S488A(AAV5衣殼VP3 S488A)並且具有SEQ ID No: 222的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A S680A T711S(AAV5衣殼VP1 S2A S680A T711S)並且具有SEQ ID No: 224的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換S544A T575S(AAV5衣殼VP2 S544A T575S)並且具有SEQ ID No: 226的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換S488A T519S(AAV5衣殼VP3 S488A T519S)並且具有SEQ ID No: 228的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換T721V(AAV5衣殼VP1 T721V)並且具有SEQ ID No: 230的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV5衣殼蛋白VP2，其包含置換T585V(AAV5衣殼VP2 T585V)並且具有SEQ ID No: 232的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換T529V(AAV5衣殼VP3 T529V)並且具有SEQ ID No: 234的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP1，其包含置換S2A T721V T711S(AAV5衣殼VP1 S2A T721V T711S)並且具有SEQ ID No: 236的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP2，其包含置換T585V T575S(AAV5衣殼VP2 T585V T575S)並且具有SEQ ID No: 238的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV5衣殼蛋白VP3，其包含置換T529V T519S(AAV5衣殼VP3 T529V T519S)並且具有SEQ ID No: 240的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換Q351K(AAV9衣殼VP1 Q351K)並且具有SEQ ID No: 242的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換Q214K(AAV9衣殼VP2 Q214K)並且具有SEQ ID No: 244的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP3，其包含置換Q149K(AAV9衣殼VP3 Q149K)並且具有SEQ ID No: 246的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換Q351R(AAV9衣殼VP1 Q351R)並且具有SEQ ID No: 248的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換Q214R(AAV9衣殼VP2 Q214R)並且具有SEQ ID No: 250的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換Q149R(AAV9衣殼VP3 Q149R)並且具有SEQ ID No: 252的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換S232T(AAV9衣殼VP1 S232T)並且具有SEQ ID No: 254的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換S95T(AAV9衣殼VP2 S95T)並且具有SEQ ID No: 256的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換S30T(AAV9衣殼VP3 S30T)並且具有SEQ ID No: 258的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換D297E(AAV9衣殼VP1 D297E)並且具有SEQ ID No: 260的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP2，其包含置換D160E(AAV9衣殼VP2 D160E)並且具有SEQ ID No: 262的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換D95E(AAV9衣殼VP3 D95E)並且具有SEQ ID No: 264的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換S692T(AAV9衣殼VP1 S692T)並且具有SEQ ID No: 266的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換S555T(AAV9衣殼VP2 S555T)並且具有SEQ ID No: 268的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換S490T(AAV9衣殼VP3 S490T)並且具有SEQ ID No: 270的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換D433Y(AAV9衣殼VP1 D433Y)並且具有SEQ ID No: 272的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換D296Y(AAV9衣殼VP2 D296Y)並且具有SEQ ID No: 274的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換D231Y(AAV9衣殼VP3 D231Y)並且具有SEQ ID No: 276的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP1，其包含置換T625L(AAV9衣殼VP1 T625L)並且具有SEQ ID No: 278的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換T488L(AAV9衣殼VP2 T488L)並且具有SEQ ID No: 280的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換T423L(AAV9衣殼VP3 T423L)並且具有SEQ ID No: 282的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換T625V(AAV9衣殼VP1 T625V)並且具有SEQ ID No: 284的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換T488V(AAV9衣殼VP2 T488V)並且具有SEQ ID No: 286的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換T423V(AAV9衣殼VP3 T423V)並且具有SEQ ID No: 288的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換N691A(AAV9衣殼VP1 N691A)並且具有SEQ ID No: 290的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換N554A(AAV9衣殼VP2 N554A)並且具有SEQ ID No: 292的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP3，其包含置換N489A(AAV9衣殼VP3 N489A)並且具有SEQ ID No: 294的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換C396V(AAV9衣殼VP1 C396V)並且具有SEQ ID No: 296的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換C259V(AAV9衣殼VP2 C259V)並且具有SEQ ID No: 298的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換C194V(AAV9衣殼VP3 C194V)並且具有SEQ ID No: 300的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換Y478F(AAV9衣殼VP1 Y478F)並且具有SEQ ID No: 302的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換Y341F(AAV9衣殼VP2 Y341F)並且具有SEQ ID No: 304的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換Y276F(AAV9衣殼VP3 Y276F)並且具有SEQ ID No: 306的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換R296L(AAV9衣殼VP1 R296L)並且具有SEQ ID No: 308的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP2，其包含置換R159L(AAV9衣殼VP2 R159L)並且具有SEQ ID No: 310的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換R94L(AAV9衣殼VP3 R94L)並且具有SEQ ID No: 312的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換T733V(AAV9衣殼VP1 T733V)並且具有SEQ ID No: 314的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換T596V(AAV9衣殼VP2 T596V)並且具有SEQ ID No: 316的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換T531V(AAV9衣殼VP3 T531V)並且具有SEQ ID No: 318的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換A427R(AAV9衣殼VP1 A427R)並且具有SEQ ID No: 320的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換A290R(AAV9衣殼VP2 A290R)並且具有SEQ ID No: 322的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換A225R(AAV9衣殼VP3 A225R)並且具有SEQ ID No: 324的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP1，其包含置換M635D(AAV9衣殼VP1 M635D)並且具有SEQ ID No: 326的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換M498D(AAV9衣殼VP2 M498D)並且具有SEQ ID No: 328的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換M433D(AAV9衣殼VP3 M433D)並且具有SEQ ID No: 330的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換L444R(AAV9衣殼VP1 L444R)並且具有SEQ ID No: 332的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換L307R(AAV9衣殼VP2 L307R)並且具有SEQ ID No: 334的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換L242R(AAV9衣殼VP3 L242R)並且具有SEQ ID No: 336的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換G604N(AAV9衣殼VP1 G604N)並且具有SEQ ID No: 338的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換G467N(AAV9衣殼VP2 G467N)並且具有SEQ ID No: 340的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP3，其包含置換G402N(AAV9衣殼VP3 G402N)並且具有SEQ ID No: 342的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換G627K(AAV9衣殼VP1 G627K)並且具有SEQ ID No: 344的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換G490K(AAV9衣殼VP2 G490K)並且具有SEQ ID No: 346的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換G425K(AAV9衣殼VP3 G425K)並且具有SEQ ID No: 348的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換W695R(AAV9衣殼VP1 W695R)並且具有SEQ ID No: 350的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換W558R(AAV9衣殼VP2 W558R)並且具有SEQ ID No: 352的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換W493R(AAV9衣殼VP3 W493R)並且具有SEQ ID No: 354的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換Q351A(AAV9衣殼VP1 Q351A)並且具有SEQ ID No: 356的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修

飾的AAV9衣殼蛋白VP2，其包含置換Q214A(AAV9衣殼VP2 Q214A)並且具有SEQ ID No: 358的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換Q149A(AAV9衣殼VP3 Q149A)並且具有SEQ ID No: 360的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換Y395F(AAV9衣殼VP1 Y395F)並且具有SEQ ID No: 362的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換Y258F(AAV9衣殼VP2 Y258F)並且具有SEQ ID No: 364的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換Y193F(AAV9衣殼VP3 Y193F)並且具有SEQ ID No: 366的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP1，其包含置換R434L(AAV9衣殼VP1 R434L)並且具有SEQ ID No: 368的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP2，其包含置換R297L(AAV9衣殼VP2 R297L)並且具有SEQ ID No: 370的氨基酸序列。

在本發明的一些實施方案中，修飾的AAV衣殼包括修飾的AAV9衣殼蛋白VP3，其包含置換(AAV9衣殼VP3 R232L)並且具有SEQ ID No: 372的氨基酸序列。

相對於相應的野生型衣殼計算修飾的AAV衣殼中的所

有上述一個或多個置換。

野生型AAV5衣殼蛋白VP1(AAV5衣殼VP1 WT)的天然存在序列由SEQ ID No: 2的氨基酸序列表示，野生型AAV5衣殼蛋白VP2(AAV5衣殼VP2 WT)的天然存在序列由SEQ ID No: 4的氨基酸序列表示，野生型AAV5衣殼蛋白VP3(AAV5衣殼VP3 WT)的天然存在序列由SEQ ID No: 6的氨基酸序列表示。

野生型AAV9衣殼蛋白VP1(AAV9衣殼VP1 WT)的天然存在序列由SEQ ID No: 373的氨基酸序列表示，野生型AAV9衣殼蛋白VP2(AAV9衣殼VP2 T)的天然存在序列由SEQ ID No: 375的氨基酸序列表示，野生型AAV9衣殼蛋白VP3(AAV9衣殼VP3 WT)的天然存在序列由SEQ ID No: 377的氨基酸序列表示。

本發明的作者發現，上述修飾的AAV衣殼與不含所述修飾的AAV衣殼(野生型AAV衣殼)相比具有一種或多種改進的性質，所述一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

*編碼根據本發明的修飾衣殼的分離的核酸*

一方面，本發明涉及編碼任何上述修飾衣殼的核酸。

在本發明的任何實施方案中，核酸分子可以是分離的。

在本說明書中可互換使用的術語“核酸”、“核序列”、“核酸序列”、“多核苷酸”、“寡核苷酸”、“多核苷酸序列”和“核苷酸序列”是指核苷酸的精確序列，不論是否被修飾，確定核酸的片段或區域，不論是否含有非天然核苷酸，並且是雙鏈DNA或RNA，單鏈DNA或RNA，或所述DNA的轉錄產物。

如在本說明書中使用的，作為非限制性實例，多核苷酸包括通過本領域可用的任何手段獲得的所有核酸序列，作為非限制性實例，包括重組手段，即從重組文庫或細胞基因組，採用普通的克隆技術和PCR等克隆核酸序列，以及通過合成手段。

此處還應包括，本發明不涉及在其天然染色體環境中，即在天然狀態下的核苷酸序列。本發明的序列已經被分離和/或純化，即它們被直接或間接地取樣，例如通過複製。因此，此處還應提及通過重組遺傳學，例如通過宿主細胞獲得或通過化學合成獲得的分離的核酸。

除非另有說明，否則對核苷酸序列的提及包括其互補序列。因此，提及具有特定序列的核酸應理解為包含其具有其互補序列的互補鏈的核酸。

“分離的”核酸分子是從至少一種核酸分子-雜質中鑒定和分離的核酸分子，前者在抗體核酸的天然來源中結合至雜質。分離的核酸分子不同於在自然條件下發現的形式或集合。因此，分離的核酸分子不同於在自然條件下存在於細胞中的核酸分子。

在本發明的一些實施方案中，分離的核酸是DNA。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G226A的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 G226A)的核酸，所述核酸具有SEQ ID No: 13的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G90A的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 G90A)的核酸，所述核酸具有SEQ ID No: 15的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G34A的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 G34A)的核酸，所述核酸具有SEQ ID No: 17的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A、G226A和T711S的修飾AAV5衣殼蛋白VP1 (AAV5衣殼VP1 S2A、G226A和T711S)的核酸，所述核酸具有SEQ ID No: 19的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G90A和T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 G90A和T575S)的核酸，所述核酸具有

SEQ ID No: 21的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G34A T519S的修飾AAV5衣殼蛋白VP3(AAV5 衣殼VP3 G34A T519S)的核酸，所述核酸具有SEQ ID No: 23的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D286E的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 D286E)的核酸，所述核酸具有SEQ ID No: 25的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D150E的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 D150E)的核酸，所述核酸具有SEQ ID No: 27的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D94E的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 D94E)的核酸，所述核酸具有SEQ ID No: 29的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A、D286E和T711S的修飾AAV5衣

殼蛋白VP1(AAV5衣殼VP1 S2A、D286E和T711S)的核酸，所述核酸具有SEQ ID No: 31的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D150E和T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 D150E和T575S)的核酸，所述核酸具有SEQ ID No: 33的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D94E和T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 D94E和T519S)的核酸，所述核酸具有SEQ ID No: 35的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L341Y的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 L341Y)的核酸，所述核酸具有SEQ ID No: 37的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L205Y的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 L205Y)的核酸，所述核酸具有SEQ ID No: 39的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換L149Y的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 L149Y)的核酸，所述核酸具有SEQ ID No: 41的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A、L341Y、T711S的修飾AAV5衣殼蛋白VP1 (AAV5衣殼VP1 S2A、L341Y、T711S)的核酸，所述核酸具有SEQ ID No: 43的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L205Y、T575S的修飾AAV5衣殼蛋白VP2 (AAV5衣殼VP2 L205Y、T575S)的核酸，所述核酸具有SEQ ID No: 45的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L149Y、T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 L149Y、T519S)的核酸，所述核酸具有SEQ ID No: 47的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C387V的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 C387V)的核酸，所述核酸具有SEQ ID No: 49的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C251V的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 C251V)的核酸，所述核酸具有SEQ ID No: 51的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C195V的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 C195V)的核酸，所述核酸具有SEQ ID No: 53的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A、C387V、T711S的修飾AAV5衣殼蛋白VP1 (AAV5衣殼VP1 S2A、C387V、T711S)的核酸，所述核酸具有SEQ ID No: 55的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C251V、T575S的修飾AAV5衣殼蛋白VP2 (AAV5衣殼VP2 C251V、T575S)的核酸，所述核酸具有SEQ ID No: 57的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C195V、T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 C195V、T519S)的核酸，所述核酸具有SEQ ID No: 59的核苷酸序列或編碼給定修飾蛋白的相應

氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q421H的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 Q421H)的核酸，所述核酸具有SEQ ID No: 61的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q285H的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 Q285H)的核酸，所述核酸具有SEQ ID No: 63的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q229H的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 Q229H)的核酸，所述核酸具有SEQ ID No: 65的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A Q421H T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A Q421H T711S)的核酸，所述核酸具有SEQ ID No: 67的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q285H T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 Q285H T575S)的核酸，所述核酸具有

SEQ ID No: 69的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q229H T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 Q229H T519S)的核酸，所述核酸具有SEQ ID No: 71的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P466T的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 P466T)的核酸，所述核酸具有SEQ ID No: 73的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P330T的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 P330T)的核酸，所述核酸具有SEQ ID No: 75的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P274T的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 P274T)的核酸，所述核酸具有SEQ ID No: 77的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A P466T T711S的修飾AAV5衣殼蛋

白VP1(AAV5衣殼VP1 S2A P466T T711S)的核酸，所述核酸具有SEQ ID No: 79的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換 P330T T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 P330T T575S)的核酸，所述核酸具有SEQ ID No: 81的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P274T T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 P274T T519S)的核酸，所述核酸具有SEQ ID No: 83的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S594Q的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S594Q)的核酸，所述核酸具有SEQ ID No: 85的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S458Q的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S458Q)的核酸，所述核酸具有SEQ ID No: 87的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換S402Q的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 S402Q)的核酸，所述核酸具有SEQ ID No: 89的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A S594Q T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A S594Q T711S)的核酸，所述核酸具有SEQ ID No: 91的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S458Q T575S 的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S458Q T575S)的核酸，所述核酸具有SEQ ID No: 93的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S402Q T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 S402Q T519S)的核酸，所述核酸具有SEQ ID No: 95的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T614L的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 T614L)的核酸，所述核酸具有SEQ ID No: 97的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T478L的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 T478L)的核酸，所述核酸具有SEQ ID No: 99的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T422L的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 T422L)的核酸，所述核酸具有SEQ ID No: 101的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A T614L T711S 的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A T614L T711S)的核酸，所述核酸具有SEQ ID No: 103的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T478L T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 T478L T575S)的核酸，所述核酸具有SEQ ID No: 105的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T422L T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 T422L T519S)的核酸，所述核酸具有SEQ ID No: 107的核苷酸序列或編碼給定修飾蛋白的相應

氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N679K的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 N679K)的核酸，所述核酸具有SEQ ID No: 109的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N543K的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 N543K)的核酸，所述核酸具有SEQ ID No: 111的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N487K的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 N487K)的核酸，所述核酸具有SEQ ID No: 113的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A N679K T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A N679K T711S)的核酸，所述核酸具有SEQ ID No: 115的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N543K T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 N543K T575S)的核酸，所述核酸具有

SEQ ID No: 117的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N487K T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 N487K T519S)的核酸，所述核酸具有SEQ ID No: 119的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P723V的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 P723V)的核酸，所述核酸具有SEQ ID No: 121的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P587V的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 P587V)的核酸，所述核酸具有SEQ ID No: 123的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P531V的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 P531V)的核酸，所述核酸具有SEQ ID No: 125的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換P723V T711S的修飾AAV5衣殼蛋白

VP1(AAV5衣殼VP1 S2A P723V T711S)的核酸，所述核酸具有SEQ ID No: 127的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換 P587V T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 P587V T575S)的核酸，所述核酸具有SEQ ID No: 129的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換 P531V T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 P531V T519S)的核酸，所述核酸具有SEQ ID No: 131的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換V431Y的修飾 AAV5衣殼蛋白VP1(AAV5衣殼VP1 V431Y)的核酸，所述核酸具有SEQ ID No: 133的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換V295Y的修飾 AAV5衣殼蛋白VP2(AAV5衣殼VP2 V295Y)的核酸，所述核酸具有SEQ ID No: 135的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換V239Y的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 V239Y)的核酸，所述核酸具有SEQ ID No: 137的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A V431Y T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A V431Y T711S)的核酸，所述核酸具有SEQ ID No: 139的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換V295Y T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 V295Y T575S)的核酸，所述核酸具有SEQ ID No: 141的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換V239Y T519S的修飾AAV5衣殼蛋白VP3 AAV5衣殼VP3 V239Y T519S)的核酸，所述核酸具有SEQ ID No: 143的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A616D的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 A616D)的核酸，所述核酸具有SEQ ID No: 145的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A480D的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 A480D)的核酸，所述核酸具有SEQ ID No: 147的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A424D的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 A424D)的核酸，所述核酸具有SEQ ID No: 149的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A A616D T711S的修飾 AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A A616D T711S)的核酸，所述核酸具有SEQ ID No: 151的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A480D T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 A480D T575S)的核酸，所述核酸具有SEQ ID No: 153的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A424D T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 A424D T519S)的核酸，所述核酸具有SEQ ID No: 155的核苷酸序列或編碼給定修飾蛋白的相應

氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W683R的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 W683R)的核酸，所述核酸具有SEQ ID No: 157的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W547R的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 W547R)的核酸，所述核酸具有SEQ ID No: 159的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W491R的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 W491R)的核酸，所述核酸具有SEQ ID No: 161的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A W683R T711S的修飾 AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A W683R T711S)的核酸，所述核酸具有SEQ ID No: 163的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W547R T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 W547R T575S)的核酸，所述核酸具有

SEQ ID No: 165的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W491R T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 W491R T519S)的核酸，所述核酸具有SEQ ID No: 167的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S222A的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S222A)的核酸，所述核酸具有SEQ ID No: 169的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S86A的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S86A)的核酸，所述核酸具有SEQ ID No: 171的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S30A的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 S30A)的核酸，所述核酸具有SEQ ID No: 173的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A S222A T711S的修飾 AAV5衣殼

蛋白VP1(AAV5衣殼VP1 S2A S222A T711S)的核酸，所述核酸具有SEQ ID No: 175的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S86A T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S86A T575S)的核酸，所述核酸具有SEQ ID No: 177的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S30A T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 S30A T519S)的核酸，所述核酸具有SEQ ID No: 179的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R285L的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 R285L)的核酸，所述核酸具有SEQ ID No: 181的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R149L的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 R149L)的核酸，所述核酸具有SEQ ID No: 183的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換R93L的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 R93L)的核酸，所述核酸具有SEQ ID No: 185的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A R285L T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A R285L T711S)的核酸，所述核酸具有SEQ ID No: 187的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R149L T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 R149L T575S)的核酸，所述核酸具有SEQ ID No: 189的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R93L T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 R93L T519S)的核酸，所述核酸具有SEQ ID No: 191的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q340A的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 Q340A)的核酸，所述核酸具有SEQ ID No: 193的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q204A的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 Q204A)的核酸，所述核酸具有SEQ ID No: 195的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q148A的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 Q148A)的核酸，所述核酸具有SEQ ID No: 197的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A Q340A T711S的修飾AAV5衣殼蛋白VP1 (AAV5衣殼VP1 S2A Q340A T711S)的核酸，所述核酸具有SEQ ID No: 199的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q204A T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 Q204A T575S)的核酸，所述核酸具有SEQ ID No: 201的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q148A T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 Q148A T519S)的核酸，所述核酸具有SEQ ID No: 203的核苷酸序列或編碼給定修飾蛋白的相應

氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S420F的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S420F)的核酸，所述核酸具有SEQ ID No: 205的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S284F的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S284F)的核酸，所述核酸具有SEQ ID No: 207的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S228F的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 S228F)的核酸，所述核酸具有SEQ ID No: 209的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A S420F T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A S420F T711S)的核酸，所述核酸具有SEQ ID No: 211的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S284F T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S284F T575S)的核酸，所述核酸具有

SEQ ID No: 213的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S228F T519S的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 S228F T519S)的核酸，所述核酸具有SEQ ID No: 215的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S680A的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S680A)的核酸，所述核酸具有SEQ ID No: 217的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S544A的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S544A)的核酸，所述核酸具有SEQ ID No: 219的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S488A的修飾AAV5衣殼蛋白VP3 (AAV5衣殼VP3 S488A)的核酸，所述核酸具有SEQ ID No: 221的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A S680A T711S的修飾AAV5衣殼蛋

白VP1(AAV5衣殼VP1 S2A S680A T711S)的核酸，所述核酸具有SEQ ID No: 223的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S544A T575S的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 S544A T575S)的核酸，所述核酸具有SEQ ID No: 225的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S488A T519S的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 S488A T519S)的核酸，所述核酸具有SEQ ID No: 227的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T721V的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 T721V)的核酸，所述核酸具有SEQ ID No: 229的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T585V的修飾AAV5衣殼蛋白VP2(AAV5衣殼VP2 T585V)的核酸，所述核酸具有SEQ ID No: 231的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換T529V的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 T529V)的核酸，所述核酸具有SEQ ID No: 233的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S2A T721V T711S的修飾AAV5衣殼蛋白VP1(AAV5衣殼VP1 S2A T721V T711S)的核酸，所述核酸具有SEQ ID No: 235的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T585V T575S的修飾 AAV5衣殼蛋白VP2(AAV5衣殼VP2 T585V T575S)的核酸，所述核酸具有SEQ ID No: 237的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T529V T519S 的修飾AAV5衣殼蛋白VP3(AAV5衣殼VP3 T529V T519S)的核酸，所述核酸具有SEQ ID No: 239的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q351K的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 Q351K)的核酸，所述核酸具有SEQ ID No: 241的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換 Q214K的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 Q214K)的核酸，所述核酸具有SEQ ID No: 243的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q149K的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 Q149K)的核酸，所述核酸具有SEQ ID No: 245的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q351R的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 Q351R)的核酸，所述核酸具有SEQ ID No: 247的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q214R的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 Q214R)的核酸，所述核酸具有SEQ ID No: 249的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q149R的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 Q149R)的核酸，所述核酸具有SEQ ID No: 251的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列

的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S232T的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 S232T)的核酸，所述核酸具有SEQ ID No: 253的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S95T的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 S95T)的核酸，所述核酸具有SEQ ID No: 255的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S30T的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 S30T)的核酸，所述核酸具有SEQ ID No: 257的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D297E的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 D297E)的核酸，所述核酸具有SEQ ID No: 259的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D160E的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 D160E)的核酸，所述核酸具有SEQ ID

No: 261的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D95E的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 D95E)的核酸，所述核酸具有SEQ ID No: 263的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S692T的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 S692T)的核酸，所述核酸具有SEQ ID No: 265的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S555T的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 S555T)的核酸，所述核酸具有SEQ ID No: 267的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換S490T的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 S490T)的核酸，所述核酸具有SEQ ID No: 269的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D433Y的修飾AAV9衣殼蛋白

VP1(AAV9衣殼VP1 D433Y)的核酸，所述核酸具有SEQ ID No: 271的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D296Y的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 D296Y)的核酸，所述核酸具有SEQ ID No: 273的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換D231Y的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 D231Y)的核酸，所述核酸具有SEQ ID No: 275的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T625L的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 T625L)的核酸，所述核酸具有SEQ ID No: 277的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T488L的修飾 AAV9衣殼蛋白VP2(AAV9衣殼VP2 T488L)的核酸，所述核酸具有SEQ ID No: 279的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換T423L的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 T423L)的核酸，所述核酸具有SEQ ID No: 281的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T625V的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 T625V)的核酸，所述核酸具有SEQ ID No: 283的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T488V的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 T488V)的核酸，所述核酸具有SEQ ID No: 285的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換 T423V的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 T423V)的核酸，所述核酸具有SEQ ID No: 287的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N691A的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 N691A)的核酸，所述核酸具有SEQ ID No: 289的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N554A的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 N554A)的核酸，所述核酸具有SEQ ID No: 291的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換N489A的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 N489A)的核酸，所述核酸具有SEQ ID No: 293的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C396V的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 C396V)的核酸，所述核酸具有SEQ ID No: 295的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C259V的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 C259V)的核酸，所述核酸具有SEQ ID No: 297的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換C194V的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 C194V)的核酸，所述核酸具有SEQ ID No: 299的核苷酸序列或編碼給定修飾蛋白的相應氨基酸

序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y478F的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 Y478F)的核酸，所述核酸具有SEQ ID No: 301的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y341F的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 Y341F)的核酸，所述核酸具有SEQ ID No: 303的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y276F的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 Y276F)的核酸，所述核酸具有SEQ ID No: 305的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R296L的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 R296L)的核酸，所述核酸具有SEQ ID No: 307的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R159L的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 R159L)的核酸，所述核酸具有SEQ ID

No: 309的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R94L的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 R94L)的核酸，所述核酸具有SEQ ID No: 311的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T733V的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 T733V)的核酸，所述核酸具有SEQ ID No: 313的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T596V的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 T596V)的核酸，所述核酸具有SEQ ID No: 315的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換T531V的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 T531V)的核酸，所述核酸具有SEQ ID No: 317的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A427R的修飾AAV9衣殼蛋白

VP1(AAV9衣殼VP1 A427R)的核酸，所述核酸具有SEQ ID No: 319的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A290R的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 A290R)的核酸，所述核酸具有SEQ ID No: 321的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換A225R的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 A225R)的核酸，所述核酸具有SEQ ID No: 323的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換M635D的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 M635D)的核酸，所述核酸具有SEQ ID No: 325的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換M498D的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 M498D)的核酸，所述核酸具有SEQ ID No: 327的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核

酸包括編碼含有置換M433D的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 M433D)的核酸，所述核酸具有SEQ ID No: 329的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L444R的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 L444R)的核酸，所述核酸具有SEQ ID No: 331的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L307R的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 L307R)的核酸，所述核酸具有SEQ ID No: 333的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換L242R的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 L242R)的核酸，所述核酸具有SEQ ID No: 335的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G604N的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 G604N)的核酸，所述核酸具有SEQ ID No: 337的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G467N的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 G467N)的核酸，所述核酸具有SEQ ID No: 339的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G402N的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 G402N)的核酸，所述核酸具有SEQ ID No: 341的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G627K的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 G627K)的核酸，所述核酸具有SEQ ID No: 343的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G490K的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 G490K)的核酸，所述核酸具有SEQ ID No: 345的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換G425K的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 G425K)的核酸，所述核酸具有SEQ ID No: 347的核苷酸序列或編碼給定修飾蛋白的相應氨基酸

序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W695R的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 W695R)的核酸，所述核酸具有SEQ ID No: 349的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W558R的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 W558R)的核酸，所述核酸具有SEQ ID No: 351的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換W493R的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 W493R)的核酸，所述核酸具有SEQ ID No: 353的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q351A的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 Q351A)的核酸，所述核酸具有SEQ ID No: 355的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q214A的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 Q214A)的核酸，所述核酸具有SEQ ID

No: 357的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Q149A的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 Q149A)的核酸，所述核酸具有SEQ ID No: 359的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y395F的修飾AAV9衣殼蛋白VP1(AAV9衣殼VP1 Y395F)的核酸，所述核酸具有SEQ ID No: 361的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y258F的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 Y258F)的核酸，所述核酸具有SEQ ID No: 363的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換Y193F的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 Y193F)的核酸，所述核酸具有SEQ ID No: 365的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R434L的修飾AAV9衣殼蛋白

VP1(AAV9衣殼VP1 R434L)的核酸，所述核酸具有SEQ ID No: 367的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換R297L的修飾AAV9衣殼蛋白VP2(AAV9衣殼VP2 R297L)的核酸，所述核酸具有SEQ ID No: 369的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

在本發明的一些實施方案中，編碼修飾衣殼的分離核酸包括編碼含有置換的修飾AAV9衣殼蛋白VP3(AAV9衣殼VP3 R232L)的核酸，所述核酸具有SEQ ID No: 371的核苷酸序列或編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列。

“編碼給定修飾蛋白的相應氨基酸序列的任何其他核苷酸序列”是指可替換所述核酸序列的核酸序列，因為由於遺傳密碼的簡並性，多種不同的DNA序列可以編碼相同的氨基酸序列。產生編碼相同氨基酸序列的這些備選DNA序列完全在本領域技術人員的技能範圍內。這種變體DNA序列在本發明的範圍內。

在一些實施方案中，編碼任何上述修飾衣殼的分離核酸包含任何上述核酸序列或其組合。

*基於重組腺相關病毒的載體*

一方面，本發明涉及一種用於向受試者遞送異源核酸

序列的基於重組腺相關病毒的載體，其包括：

1)任何上述修飾的衣殼，和

2)異源核酸序列，其包含促進由異源核酸序列編碼的產物在靶細胞中表達的調節序列。

本說明書中使用的術語“基於重組腺相關病毒的載體”、“基於AAV的重組病毒”、“基於AAV的病毒樣顆粒”、“重組病毒AAV毒株”、“重組AAV載體”或“rAAV載體”具有相同的含義。

在本發明的一些實施方案中，rAAV載體包括包含提供產物表達的調節序列的異源核酸序列，其中異源核酸序列的表達產物是治療性多肽或報告多肽。

根據本發明的rAAV載體不包含編碼病毒生命週期必需的蛋白質(Rep)序列以及重疊衣殼蛋白(Cap)序列的基因的核苷酸序列。

在說明書的上述部分中詳細表徵了衣殼。

如本發明所用的，“提供由異源核酸序列編碼的產物在靶細胞中表達的調節序列”是指影響它們所克隆到的編碼序列的表達和加工所必需的多核苷酸序列。表達控制序列包括適當的轉錄起始、終止、啟動子和增強子序列；有效的RNA加工信號如剪接和多聚腺苷酸化信號；穩定細胞質mRNA的序列；提高翻譯效率的序列(即Kozak共有序列)；增強蛋白質穩定性的序列；並且當需要時，增強蛋白質分泌的序列。這種控制序列的性質因宿主生物而異；在原核生物中，此類控制序列一般包括核糖體結合位點的

啟動子和轉錄終止序列；在真核生物中，此類控制序列通常包括啟動子和轉錄終止序列。術語“調節序列”至少包括所有組分，其存在對於表達和加工是必需的，並且還可以包括附加組分，其存在是有利的，例如前導肽序列。

如在本說明書中使用的，術語“啟動子”是指控制一個或多個編碼序列的轉錄的核酸片段，並且通過存在DNA依賴性RNA聚合酶的結合位點、轉錄起始位點和任何其他DNA序列在結構上進行鑒定，包括但不限於轉錄因數結合位點、阻遏蛋白和啟動蛋白結合位點，以及本領域技術人員已知的直接或間接調節所述啟動子的轉錄水準的任何其他核苷酸序列。“組成型”啟動子是在典型生理和發育條件下在大多數組織中具有活性的啟動子。“誘導型”啟動子是生理或發育調節的啟動子，例如在化學誘導劑的影響下。“組織特異性”啟動子僅在特定類型的組織或細胞中具有活性。

用於在真核細胞中，特別是在哺乳動物細胞中高水準產生多肽的啟動子應該是強的並且較佳在廣泛的細胞類型中具有活性。能夠在許多細胞類型中驅動表達的強組成型啟動子在本領域中是眾所周知的，因此在本文中沒有必要對其進行詳細描述。根據本發明的思想，較佳使用巨細胞病毒(CMV)啟動子。源自人巨細胞病毒(hCMV)的立即早期(IE)區的啟動子或啟動子/增強子特別適合作為本發明的rAAV5載體的啟動子。人巨細胞病毒(hCMV)立即早期(IE)區和由其衍生的功能性表達誘導和/或功能性表達增強片段已在例如EP0173177和EP0323997中公開，並且是本領

域公知的。因此，hCMV立即早期(IE)區的幾個片段可以用作啟動子和/或啟動子/增強子。

如本文所用，術語“增強子”或“增強子”可以指位於與編碼重組產物的DNA序列相鄰的DNA序列。增強子元件通常位於啟動子元件的5'方向，或者可以位於編碼DNA序列(例如轉錄或翻譯成一種或多種重組產物的DNA序列)的下游或內部。因此，增強子元件可以位於編碼重組產物的DNA序列上游或所述序列下游的100個堿基對、200個堿基對或300個或更多堿基對處。增強子元件可以增加從DNA序列表達的重組產物的量高於與單個啟動子元件相關的表達水準。本領域普通技術人員容易獲得多種增強子元件。

在本發明的一些實施方案中，包含提供由異源核酸序列編碼的產物在靶細胞中表達的調節序列的異源核酸序列可以在5'-端至3'-端方向上包括以下元件：

左手(第一)ITR(反向末端重複序列)；

CMV(巨細胞病毒)增強子；

CMV(巨細胞病毒)啟動子；

hBG1基因的內含子(血紅蛋白亞單位γ1基因)；

編碼產物的核酸；

hGH1多聚腺苷酸化信號(人生長激素基因多聚腺苷酸化信號)；

右手(第二)ITR。

在一些實施方案中，編碼產物(轉基因)的核酸是至少一種編碼蛋白質的基因。在一些實施方案中，轉基因編碼

至少一種小的抑制性核酸。在一些實施方案中，轉基因編碼至少一種報導分子。在一些實施方案中，小抑制性核酸是miRNA。在一些實施方案中，小抑制性核酸是海綿miRNA或TuD-RNA，其抑制動物中至少一種miRNA的活性。在一些實施方案中，miRNA在靶組織的細胞中表達。在一些實施方案中，靶組織是肝臟、中樞神經系統(CNS)、眼睛、胃腸道、呼吸道、乳房、胰腺、泌尿道或子宮組織的組織。

在一些實施方案中，rAAV載體具有異源核酸序列的表達產物，其是治療性多肽或報告多肽。

在一些實施方案中，rAAV載體包含編碼作為治療性多肽的產物的異源核酸序列，其中治療性多肽是選自因數VIII、因數IX或其功能變體的凝血因數。

在一些實施方案中，rAAV載體包含編碼作為因數VIII或其功能變體的產物的異源核酸序列。

在一些實施方案中，rAAV載體包含編碼作為因數IX或其功能變體的產物的異源核酸序列。

在一些實施方案中，rAAV載體包含編碼作為SMN1蛋白(存活運動神經元蛋白)的產物的異源核酸序列。

在一些實施方案中，rAAV載體包含編碼作為SARS-cov2 (嚴重急性呼吸綜合征冠狀病毒2)的RBD-S多肽(S糖蛋白的重組受體結合結構域)的產物的異源核酸序列。

## 實施例

提供以下實施例以更好地理解本發明。這些實施例僅用於說明的目的，並且不應解釋為以任何方式限制本發明的範圍。

本說明書中引用的所有出版物、專利和專利申請均通過引用併入本文。儘管為了清楚理解的目的已經通過說明和示例的方式對前述發明進行了一些詳細的描述，但是根據本發明的教導，對於本領域的普通技術人員將很容易明白，可以在不背離所附實施方案的精神或範圍的情況下進行某些變化和修改。

## 材料和一般方法

*重組DNA技術*

標準方法用於操作DNA，如Sambrook等人，Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2012所述。根據製造商方案使用分子生物學試劑。

*基因合成*

所需的基因區段由化學合成的寡核苷酸製備。長度為300-4,000bp的基因區段兩側帶有單個限制性位點，通過寡核苷酸的退火和連接(包括PCR擴增)組裝，隨後通過指定的限制性位點進行克隆。通過DNA測序確認亞克隆基因片段的DNA序列。

*DNA序列測定*

DNA序列通過Sanger測序確定。

*DNA和蛋白質序列分析和序列資料管理*

Infomax的VectorNTI Advance套件版本8.0和SnapGene版本5.1.4.1用於序列創建、作圖、分析、注釋和說明。

*統計資料分析*

結果表明平均值±標準差(SD)，方差分析(ANOVA)用於比較測試和對照結果，並確定它們具有統計學顯著性。

**實施例1**

**基於細小病毒科衣殼結構相似性的AAV5衣殼突變變體的選擇**

AAV5衣殼突變的設計基於細小病毒科(包括非AAV病毒)內衣殼蛋白的高度結構相似性。置換的選擇包括三個步驟：

1)確定AAV5殼粒蛋白中位於衣殼相鄰五聚體亞單位相互作用介面區域的氨基酸；

2)將修飾的AAV5衣殼與非AAV細小病毒之一的衣殼(下文稱為“範本衣殼”)進行結構比對；

3)成對比較AAV5衣殼和範本衣殼的氨基酸殘基。

預先製備AAV5和範本衣殼的三維模型結構；預先製備包括使用Schrodinger Suite中的Prepwizard utility補充結

構中缺失的原子。

在步驟1中，相互作用介面是指一組與相鄰五聚體亞單位的氨基酸相互作用的殼粒蛋白多肽鏈片段，其中五聚體亞單位是指具有正五邊形形狀且由位於中心孔周圍的5種殼粒蛋白組成的衣殼結構元件(L. M. Drouin and M. Agbandje-McKenna. Adeno-associated virus structural biology as a tool in vector development. Future Virol., vol. 8, no. 12, pp. 1183-1199, 2013)。

第二步包括使用Schrodinger Suite的Structure Alignment utility將AAV5衣殼與範本衣殼進行結構比對。根據比對結果，對於來自相鄰五聚體亞單位相互作用介面的每個AAV5氨基酸，確定最接近範本衣殼的氨基酸(初始氨基酸的結構類似物)，這被認為是潛在的置換。如果在離範本衣殼最近的氨基酸附近存在另一個AAV5氨基酸(其距離小於與初始氨基酸的距離)，則不存在初始氨基酸的結構類似物。這裡，氨基酸之間的距離通過α-碳原子之間的距離來估計。

在確定了相鄰五聚體亞單位相互作用介面的所有氨基酸的結構類似物後，根據實施例2、3和4做出置換決定。

**實施例2. 基於增加氨基酸殘基體積的原則設計突變**

在將AAV5衣殼與範本衣殼進行結構比對後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，根據比較氨基酸側取代基的范德華體積的結果，決定

將初始氨基酸置換為範本衣殼的相應氨基酸(N. J. Darby, S. S. N. J. Darby, and T. E. Creighton, Protein Structure. IRL Press at Oxford University Press, 1993; 表1)。如果滿足以下條件，則引入突變：

1. 範本衣殼中氨基酸側取代基的體積大於初始氨基酸側取代基的體積，

2. 來自範本衣殼的氨基酸不是半胱氨酸或甲硫氨酸。

從潛在置換的氨基酸中排除半胱氨酸和甲硫氨酸是由於它們具有顯著的反應性，特別是可氧化性。

表1. 氨基酸側取代基的范德華體積(N. J. Darby, S. S. N. J. Darby, and T. E. Creighton, Protein Structure. IRL Press at Oxford University Press, 1993.)

| 三字母代碼 | 單字母代碼 | 體積, Å 3 |
|---|---|---|
| Ala | A | 67 |
| Arg | R | 148 |
| Asp | D | 96 |
| Asn | N | 91 |
| Cys | C | 86 |
| Gln | Q | 114 |
| Glu | E | 109 |
| Gly | G | 48 |
| His | H | 118 |
| Ile | I | 124 |
| Leu | L | 124 |
| Lys | K | 135 |
| Met | M | 124 |
| Phe | F | 135 |
| Pro | P | 90 |
| Ser | S | 73 |
| Thr | T | 93 |
| Trp | W | 163 |
| Tyr | Y | 141 |
| Val | V | 105 |

表2顯示了應用本主題方法使用三種範本衣殼設計AAV5衣殼中的突變的結果：HBoV1(PDBid 5urf)、B19(PDBid 1s58)、BPV(PDBid 4qc8)。第一列包含來自AAV5衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，其餘列包含AAV5與每個衣殼的成對結構比對後產生的氨基酸：HBoV1、B19和BPV。星號表示根據側取代基的大小標準以及半胱氨酸和甲硫氨酸的禁止置換而適合置換的氨基酸。

給定亞單位的相互作用介面旨在指所有氨基酸，其中至少一個原子與相鄰五聚體亞單位的任何原子的距離小於5Å。

對於AAV5，計算基於衣殼結構PDB ID：6JCT。

根據計算，其AAV5相鄰五聚體亞單位之間的相互作用介面包括以下範圍的AAV5衣殼蛋白VP1殘基：220-226、283-293、339-342、385-392、412-435、462-466、590-598、611-626、677-685、721-724。

表2. 來自AAV5衣殼的相鄰五聚體亞單位相互作用介面的氨基酸以及與其結構上對應的殼粒B19、HBoV1和BPV的氨基酸。星號表示側取代基大於AAV5殼粒的起始氨基酸的氨基酸。破折號表明範本衣殼中沒有結構相似的氨基酸。

| AAV5 | B19 | HBoV1 | BPV |
|---|---|---|---|
| C220 | E* | G | G |
| D221 | G | G | G |
| S222 | A | T* | S |
| T223 | T | I* | H* |
| W224 | F | F | F |

| | | | |
|---|---|---|---|
| M225 | S | S | S |
| G226 | A* | E* | D* |
| S283 | S | S | S |
| P284 | P | P | P |
| R285 | L | N | Q |
| D286 | E* | D | D |
| W287 | F | W | W |
| Q288 | Q | Q | Q |
| R289 | H | H | R |
| L290 | L | L | L |
| I291 | I | V | T |
| N292 | E* | N | N |
| N293 | N | D* | E* |
| Y339 | Y | H | H |
| Q340 | K* | R* | A |
| L341 | Y* | Y* | Y* |
| P342 | P | P | P |
| F385 | F | L | F |
| F386 | Y* | Y* | F |
| C387 | V* | M | L* |
| L388 | L | L | L |
| E389 | E | E | E |
| Y390 | H | N | N |
| F391 | S | S | S |
| P392 | S | D* | D* |
| P412 | P | E* | E* |
| F413 | P | W* | W* |
| H414 | E | I* | V |
| S415 | N* | E* | N* |
| S416 | L* | N* | N* |
| F417 | E | N | E |
| A418 | G | I* | R* |
| P419 | C | T* | A |
| S420 | Q* | F* | Y* |
| Q421 | H* | S | I* |
| N422 | - | M | P |
| L423 | F* | P | P |

| | | | |
|---|---|---|---|
| F424 | Y* | Q | G |
| K425 | E | M | L |
| L426 | M | M | M |
| A427 | Y* | Y* | F* |
| N428 | N | N | N |
| P429 | P | P | P |
| L430 | L | L | K* |
| V431 | Y* | V | V |
| D432 | G | R* | P |
| Q433 | S | S | T |
| Y434 | R* | R* | R* |
| L435 | L | R* | R* |
| K462 | Q | S | T |
| N463 | N | N | S |
| W464 | F | W | W |
| F465 | M | M | M |
| P466 | P | S | T* |
| I590 | L | M | M |
| V591 | M | L* | F* |
| P592 | V* | P | P |
| G593 | G | N* | N* |
| S594 | S | Q* | Q* |
| V595 | V | M | V |
| W596 | W | W | W |
| M597 | N | D | D |
| E598 | R* | S | R* |
| I611 | I | V | K* |
| P612 | P | P | P |
| E613 | N | R* | R* |
| T614 | L* | V* | A |
| G615 | D* | N* | D* |
| A616 | D* | R* | K* |
| H617 | S | K* | H |
| F618 | F | T | T |
| H619 | K* | L* | I* |
| P620 | T* | L* | M |
| S621 | F* | D* | D* |

| | | | |
|---|---|---|---|
| P622 | A | T* | P |
| A623 | A | Q* | F* |
| M624 | L | D | D |
| G625 | G | G | G |
| G626 | G | S* | S* |
| K677 | P | K | R* |
| E678 | R* | R* | Y* |
| N679 | K* | G | A |
| S680 | A | T* | T* |
| K681 | T | K | K |
| R682 | G | N | N |
| W683 | R | W | W |
| N684 | N | R* | R* |
| P685 | P | P | P |
| T721 | V* | H* | N |
| R722 | H | K | K |
| P723 | P | V* | V* |
| L724 | L | L | L |

**實施例3. 基於增加殼粒之間極性接觸數量的原則設計突變。**

根據實施例1，在結構上比對AAV5衣殼和範本衣殼後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，基於對初始氨基酸及其結構類似物的微環境的分析，做出將初始氨基酸置換為範本衣殼的相應氨基酸的決定。特別是，如果在範本衣殼中的一對相應氨基酸中存在具有極性側取代基的氨基酸，並且在AAV5中存在具有非極性取代基的氨基酸，則做出積極的置換決定，其中兩種氨基酸與衣殼的相鄰五聚體亞單位具有至少一個極性接觸。如果在距側取代基的除氫以外的任何原子不超過

5Å的距離處，存在另一殼粒的另一氨基酸的側取代基的極性基團的至少一個原子，則假定該氨基酸具有極性接觸(表3)。與實施例2類似，半胱氨酸和甲硫氨酸的置換也是不允許的。

表3. 具有極性側取代基的氨基酸列表及其極性基團的原子(根據PDB規範命名)，但氫原子除外。

| 氨基酸 | 極性側基中包含的原子(根據 PDB 規範命名) |
|---|---|
| Arg | NH1, NH2, CZ, NE |
| Asn | CG, OD1, ND2, |
| Asp | CG, OD1, OD2 |
| Cys | SG |
| Gln | CD, OE1, NE2 |
| Glu | CD, OE1, OE2 |
| His | ND1, CD2, CE1, NE2 |
| Lys | NZ |
| Ser | OG |
| Thr | OG1 |
| Tyr | OH |

表4顯示了應用本主題方法使用三種範本衣殼設計AAV5衣殼中的突變的結果：HBoV1(PDBid 5urf)、B19(PDBid 1s58)、BPV(4qc8)。第一列包含來自AAV5衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，在三種衣殼之一中發現了合適的置換：HBoV1、B19和BPV。其餘列包含AAV5與每個衣殼的成對結構比對後產生的氨基酸。星號表示根據極性接觸的增加數量標準以及半胱氨酸和甲硫氨酸的禁止置換，適合置換的氨基酸。

表4. 顯示具有合適置換的AAV5衣殼位置及衣殼HBoV1、B19和BPV與其對應的氨基酸。星號表示根據殼粒之間極性接觸的增加數量標準而適合置換的氨基酸。

| AAV5 | B19 | HBoV1 | BPV |
|---|---|---|---|
| 431V | Y* | V | V |
| 616A | D* | K | R |
| 683W | R* | W | W |

**實施例4. 基於減少殼粒之間極性接觸數量原則的突變設計**

根據實施例1，在結構上比對AAV5衣殼和範本衣殼後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，基於對初始氨基酸及其結構類似物的微環境的分析，做出將初始氨基酸置換為範本衣殼的相應氨基酸的決定。特別是，如果在AAV5衣殼中的一對相應氨基酸中存在與相鄰五聚體亞單位形成至少單一極性接觸的極性氨基酸殘基，而在範本衣殼中存在非極性氨基酸殘基，則做出積極的置換決定。此外，與實施例2類似，半胱氨酸或甲硫氨酸殘基不考慮用於置換。

表5顯示了應用本主題方法使用三種範本衣殼設計AAV5衣殼中的突變的結果：HBoV1(PDBid 5urf)、B19(PDBid 1s58)、BPV(4qc8)。第一列包含來自AAV5衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，在三種衣殼之一中發現了合適的置換：HBoV1、B19和BPV。剩餘的列包含AAV5與每個範本衣殼的成對結構比對後產生的

氨基酸。星號表示根據極性接觸的增加數量標準以及半胱氨酸和甲硫氨酸的禁止置換，適合置換的氨基酸。

表5. 顯示具有合適置換的AAV5衣殼位置及衣殼HBoV1、B19和BPV與其對應的氨基酸。星號表示根據殼粒之間極性接觸的減少數量標準適合置換的氨基酸。

| AAV5 | B19 | HBoV1 | BPV |
|---|---|---|---|
| S222 | A* | S | T |
| R285 | L* | Q | N |
| Q340 | K | A* | R |
| S420 | Q | Y | F* |
| K425 | E | L* | M |
| N679 | K | A* | G |
| S680 | A* | T | T |
| T721 | V* | N | H |

**實施例5. 基於細小病毒科衣殼結構相似性的AAV9衣殼突變變體的選擇**

AAV9衣殼突變的設計基於細小病毒科(包括非AAV病毒)內殼粒蛋白的高度結構相似性。置換的選擇包括三個步驟：

1)確定AAV9殼粒蛋白中位於衣殼相鄰五聚體亞單位相互作用介面區域的氨基酸；

2)將修飾的AAV9衣殼與非AAV細小病毒之一的衣殼(下文稱為“範本衣殼”)進行結構比對；

3)成對比較AAV9衣殼和範本衣殼的氨基酸殘基。

預先製備AAV9和範本衣殼的三維模型結構；預先製備包括使用Schrodinger Suite中的Prepwizard utility補充結

構中缺失的原子。

在步驟1中，相互作用介面是指一組與相鄰五聚體亞單位的氨基酸相互作用的殼粒蛋白多肽鏈片段，其中五聚體亞單位是指具有正五邊形形狀且由位於中心孔周圍的5種殼粒蛋白組成的衣殼結構元件(L. M. Drouin and M. Agbandje-McKenna. Adeno-associated virus structural biology as a tool in vector development. Future Virol., vol. 8, no. 12, pp. 1183-1199, 2013)。

第二步包括使用Schrodinger Suite的Structure Alignment utility將AAV9衣殼與範本衣殼進行結構比對。根據比對結果，對於來自相鄰五聚體亞單位相互作用介面的每個AAV9氨基酸，確定最接近範本衣殼的氨基酸(初始氨基酸的結構類似物)，這被認為是潛在的置換。如果在離範本衣殼最近的氨基酸附近存在另一個AAV9氨基酸(其距離小於與初始氨基酸的距離)，則不存在初始氨基酸的結構類似物。這裡，氨基酸之間的距離通過α-碳原子之間的距離來估計。

在確定了相鄰五聚體亞單位相互作用介面的所有氨基酸的結構類似物後，根據實施例6、7和8做出置換決定。

**實施例6. 基於增加氨基酸殘基體積的原則設計突變**

在將AAV9衣殼與範本衣殼進行結構比對後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，根據比較氨基酸側取代基的范德華體積的結果，決定

將初始氨基酸置換為範本衣殼的相應氨基酸(N. J. Darby, S. S. N. J. Darby, and T. E. Creighton, Protein Structure. IRL Press at Oxford University Press, 1993; 表1)。如果滿足以下條件，則引入突變：

1. 範本衣殼中氨基酸側取代基的體積大於初始氨基酸側取代基的體積，

2. 來自範本衣殼的氨基酸不是半胱氨酸或甲硫氨酸。

從潛在置換的氨基酸中排除半胱氨酸和甲硫氨酸是由於它們具有顯著的反應性，特別是可氧化性。

表6顯示了應用本主題方法使用三種範本衣殼設計AAV9衣殼中的突變的結果：HBoV1(PDBid 5urf)、B19(PDBid 1s58)、BPV(PDBid 4qc8)。第一列包含來自AAV9衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，其餘列包含AAV9與每個衣殼的成對結構比對後產生的氨基酸：HBoV1、B19和BPV。星號表示根據側取代基的大小標準以及半胱氨酸和甲硫氨酸的禁止置換而適合置換的氨基酸。

給定亞單位的相互作用介面旨在指所有氨基酸，其中至少一個原子與相鄰五聚體亞單位的任何原子的距離小於5Å。

對於AAV9，計算基於結構PDB ID：3UX1。

根據計算，其AAV9相鄰五聚體亞單位之間的相互作用介面包括以下範圍的AAV9衣殼蛋白VP1殘基：230-236、294-304、350-353、394-401、421-444、476-480、601-609、622-637、689-697、733-736。

表6. 來自AAV9衣殼的相鄰五聚體亞單位相互作用介面的氨基酸以及與其結構上對應的殼粒B19、HBoV1和BPV的氨基酸。星號表示側取代基大於AAV9殼粒的起始氨基酸的氨基酸。破折號表明範本衣殼中沒有結構相似的氨基酸。

| **AAV9** | **B19** | **HBoV1** | **BPV** |
|---|---|---|---|
| C230 | E* | G | G |
| D231 | G | G | G |
| S232 | A | T* | S |
| Q233 | T | I* | H* |
| W234 | F | F | F |
| L235 | S | S | S |
| G236 | A* | E* | D* |
| S294 | S | S | S |
| P295 | P | P | P |
| R296 | L | N | Q |
| D297 | E* | D | D |
| W298 | F | W | W |
| Q299 | Q | Q | Q |
| R300 | H | H | R |
| L301 | L | L | L |
| I302 | I | V | T |
| N303 | E* | N | N |
| N304 | N | D* | E* |
| Y350 | Y | H | H |
| Q351 | K* | R* | A |
| L352 | Y* | Y* | Y* |
| P353 | P | P | P |
| F394 | F | L | F |
| Y395 | Y | Y | F |
| C396 | V* | M | L* |
| L397 | L | L | L |
| E398 | E | E | E |
| Y399 | H | N | N |
| F400 | S | S | S |
| P401 | S | D* | D* |
| P421 | P | E* | E* |
| F422 | P | W* | W* |

| | | | |
|---|---|---|---|
| H423 | E | I* | V |
| S424 | N* | E* | N* |
| S425 | L* | N* | N* |
| Y426 | E | N | E |
| A427 | G | I* | R* |
| H428 | C | T | A |
| S429 | Q* | F* | Y* |
| Q430 | H* | S | I* |
| S431 | F* | M | P* |
| L432 | - | P | P |
| D433 | Y* | Q* | G |
| R434 | E | M | L |
| L435 | M | M | M |
| M436 | Y* | Y* | F* |
| N437 | N | N | N |
| P438 | P | P | P |
| L439 | Y* | L | K* |
| I440 | G | V | V |
| D441 | S | R* | P |
| Q442 | R* | S | T |
| Y443 | - | R* | R* |
| L444 | - | R* | R* |
| R476 | Q | S | T |
| N477 | N | N | S |
| Y478 | F | W* | W* |
| I479 | M | M | M |
| P480 | P | S | T* |
| I601 | L | M | M |
| L602 | M | L | F* |
| P603 | V* | P | P |
| G604 | G | N* | N* |
| M605 | S | Q | Q |
| V606 | V | M | V |
| W607 | W | W | W |
| Q608 | N | D | D |
| D609 | R* | S | R* |
| I622 | I | V | K* |

| | | | |
|---|---|---|---|
| P623 | P | P | P |
| H624 | N | R* | R* |
| T625 | L* | V* | A |
| D626 | D | N | D |
| G627 | D* | R* | K* |
| N628 | S | K* | H* |
| F629 | F | T | T |
| H630 | K* | L* | I* |
| P631 | T* | L* | M |
| S632 | Q* | D* | D* |
| P633 | A | T* | - |
| L634 | A | Q | P |
| M635 | L | D | D |
| G636 | G | G | G |
| G637 | G | S* | S* |
| K689 | P | K | R* |
| E690 | R* | R* | Y* |
| N691 | K* | G | A |
| S692 | A | T* | T* |
| K693 | T | K | K |
| R694 | G | N | N |
| W695 | R | W | W |
| N696 | N | R* | R* |
| P697 | P | P | P |
| T733 | V* | H* | N |
| R734 | H | K | K |
| N735 | L* | V* | V* |
| L736 | - | L | L |

**實施例7. 基於增加殼粒之間極性接觸數量的原則設計突變。**

根據實施例5，在結構上比對AAV9衣殼和範本衣殼的結果後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，基於對初始氨基酸及其結構類似物的微環境的分析，做出將初始氨基酸置換為範本衣殼的相應

氨基酸的決定。特別是，如果在範本衣殼中的一對相應氨基酸中存在具有極性側取代基的氨基酸，並且在AAV9中存在具有非極性取代基的氨基酸，則做出積極的置換決定，其中兩種氨基酸與衣殼的相鄰五聚體亞單位具有至少一個極性接觸。如果在距側取代基的除氫以外的任何原子不超過5Å的距離處，存在另一殼粒的另一氨基酸的側取代基的極性基團的至少一個原子，則假定該氨基酸具有極性接觸(表3)。與實施例6類似，半胱氨酸和甲硫氨酸的置換也是不允許的。

表7顯示了應用本主題方法使用三種範本衣殼設計AAV9衣殼中的突變的結果：HBoV1(PDBid5urf)、B19(PDBid1s58)、BPV(4qc8)。第一列包含來自AAV9衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，在三種衣殼之一中發現了合適的置換：HBoV1、B19和BPV。剩餘的列包含AAV9與每個衣殼的成對結構比對後產生的氨基酸。星號表示根據極性接觸的增加數量標準和半胱氨酸和甲硫氨酸的禁止置換，適合置換的氨基酸。

表7. 顯示具有合適置換的AAV9衣殼位置及其對應的衣殼氨基酸：HBoV1、B19和BPV。星號表示根據殼粒之間極性接觸的增加數量標準適合置換的氨基酸。

| AAV9 | B19 | HBoV1 | BPV |
|---|---|---|---|
| A427 | G | R* | I |
| L439 | Y* | K* | L |
| M635 | L | D* | D |
| W695 | R* | W | W |

**實施例8. 基於減少殼粒之間極性接觸數量的原則設計突變**

根據實施例5，在結構上比對AAV9衣殼和範本衣殼的結果後，我們得到其氨基酸殘基之間的成對結構對應性。在其中一種情況下，基於對初始氨基酸及其結構類似物的微環境的分析，做出將初始氨基酸置換為範本衣殼的相應氨基酸的決定。特別是，如果在AAV9衣殼中的一對相應氨基酸中存在與相鄰五聚體亞單位形成至少單一極性接觸的極性氨基酸殘基，而在範本衣殼中存在非極性氨基酸殘基，則做出積極的置換決定。此外，與實施例6類似，半胱氨酸或甲硫氨酸殘基不考慮用於置換。

表8顯示了應用本主題方法使用三種範本衣殼設計AAV9衣殼中的突變的結果：HBoV1(PDBid 5urf)、B19(PDBid 1s58)、BPV(4qc8)。第一列包含來自AAV9衣殼的相鄰五聚體亞單位相互作用介面的氨基酸，在三種衣殼之一中發現了合適的置換：HBoV1、B19和BPV。剩餘的列包含AAV9與每個範本衣殼的成對結構比對後產生的氨基酸。星號表示根據極性接觸的增加數量標準以及半胱氨酸和甲硫氨酸的禁止置換，適合置換的氨基酸。

表8. 顯示具有合適置換的AAV9衣殼位置及衣殼HBoV1、B19和BPV與其對應的氨基酸。星號表示根據殼粒之間極性接觸的減少數量標準適合置換的氨基酸。

| AAV9 | B19 | HBoV1 | BPV |
|---|---|---|---|
| R296 | L* | Q | N |
| Q351 | K | A* | R |
| Y395 | Y | F* | Y |
| S429 | Q | Y | F* |
| S431 | F* | P | M |
| R434 | E | L* | M |
| T625 | L* | A* | V* |
| N691 | K | A* | G |
| S692 | A* | T | T |
| T733 | V* | N | H |
| N735 | L* | V* | V* |

## 實施例9. AAV5衣殼變體文庫的產生

AAV5衣殼變體文庫通過Cap基因序列的隨機誘變產生(Davidsson M等人, 2016)。簡而言之，從頭組裝Cap基因血清型5(GenBank ID AF085716.1)的野生型序列，然後使用尿嘧啶-DNA糖基化酶將合成的野生型AAV5衣殼基因片段化，使用不具有校對活性的DNA聚合酶將所得片段組裝成全長Cap基因(導致序列中的隨機突變)。將全長突變變體克隆到載體質粒pAAV-接頭(圖1)中的AscI/EcoRI限制性位點，與綠色螢光蛋白(GFP)處於共同閱讀框中，從而產生多樣化的AAV5衣殼隨機文庫，此後使用該文庫來選擇具有增加的轉導活性的衣殼變體。

在CHO-K1-S細胞上體外陽性選擇了轉導活性增加的病毒顆粒。此外，對於轉導，我們使用了在碘克沙醇梯度中使用超速離心純化的顆粒。48小時後，收穫細胞並分離

基因組DNA用於隨後擴增能夠有效轉導的病毒基因組序列。所得序列隨後被重新克隆並重新產生以用於隨後的選擇反覆運算以富集具有最高轉導效率的變體文庫。經過5輪選擇後，對30個克隆的衣殼基因進行測序，以確定最成功的突變及其組合。測序結果顯示，AAV5 VP1中的主要突變組合為S2A、T711S以及AAV5 VP1中含有S2A、T711S、S651A的衣殼變體，約占克隆的20%。還選擇了在AAV5 VP1中包含突變S651A的衣殼變體。這些衣殼變體被克隆到用於產生病毒顆粒的載體中，並進一步用於視覺化和比較相對於野生型AAV5的轉導譜。

**實施例10. 生成然後從所得序列文庫中選擇重組病毒顆粒**

為了產生並隨後從所得序列文庫中選擇重組病毒顆粒，開發了如下一系列質粒：載體質粒、包含Rep基因序列的質粒，以及包含病毒顆粒複製所需的腺病毒基因的構建體。

載體質粒pAAV-接頭(圖1)(旨在將AAV血清型5衣殼基因的隨機變體文庫與報告蛋白克隆到一個閱讀框中)如下產生：使用在HindIII/EcoRI位點的限制酶-連接酶克隆方法，將原始構建體pAAV-GFP中修飾的綠色螢光蛋白的序列置換為從頭合成的序列T2A-GFP，且從5'端添加EcoRI限制性位點和從3'端添加HindIII限制性位點。

包含Rep基因序列的質粒pAAV-Rep(圖2)如下產生：從頭克隆AAV血清型2(GenBank ID AF043303.1)的合成Rep

基因序列，且將5'端的PciI限制性位點和3'端的PsiI限制位點添加到質粒pGEM-T Easy(Promega, USA)，該質粒也用PciI/PsiI限制酶(New England Biolabs, USA)處理。

用於產生重組病毒顆粒的腺病毒基因來源於構建體pHelper(圖3)，其包含AmpR-一種提供對氨苄青黴素的抗性的β-內醯胺酶基因，Ori-細菌中的複製起點，Adeno E2A-參與病毒DNA複製的輔助腺病毒基因序列，Adeno E4-參與病毒DNA複製的輔助腺病毒基因序列，Adeno VARNA-負責翻譯早期和晚期病毒基因的輔助腺病毒基因序列。

**實施例11. AAV5和AAV9衣殼的突變變體的產生**

野生型AAV血清型5衣殼(GenBank ID AF085716.1)和野生型AAV血清型9衣殼(GenBank ID AY530579.1)的基因序列是從頭合成的，其中將5'端的SwaI位點和3'端的NotI位點分別納入每個合成序列；在用SwaI/NotI限制性內切酶(New England Biolabs, USA)處理後，將每個序列克隆到質粒載體pAAV-Rep(圖2)中，該載體也用SwaI/NotI限制酶(New England Biolabs, USA)預處理，產生以下質粒：pAAV-RC5(圖4)，包含野生型AAV血清型5衣殼序列並進一步包含AAV Rep基因序列；和pAAV-RC9(圖5)，包含野生型AAV血清型9衣殼序列並進一步包含AAV Rep基因序列。

根據製造商的說明，使用商業QuikChange II定點誘變試劑盒(Agilent，USA)將突變引入野生型AAV衣殼序列。使用QuikChange Primer Design軟體(Agilent，USA)開發

PCR引物(表9、表10)。簡而言之，分別使用質粒pAAV-RC5或pAAV-RC9作為範本進行基於定點誘變的程式。為了引入突變，第一步包括在單獨的試管中對每個突變體和補充有相應寡核苷酸的對照樣品進行PCR反應。第二步包括根據製造商的方案用限制酶處理以去除範本質粒DNA，用所得混合物轉化感受態大腸桿菌XL-1 Blue細胞。使用PCR分析所得克隆的目標質粒中的特定區域，選擇的突變體在使用前進行測序。

表9. 用於AAV衣殼血清型5基因序列的定點誘變的引物核苷酸序列。

| 置換 | 正向序列 | 反向序列 |
|---|---|---|
| G226A | cgattccacgtggatg**gcc**gacagagtcgtcacc | ggtgacgactctgtc**ggc**catccacgtggaatcg |
| D286E | ccactggagcccccga**gag**tggcaaagactcatcaac | gttgatgagtctttg**cca**ctctcgggggctccagtgg |
| L341Y | ggacgacgactaccag**tac**ccctacgtcgtcggc | gccgacgacgtaggg**gta**ctggtagtcgtcgtcc |
| C387V | cgagaggagcagcttcttc**gtc**ctagagtactttcccagc | gctgggaaagtactctag**gac**gaagaagctgctcctctcg |
| Q421H | cagcttcgctcccagt**cac**aacctcttcaagctgg | ccagcttgaagaggtt**gtg**actgggagcgaagctg |
| P466T | ctacaaaaactggttc**acc**gggcccatgggccgaac | gttcggcccatgggccc**ggt**gaaccagtttttgtag |
| S594Q | caggaaatcgtgcccggc**cag**gtgtggatggagagggac | gtccctctccatccacac**ctg**gccgggcacgatttcctg |
| T614L | gccaagatcccagag**ctc**ggggcgcactttcac | gtgaaagtgcgcccc**gag**ctctgggatcttggc |
| N679K | gagatggagtgggagctcaagaaggaa**aag**tccaagaggtggaacc | ggttccacctcttgga**ctt**ttccttcttgagctcccactccatctc |
| P723V | ctatcggaacccgataccttacccga**gtg**ctttaacccattcatg | catgaatgggttaaag**cac**tcgggtaaggtatcgggttccgatag |
| V431Y | gctggccaacccgctg**tac**gaccagtacttgtacc | ggtacaagtactggtc**gta**cagcgggttggccagc |
| A616D | gatcccagagacgggg**gac**cactttcacccctctc | gagaggggtgaaagtg**gtc**ccccgtctctgggatc |
| W683R | gaaaactccaagagg**aga**aacccagagatccagtac | gtactggatctctgggtt**tct**cctcttggagttttc |
| S222A | gagattggcattgcgat**gcc**acgtggatgggggacag | ctgtcccccatccacgt**ggc**atcgcaatgccaatctc |
| R285L | cagccactggagcccc**ctg**gactggcaaagactcatc | gatgagtctttgccagtc**cag**gggggctccagtggctg |
| Q340A | cggacgacgactac**gcc**ctgccctacgtcgtcg | cgacgacgtagggcag**ggc**gtagtcgtcgtccg |
| S420F | ctccagcttcgctccc**ttc**cagaacctcttcaagc | gcttgaagaggttctg**gaa**gggagcgaagctggag |
| S680A | gctcaagaaggaaaac**gcc**aagaggtggaacccag | ctgggttccacctctt**ggc**gttttccttcttgagc |
| T721V | ggaacccgatacctt**gtg**cgacccctttaacccattc | gaatgggttaaaggggtcg**cac**aaggtatcgggttcc |

帶有置換的密碼子以粗體突出顯示。

以類似的方式，將突變引入AAV血清型5衣殼的前導變體，其同時包含突變S2A和T711S，通過實施例9和10中描述的靶向進化產生。

表10. 用於AAV衣殼血清型9基因序列的定點誘變的引物核苷酸序列。

| 置換 | 正向序列 | 反向序列 |
|---|---|---|
| Q351K | cttcacggactcagactat**aag**ctcccgtacgtgctc | gagcacgtacgggag**ctt**atagtctgagtccgtgaag |
| Q351R | cttcacggactcagactat**aga**ctcccgtacgtgctc | gagcacgtacgggag**tct**atagtctgagtccgtgaag |
| S232T | gaaattggcattgcgat**acc**caatggctgggggacag | ctgtcccccagccattg**ggt**atcgcaatgccaatttc |
| D297E | ccacttctcaccacgt**gag**tggcagcgactcatcaac | gttgatgagtcgctgcca**ctc**acgtggtgagaagtgg |
| S692T | gctgcagaaggaaaac**act**aagcgctggaacccg | cgggttccagcgct**agt**gttttccttctgcagc |
| D433Y | ctcacagccaaagcctg**tat**cgactaatgaatccac | gtggattcattagtcg**ata**caggctttggctgtgag |
| T625L | gccaaaattcctcac**ctg**gacggcaactttcacc | ggtgaaagttgccgtc**cag**gtgaggaattttggc |
| T625V | ggccaaaattcctcac**gtt**gacggcaactttcac | gtgaaagttgccgtc**aac**gtgaggaattttggcc |
| N691A | gagctgcagaaggaa**gcc**agcaagcgctggaacc | ggttccagcgcttgct**ggc**ttccttctgcagctc |
| C396V | gtcgttcgtccttttac**gtg**ctggaatatttcccgtc | gacgggaaatattccag**cac**gtaaaaggacgaacgac |
| Y478F | ctgtccagggaagaaac**ttc**atacctggacccagc | gctgggtccaggtat**gaa**gtttcttccctggacag |
| R296L | ctgccacttctcacca**ctt**gactggcagcgactc | gagtcgctgccagtc**aag**tggtgagaagtggcag |
| T733V | gcaccagatacctg**gtc**cgtaatctgtaacgtac | gtacgttacagattacg**gac**caggtatctggtgc |
| A427R | ctttccatagcagctac**aga**cacagccaaagcctggac | gtccaggctttggctgtg**tct**gtagctgctatggaaag |
| M635D | caccettctccgctg**gat**ggagggtttggaatg | cattccaaaccctcc**atc**cagcggagaagggtg |
| L444R | cactcatcgaccaatac**aga**tactatctctcaaag | ctttgagagatagta**tct**gtattggtcgatgagtg |
| G604N | ccaaggaatacttccg**aat**atggtttggcaggac | gtcctgccaaaccat**att**cggaagtattccttgg |
| G627K | caaaattcctcacacggac**aag**aactttcacccttctc | gagaagggtgaaagtt**ctt**gtccgtgtgaggaattttg |
| W695R | ggaaaacagcaagcgc**aga**aacccggagatccagtac | gtactggatctccgggtt**tct**gcgcttgctgttttcc |
| Q351A | cacggactcagactat**gct**ctcccgtacgtgctcg | cgagcacgtacgggag**agc**atagtctgagtccgtg |
| Y395F | gtgggtcgttcgtcctt**ttc**tgcctggaatatttcc | ggaaatattccaggc**agaa**aaaggacgaacgacccac |
| R434L | cagccaaagcctggac**ctg**ctaatgaatccactcatc | gatgagtggattcattag**cag**gtccaggctttggctg |

帶有置換的密碼子以粗體突出顯示。

## 實施例11.基於修飾的腺相關病毒血清型5(rAAV5)產生載體的效率

為了產生具有修飾的血清型5衣殼的rAAV顆粒，使用

聚乙烯亞胺(PEI，線性，MW25000，Polyscinces, Inc.)同時用以下3種質粒轉染HEK293生產細胞：

1) 用包含腺病毒核苷酸序列的質粒(輔助質粒)，所述核苷酸序列編碼組裝rAAV顆粒所需的蛋白質和RNA；

2) 用包含腺相關病毒血清型2的Rep基因的天然核苷酸序列，以及修飾Cap基因序列的質粒，所述修飾Cap基因序列選自：SEQ ID NO: 7、13、19、25、31、37、43、49、55、61、67、73、79、85、91、97、103、109、115、121、127、133、139、145、151、157、163、169、175、181、187、193、199、205、211、217、223、229、235的核苷酸序列或編碼具有SEQ ID No: 8、14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230或236的氨基酸序列的蛋白質VP1，以及來自所用核苷酸序列的可變閱讀框的蛋白質VP2和VP3的任何其他核苷酸序列，其中

VP2可具有SEQ ID No: 10、16、22、28、34、40、46、52、58、64、70、76、82、88、94、100、106、112、118、124、130、136、142、148、154、160、166、172、178、184、190、196、202、208、214、220、226、232或238的任一氨基酸序列；

並且VP3可具有SEQ ID No: 12、18、24、30、36、42、48、54、60、66、72、78、84、90、96、102、108、

114、120、126、132、138、144、150、156、162、168、174、180、186、192、198、204、210、216、222、228、234或240的任一氨基酸序列；

3) 用包含異源rAAV顆粒基因組的質粒，該基因組編碼旨在遞送到患者細胞中的目標基因。

這組基因在72小時內提供了rAAV病毒顆粒的組裝和目標基因組在其中的包殼化。轉染後72小時，對生產細胞進行裂解(TAKARA BIO INC, Japan)以釋放rAAV顆粒，所得載體在37℃下用DNAaseI (Invitrogen, USA)處理2小時，然後在56℃下用蛋白酶K(Invitrogen, USA)再處理2小時。使用由特異於GFP序列的正向引物5'- ACCACATGAAGCAGCACGAC -3'、反向引物5'- TCAGCTCGATGCGGTTCAC -3'和探針5'- HEX-CATGCCCGAAGGCTACGTCCAG-BHQ1 -3'組成的寡核苷酸組，通過定量PCR (TaqMan, Applied Biosystems, USA)證實所得rAAV顆粒的滴度。

本發明的作者意想不到地發現在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中存在一種或多種選自G226A、D286E、L341Y、C387V、Q421H、P466T、S594Q、T614L、N679K、P723V、V431Y、A616D、W683R、S222A、R285L、Q340A、S420F、S680A、T721V的突變導致具有上述突變的rAAV載體的轉基因包裝效率在統計學上可靠地增加。例如，定量PCR顯示編碼目標GFP基因的包裝異源rAAV顆粒基因組的拷貝數發生變化(圖6、圖7)。

在同時存在突變S2A和T711S (AAV5-01Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到7.1倍，從2.51E+09 vg/ml至1.78E+10 vg/ml。

在同時存在突變S2A、G226A和T711S (AAV5-02Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到5.4倍，從2.51E+09 vg/ml至1.36E+10 vg/ml。

在同時存在突變S2A、D286E和T711S (AAV5-03Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到3.5倍，從2.51E+09 vg/ml至8.79E+09 vg/ml。

在同時存在突變S2A、L341Y和T711S (AAV5-04Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到5.8倍，從2.51E+09 vg/ml至1.46E+10 vg/ml。

在同時存在突變S2A、C387V和T711S (AAV5-05Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到4.8倍，從2.51E+09 vg/ml至1.21E+10 vg/ml。

在同時存在突變S2A、Q421H和T711S (AAV5-06Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到2.1倍，從2.51E+09 vg/ml至5.27E+09 vg/ml。

在同時存在突變S2A、P466T和T711S (AAV5-07Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到6.2倍，從2.51E+09 vg/ml至1.56E+10 vg/ml。

在同時存在突變S2A、S594Q和T711S (AAV5-08Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到5.9倍，從2.51E+09 vg/ml至1.48E+10 vg/ml。

在同時存在突變S2A、T614L和T711S (AAV5-09Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到6.8倍，從2.51E+09 vg/ml至1.71E+10 vg/ml。

在同時存在突變S2A、N679K和T711S (AAV5-10Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到3.3倍，從2.51E+09 vg/ml至8.28E+09 vg/ml。

在同時存在突變S2A、P723V和T711S (AAV5-11Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到5.0倍，從2.51E+09 vg/ml至1.26E+10 vg/ml。

在同時存在突變S2A、V431Y和T711S (AAV5-12Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到2.4倍，從2.51E+09 vg/ml至6.03E+09 vg/ml。

在同時存在突變S2A、A616D和T711S (AAV5-13Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到3.6倍，從2.51E+09 vg/ml至9.04E+09 vg/ml。

在同時存在突變S2A、W683R和T711S (AAV5-14Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到4.6倍，從2.51E+09 vg/ml至1.15E+10 vg/ml。

在同時存在突變S2A、S222A和T711S (AAV5-15Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到1.5倍，從2.51E+09 vg/ml至3.77E+09 vg/ml。

在同時存在突變S2A、R285L和T711S (AAV5-16Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到2.5倍，從2.51E+09 vg/ml至6.28E+09 vg/ml。

在同時存在突變S2A、Q340A和T711S (AAV5-17Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到1.8倍，從2.51E+09 vg/ml至4.52E+09 vg/ml。

在同時存在突變S2A、S420F和T711S (AAV5-18Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到4.4倍，從2.51E+09 vg/ml至1.10E+10 vg/ml。

在同時存在突變S2A、S680A和T711S (AAV5-19Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到3.0倍，從2.51E+09 vg/ml至7.53E+09 vg/ml。

在同時存在突變S2A、T721V和T711S (AAV5-20Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組數量增加到5.2倍，從2.51E+09 vg/ml至1.31E+10 vg/ml。

在存在單突變G226A (AAV5-21Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到3,9倍，從2,74E+09 vg/ml至1,07E+10 vg/ml。

在存在單突變D286E (AAV5-22Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,4倍，從2,74E+09 vg/ml至6,58E+09 vg/ml。

在存在單突變L341Y (AAV5-23Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,7倍，從2,74E+09 vg/ml至7,40E+09 vg/ml。

在存在單突變C387V (AAV5-24Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到4,8倍，從2,74E+09 vg/ml至1,32E+10 vg/ml。

在存在單突變Q421H (AAV5-25Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到3,2倍，從2,74E+09 vg/ml至8,77E+09 vg/ml。

在存在單突變P466T (AAV5-26Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,7倍，從2,74E+09 vg/ml至4,66E+09 vg/ml。

在存在單突變S594Q (AAV5-27Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到3,1倍，從2,74E+09 vg/ml至8,50E+09 vg/ml。

在存在單突變T614L (AAV5-28Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,5倍，從2,74E+09 vg/ml至4,11E+09 vg/ml。

在存在單突變N679K (AAV5-29Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,5倍，從2,74E+09 vg/ml至6,85E+09 vg/ml。

在存在單突變P723V (AAV5-30Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到3,2倍，從2,74E+09 vg/ml至8,77E+09 vg/ml。

在存在單突變V431Y (AAV5-31Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,4倍，從2,74E+09 vg/ml至6,58E+09 vg/ml。

在存在單突變A616D (AAV5-32Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,6倍，從2,74E+09 vg/ml至4,39E+09 vg/ml。

在存在單突變W683R (AAV5-33Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,3倍，從2,74E+09 vg/ml至3,56E+09 vg/ml。

在存在單突變S222A (AAV5-34Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,8倍，從2,74E+09 vg/ml至4,93E+09 vg/ml。

在存在單突變R285L (AAV5-35Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,1倍，從2,74E+09 vg/ml至5,76E+09 vg/ml。

在存在單突變Q340A (AAV5-36Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到1,4倍，從2,74E+09 vg/ml至3,84E+09 vg/ml。

在存在單突變S420F (AAV5-37Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到3,5倍，從2,74E+09 vg/ml至9,59E+09 vg/ml。

在存在單突變S680A (AAV5-38Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到2,2倍，從2,74E+09 vg/ml至6,03E+09 vg/ml。

在存在單突變T721V (AAV5-39Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，包裝病毒基因組的量增加到5,1倍，從2,74E+09 vg/ml至1,40E+10 vg/ml。

**實施例12. 基於修飾的腺相關病毒血清型9(rAAV9)產生載體的效率**

為了產生具有修飾的血清型9衣殼的rAAV顆粒，使用聚乙烯亞胺(PEI，線性，MW25000，Polyscinces, Inc.)同時用以下3種質粒轉染HEK293生產細胞：

1) 用包含腺病毒核苷酸序列的質粒(輔助質粒)，所述核苷酸序列編碼組裝rAAV顆粒所需的蛋白質和RNA；

2) 用包含腺相關病毒血清型2的Rep基因的天然核苷酸序列，以及修飾Cap基因序列的質粒，所述修飾Cap基因序列選自：SEQ ID NO: 241、247、253、259、265、271、277、283、289、295、301、307、313、319、325、

331、337、343、349、355、361或367的核苷酸序列或編碼具有SEQ ID No: 242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368的氨基酸序列的蛋白質VP1，以及來自所用核苷酸序列的可變閱讀框的蛋白質VP2和VP3的任何其他核苷酸序列，其中

VP2可具有SEQ ID No: 244、250、256、262、268、274、280、286、292、298、304、310、316、322、328、334、340、346、352、358、364或370的任一氨基酸序列；

並且VP3可具有SEQ ID No: 246、252、258、264、270、276、282、288、294、300、306、312、318、324、330、336、342、348、354、360、366或372的任一氨基酸序列；

3) 用包含異源rAAV顆粒基因組的質粒，該基因組編碼旨在遞送到患者細胞中的目標基因。

這組基因在72小時內提供了rAAV病毒顆粒的組裝和目標基因組在其中的包殼化。轉染後72小時，對生產細胞進行裂解(TAKARA BIO INC, Japan)以釋放rAAV顆粒，所得載體在37℃下用DNAaseI (Invitrogen, USA)處理2小時，然後在56℃下用蛋白酶K(Invitrogen, USA)再處理2小時。使用由特異於GFP序列的正向引物5'- ACCACATGAAGCAGCACGAC -3'、反向引物5'- TCAGCTCGATGCGGTTCAC -3'和探針5'- HEX-CATGCCCGAAGGCTACGTCCAG-BHQ1 -3'組成

的寡核苷酸組，通過定量PCR (TaqMan, Applied Biosystems, USA)證實所得rAAV顆粒的滴度。

本發明的作者意想不到地發現在野生型rAAV9衣殼蛋白VP1中存在一種或多種選自S232T、D297E、Q351K、Q351R、C396V、D433Y、L444R、Y478F、G604N、G627K、T625L、T625V、S692T、T733V、A427R、M635D、W695R、R296L、Q351A、Y395F、R434L、N691A的突變導致具有上述突變的rAAV載體的轉基因包裝效率顯著增加。例如，定量PCR顯示編碼目標GFP基因的包裝異源rAAV顆粒基因組的拷貝數發生變化(圖8)。

在存在突變S232T (AAV9-01Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,9倍，從6,32E+09 vg/ml至1,19E+10 vg/ml。

在存在突變D297E (AAV9-02Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到3,2倍，從6,32E+09 vg/ml至2,03E+10 vg/ml。

在存在突變Q351K (AAV9-03Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,5倍，從6,32E+09 vg/ml至9,30E+09 vg/ml。

在存在突變Q351R (AAV9-04Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-

GFP)相比，包裝病毒基因組的量增加到2,5倍，從6,32E+09 vg/ml至1,60E+10 vg/ml。

在存在突變C396V (AAV9-05Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,9倍，從6,32E+09 vg/ml至1,17E+10 vg/ml。

在存在突變D433Y (AAV9-06Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到2,9倍，從6,32E+09 vg/ml至1,85E+10 vg/ml。

在存在突變L444R (AAV9-07Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,9倍，從6,32E+09 vg/ml至1,23E+10 vg/ml。

在存在突變Y478F (AAV9-08Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到5,0倍，從6,32E+09 vg/ml至3,14E+10 vg/ml。

在存在突變G604N (AAV9-09Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到2,5倍，從6,32E+09 vg/ml至1,61E+10 vg/ml。

在存在突變G627K (AAV9-10Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-

GFP)相比，包裝病毒基因組的量增加到1,8倍，從6,32E+09 vg/ml至1,14E+10 vg/ml。

在存在突變T625L (AAV9-11Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到4,6倍，從6,32E+09 vg/ml至2,91E+10 vg/ml。

在存在突變T625V (AAV9-12Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到5,7倍，從6,32E+09 vg/ml至3,62E+10 vg/ml。

在存在突變S692T (AAV9-13Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到2,1倍，從6,32E+09 vg/ml至1,32E+10 vg/ml。

在存在突變T733V (AAV9-14Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到3,0倍，從6,32E+09 vg/ml至1,89E+10 vg/ml。

在存在突變A427R (AAV9-15Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,6倍，從6,32E+09 vg/ml至9,86E+09 vg/ml。

在存在突變M635D (AAV9-16Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-

GFP)相比，包裝病毒基因組的量增加到1,9倍，從6,32E+09 vg/ml至1,21E+10 vg/ml。

在存在突變W695R (AAV9-17Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到1,5倍，從6,32E+09 vg/ml至9,41E+09 vg/ml。

在存在突變R296L (AAV9-18Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到3,0倍，從6,32E+09 vg/ml至1,93E+10 vg/ml。

在存在突變Q351A (AAV9-19Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到2,0倍，從6,32E+09 vg/ml至1,29E+10 vg/ml。

在存在突變Y395F (AAV9-20Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到4,6倍，從6,32E+09 vg/ml至2,91E+10 vg/ml。

在存在突變R434L (AAV9-21Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，包裝病毒基因組的量增加到4,0倍，從6,32E+09 vg/ml至2,50E+10 vg/ml。

在存在突變N691A (AAV9-22Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-

GFP)相比，包裝病毒基因組的量增加到4,5倍，從6,32E+09 vg/ml至2,85E+10 vg/ml。

**實施例13. 用在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中包含點突變的基於rAAV5的產物提高細胞轉導效率。**

實驗設計：

將SK-Hep1細胞接種到12孔板的孔中。將細胞接種到補充有穀氨醯胺、10%牛血清的EMEM生長培養基中。細胞接種密度為10,000個細胞/$cm^2$。在轉導運行期間，預先製備的細胞以1,250vg/細胞的MOI進行轉導。所有樣品一式三份運行。完整細胞用作陰性對照。

使用Guava EasyCyte流式細胞儀和GuavaSoft軟體通過GFP報告蛋白信號的強度測量轉導效率。

本發明的作者意想不到地發現在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中存在一種或多種選自G226A、D286E、L341Y、C387V、Q421H、P466T、S594Q、T614L、N679K、P723V、V431Y、A616D、W683R、S222A、R285L、Q340A、S420F、S680A、T721V的突變導致具有上述突變的rAAV載體的轉基因遞送效率在統計學上顯著增加。例如，流式細胞術顯示，在用具有野生型AAV5衣殼蛋白VP1或攜帶一種或多種選自以下突變的野生型AAV5衣殼蛋白VP1：G226A、D286E、L341Y、C387V、Q421H、

P466T、S594Q、T614L、N679K、P723V、V431Y、A616D、W683R、S222A、R285L、Q340A、S420F、S680A、T721V(在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中)的基於rAAV的產物轉導SK-Hep1系後48小時GFP陽性細胞量變化(圖9、圖10)。

在同時存在突變S2A和T711S (AAV5-01Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.16倍，從20.11%至43.44%。

在同時存在突變S2A、G226A和T711S (AAV5-02Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.02倍，從20.11%至40.69%。

在同時存在突變S2A、D286E和T711S (AAV5-03Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.90倍，從20.11%至38.17%。

在同時存在突變S2A、L341Y和T711S (AAV5-04Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.75倍，從20.11%至35.27%。

在同時存在突變S2A、C387V和T711S (AAV5-05Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照

AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.10倍，從20.11%至42.22%。

在同時存在突變S2A、Q421H和T711S (AAV5-06Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.64倍，從20.11%至32.97%。

在同時存在突變S2A、P466T和T711S (AAV5-07Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.41倍，從20.11%至28.35%。

在同時存在突變S2A、S594Q和T711S (AAV5-08Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.08倍，從20.11%至41.82%。

在同時存在突變S2A、T614L和T711S (AAV5-09Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.36倍，從20.11%至47.50%。

在同時存在突變S2A、N679K和T711S (AAV5-10Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.67倍，從20.11%至33.55%。

在同時存在突變S2A、P723V和T711S (AAV5-11Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照

AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.87倍，從20.11%至37.60%。

在同時存在突變S2A、V431Y和T711S (AAV5-12Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.27倍，從20.11%至25.54%。

在同時存在突變S2A、A616D和T711S (AAV5-13Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.12倍，從20.11%至42.63%。

在同時存在突變S2A、W683R和T711S (AAV5-14Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.26倍，從20.11%至25.33%。

在同時存在突變S2A、S222A和T711S (AAV5-15Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.57倍，從20.11%至31.57%。

在同時存在突變S2A、R285L和T711S (AAV5-16Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.38倍，從20.11%至27.75%。

在同時存在突變S2A、Q340A和T711S (AAV5-17Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照

AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.45倍，從20.11%至29.15%。

在同時存在突變S2A、S420F和T711S (AAV5-18Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.57倍，從20.11%至31.57%。

在同時存在突變S2A、S680A和T711S (AAV5-19Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1.68倍，從20.11%至33.78%。

在同時存在突變S2A、T721V和T711S (AAV5-20Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2.01倍，從20.11%至40.41%。

在存在單突變G226A (AAV5-21Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,29倍，從20,11%至34,10%。

在存在單突變D286E (AAV5-22Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,27倍，從20,11%至33,57%。

在存在單突變L341Y (AAV5-23Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-

GFP)相比，GFP表達細胞的量增加到1,41倍，從20,11%至37,08%。

在存在單突變C387V (AAV5-24Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,85倍，從20,11%至48,62%。

在存在單突變Q421H (AAV5-25Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,52倍，從20,11%至40,05%。

在存在單突變P466T (AAV5-26Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,33倍，從20,11%至34,94%。

在存在單突變S594Q (AAV5-27Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,60倍，從20,11%至42,26%。

在存在單突變T614L (AAV5-28Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,26倍，從20,11%至33,16%。

在存在單突變N679K (AAV5-29Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-

GFP)相比，GFP表達細胞的量增加到1,24倍，從20,11%至32,78%。

在存在單突變P723V (AAV5-30Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,58倍，從20,11%至41,67%。

在存在單突變V431Y (AAV5-31Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,27倍，從20,11%至33,46%。

在存在單突變A616D (AAV5-32Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,46倍，從20,11%至38,47%。

在存在單突變W683R (AAV5-33Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,52倍，從20,11%至40,05%。

在存在單突變S222A (AAV5-34Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,34倍，從20,11%至35,31%。

在存在單突變R285L (AAV5-35Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-

GFP)相比，GFP表達細胞的量增加到1,55倍，從20,11%至40,84%。

在存在單突變Q340A (AAV5-36Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,44倍，從20,11%至37,94%。

在存在單突變S420F (AAV5-37Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,63倍，從20,11%至42,95%。

在存在單突變S680A (AAV5-38Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到1,71倍，從20,11%至45,06%。

在存在單突變T721V (AAV5-39Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-GFP)相比，GFP表達細胞的量增加到2,06倍，從20,11%至54,40%。

**實施例14. 用在野生型rAAV9衣殼蛋白VP1中包含點突變的基於rAAV9的產物提高細胞轉導效率。**

實驗設計：

將HeLa細胞接種到12孔板的孔中。將細胞接種到補充有穀氨醯胺、10%牛血清的EMEM生長培養基中。細胞接

種密度為10,000個細胞/$cm^2$。在轉導運行期間，預先製備的細胞以100,000vg/細胞的MOI進行轉導。所有樣品一式三份運行。完整細胞用作陰性對照。

使用Guava EasyCyte流式細胞儀和GuavaSoft軟體通過GFP報告蛋白信號的強度測量轉導效率。

本發明的作者意想不到地發現在野生型rAAV9衣殼蛋白VP1中存在一種或多種選自S232T、D297E、Q351K、Q351R、C396V、D433Y、L444R、Y478F、G604N、G627K、T625L、T625V、S692T、T733V、A427R、M635D、W695R、R296L、Q351A、Y395F、R434L、N691A的突變導致具有上述突變的rAAV載體的轉基因遞送效率顯著提高。例如，流式細胞術顯示，在用具有野生型AAV9衣殼蛋白VP1或攜帶一種或多種選自以下突變的野生型AAV9衣殼蛋白VP1：S232T、D297E、Q351K、Q351R、C396V、D433Y、L444R、Y478F、G604N、G627K、T625L、T625V、S692T、T733V、A427R、M635D、W695R、R296L、Q351A、Y395F、R434L、N691A(在野生型rAAV9衣殼蛋白VP1中)的基於rAAV的產物轉導HeLa系後48小時GFP陽性細胞量變化(圖11)。

在存在突變S232T (AAV9-01Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,21倍，從27,53%至33,31%。

在存在突變D297E (AAV9-02Mut-GFP)的情況下，與

具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,25倍，從27,53%至34,41%。

在存在突變Q351K (AAV9-03Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,30倍，從27,53%至35,87%。

在存在突變Q351R (AAV9-04Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,16倍，從27,53%至31,93%。

在存在突變C396V (AAV9-05Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,37倍，從27,53%至37,74%。

在存在突變D433Y (AAV9-06Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,10倍，從27,53%至30,28%。

在存在突變L444R (AAV9-07Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,40倍，從27,53%至38,45%。

在存在突變Y478F (AAV9-08Mut-GFP)的情況下，與

具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,56倍，從27,53%至42,95%。

在存在突變G604N (AAV9-09Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,25倍，從27,53%至34,32%。

在存在突變G627K (AAV9-10Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,24倍，從27,53%至34,14%。

在存在突變T625L (AAV9-11Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,45倍，從27,53%至39,92%。

在存在突變T625V (AAV9-12Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,49倍，從27,53%至41,02%。

在存在突變S692T (AAV9-13Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,38倍，從27,53%至37,99%。

在存在突變T733V (AAV9-14Mut-GFP)的情況下，與

具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,44倍，從27,53%至39,73%。

在存在突變A427R (AAV9-15Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,23倍，從27,53%至33,86%。

在存在突變M635D (AAV9-16Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,22倍，從27,53%至33,59%。

在存在突變W695R (AAV9-17Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,47倍，從27,53%至40,47%。

在存在突變R296L (AAV9-18Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,26倍，從27,53%至34,69%。

在存在突變Q351A (AAV9-19Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,35倍，從27,53%至37,17%。

在存在突變Y395F (AAV9-20Mut-GFP)的情況下，與

具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,48倍，從27,53%至40,74%。

在存在突變R434L (AAV9-21Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,67倍，從27,53%至45,91%。

在存在突變N691A (AAV9-22Mut-GFP)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-GFP)相比，GFP表達細胞的量增加到1,72倍，從27,53%至47,47%。

**實施例15. 用在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中包含單突變的基於rAAV5的產物進行細胞轉導後增加由轉基因編碼的目標蛋白的產量。**

實驗設計：

將SK-Hep1細胞接種到12孔板的孔中。將細胞接種到補充有穀氨醯胺、10%牛血清的EMEM生長培養基中。細胞接種密度為10,000個細胞/$cm^2$。在轉導運行期間，預先製備的細胞以10,000vg/細胞的MOI進行轉導。所有樣品一式三份運行。完整細胞用作陰性對照。

使用Human Factor IX ELISA試劑盒評估轉導後7天培養液中FIX蛋白的量。我們使用1:50稀釋的樣品。該程式

根據製造商的說明進行。

本發明的作者意想不到地發現在野生型rAAV5衣殼蛋白VP1或已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中存在一種或多種選自G226A、D286E、L341Y、C387V、Q421H、P466T、S594Q、T614L、N679K、P723V、V431Y、A616D、W683R、S222A、R285L、Q340A、S420F、S680A、T721V的突變導致在用具有上述突變的rAAV5載體轉導SK-Hep1細胞後FIX蛋白的產生顯著增加。例如，酶聯免疫測定法(EIA)顯示，在用具有野生型AAV5衣殼蛋白VP1或攜帶一種或多種選自以下突變的野生型AAV5衣殼蛋白VP1：G226A、D286E、L341Y、C387V、Q421H、P466T、S594Q、T614L、N679K、P723V、V431Y、A616D、W683R、S222A、R285L、Q340A、S420F、S680A、T721V(在野生型rAAV5衣殼蛋白VP1或在已經包含突變S2A和T711S的rAAV5衣殼蛋白VP1中)的基於rAAV的產物轉導SK-Hep1細胞後7天，培養基中FIX蛋白的量增加(圖12、圖13)。

在同時存在突變的情況下S2A、T711S (AAV5-01Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到2.76倍，從628.67 ng/ml至1736.67 ng/ml。

在同時存在突變的情況下S2A、G226A和T711S (AAV5-02Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加

到1.85倍，從628.67 ng/ml至1161.83 ng/ml。

在同時存在突變的情況下S2A、D286E和T711S(AAV5-03Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.71倍，從628.67 ng/ml至1075.02 ng/ml。

在同時存在突變的情況下S2A、L341Y和T711S(AAV5-04Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.65倍，從628.67 ng/ml至1037.30 ng/ml。

在同時存在突變的情況下S2A、C387V和T711S(AAV5-05Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到2.28倍，從628.67 ng/ml至1433.36 ng/ml。

在同時存在突變的情況下S2A、Q421H和T711S(AAV5-06Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.67倍，從628.67 ng/ml至1049.87 ng/ml。

在同時存在突變的情況下S2A、P466T和T711S(AAV5-07Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.21倍，從628.67 ng/ml至763.33 ng/ml。

在同時存在突變的情況下S2A、S594Q和T711S(AAV5-08Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加

到1.26倍，從628.67 ng/ml至792.12 ng/ml。

在同時存在突變的情況下S2A、T614L和T711S(AAV5-09Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到2.17倍，從628.67 ng/ml至1362.67 ng/ml。

在同時存在突變的情況下S2A、N679K和T711S(AAV5-10Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.98倍，從628.67 ng/ml至1244.76 ng/ml。

在同時存在突變的情況下S2A、P723V和T711S(AAV5-11Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.69倍，從628.67 ng/ml至1062.45 ng/ml。

在同時存在突變的情況下S2A、V431Y和T711S(AAV5-12Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.12倍，從628.67 ng/ml至704.11 ng/ml。

在同時存在突變的情況下S2A、A616D和T711S(AAV5-13Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到2.15倍，從628.67 ng/ml至1351.63 ng/ml。

在同時存在突變的情況下S2A、W683R和T711S(AAV5-14Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加

到1.08倍，從628.67 ng/ml至678.96 ng/ml。

在同時存在突變的情況下S2A、S222A和T711S(AAV5-15Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.30倍，從628.67 ng/ml至817.27 ng/ml。

在同時存在突變的情況下S2A、R285L和T711S(AAV5-16Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.16倍，從628.67 ng/ml至729.25 ng/ml。

在同時存在突變的情況下S2A、Q340A和T711S(AAV5-17Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.36倍，從628.67 ng/ml至854.99 ng/ml。

在同時存在突變的情況下S2A、S420F和T711S(AAV5-18Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.19倍，從628.67 ng/ml至745.00 ng/ml。

在同時存在突變的情況下S2A、S680A和T711S(AAV5-19Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1.52倍，從628.67 ng/ml至955.57 ng/ml。

在同時存在突變的情況下S2A、T721V和T711S(AAV5-20Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加

到1.79倍，從628.67 ng/ml至1125.00 ng/ml。

在存在單突變G226A (AAV5-21Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,56倍，從653,19 ng/ml至1020,59 ng/ml。

在存在單突變D286E (AAV5-22Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,63倍，從653,19 ng/ml至1063,78 ng/ml。

在存在單突變L341Y (AAV5-23Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,95倍，從653,19 ng/ml至1271,00 ng/ml。

在存在單突變C387V (AAV5-24Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,81倍，從653,19 ng/ml至1184,04 ng/ml。

在存在單突變Q421H (AAV5-25Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,92倍，從653,19 ng/ml至1254,12 ng/ml。

在存在單突變P466T (AAV5-26Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,73倍，從653,19 ng/ml至

1130,02 ng/ml。

在存在單突變S594Q (AAV5-27Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,88倍，從653,19 ng/ml至1228,00 ng/ml。

在存在單突變T614L (AAV5-28Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,40倍，從653,19 ng/ml至915,81 ng/ml。

在存在單突變N679K (AAV5-29Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,21倍，從653,19 ng/ml至790,36 ng/ml。

在存在單突變P723V (AAV5-30Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,35倍，從653,19 ng/ml至881,81 ng/ml。

在存在單突變V431Y (AAV5-31Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,34倍，從653,19 ng/ml至875,27 ng/ml。

在存在單突變A616D (AAV5-32Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,11倍，從653,19 ng/ml至

727,75 ng/ml。

在存在單突變W683R (AAV5-33Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,57倍，從653,19 ng/ml至1025,51 ng/ml。

在存在單突變S222A (AAV5-34Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,78倍，從653,19 ng/ml至1162,68 ng/ml。

在存在單突變R285L (AAV5-35Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,47倍，從653,19 ng/ml至960,19 ng/ml。

在存在單突變Q340A (AAV5-36Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,64倍，從653,19 ng/ml至1071,23 ng/ml。

在存在單突變S420F (AAV5-37Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,24倍，從653,19 ng/ml至809,96 ng/ml。

在存在單突變S680A (AAV5-38Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,37倍，從653,19 ng/ml至

894,87 ng/ml。

在存在單突變T721V (AAV5-39Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV5(AAV5-NullMut-FIX)相比，蛋白產生量增加到1,96倍，從653,19 ng/ml至1280,25 ng/ml。

**實施例16.在用包含野生型rAAV9衣殼蛋白VP1突變的基於rAAV9的產物轉導細胞後增加由轉基因編碼的目標蛋白的產量。**

實驗設計：

將CHO-K1-S細胞接種到12孔板的孔中。接種於以下生長培養基：DMEM/F12，添加穀氨醯胺，葡萄糖含量為4.5g/l，5%牛血清。細胞接種密度為10,000個細胞/$cm^2$。在轉導運行期間，預先製備的細胞以200,000vg/細胞的MOI進行轉導。所有樣品一式三份運行。完整細胞用作陰性對照。

使用Human Factor IX ELISA試劑盒評估轉導後7天培養液中FIX蛋白的量。我們使用1:25稀釋的樣品。該程式根據製造商的說明進行。

本發明的作者意想不到地發現在野生型rAAV9衣殼蛋白VP1中存在一種或多種選自以下的突變：S232T、D297E、Q351K、Q351R、C396V、D433Y、L444R、Y478F、G604N、G627K、T625L、T625V、S692T、T733V、A427R、M635D、W695R、R296L、Q351A、

Y395F、R434L、N691A導致在用具有上述突變的rAAV載體轉導CHO-K1-S細胞後，FIX蛋白的產生顯著增加。例如，酶聯免疫測定法(EIA)顯示，在用攜帶一種或多種選自以下突變的野生型AAV9衣殼蛋白VP1：S232T、D297E、Q351K、Q351R、C396V、D433Y、L444R、Y478F、G604N、G627K、T625L、T625V、S692T、T733V、A427R、M635D、W695R、R296L、Q351A、Y395F、R434L、N691A轉導CHO-K1-S細胞後7天，培養基中FIX蛋白的量增加(圖14)。

在存在突變S232T (AAV9-01Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,18倍，從947,5 ng/ml至1118,05 ng/ml。

在存在突變D297E (AAV9-02Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,20倍，從947,5 ng/ml至1137,00 ng/ml。

在存在突變Q351K (AAV9-03Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,26倍，從947,5 ng/ml至1195,00 ng/ml。

在存在突變Q351R (AAV9-04Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,22倍，從947,5 ng/ml至

1157,17 ng/ml。

在存在突變C396V (AAV9-05Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,64倍，從947,5 ng/ml至1554,17 ng/ml。

在存在突變D433Y (AAV9-06Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,17倍，從947,5 ng/ml至1110,50 ng/ml。

在存在突變L444R (AAV9-07Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,20倍，從947,5 ng/ml至1141,50 ng/ml。

在存在突變Y478F (AAV9-08Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到2,01倍，從947,5 ng/ml至1908,50 ng/ml。

在存在突變G604N (AAV9-09Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,26倍，從947,5 ng/ml至1197,67 ng/ml。

在存在突變G627K (AAV9-10Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,23倍，從947,5 ng/ml至

1166,67 ng/ml。

在存在突變T625L (AAV9-11Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,77倍，從947,5 ng/ml至1677,50 ng/ml。

在存在突變T625V (AAV9-12Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,83倍，從947,5 ng/ml至1736,67 ng/ml。

在存在突變S692T (AAV9-13Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,55倍，從947,5 ng/ml至1464,83 ng/ml。

在存在突變T733V (AAV9-14Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,55倍，從947,5 ng/ml至1469,50 ng/ml。

在存在突變A427R (AAV9-15Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,65倍，從947,5 ng/ml至1563,38 ng/ml。

在存在突變M635D (AAV9-16Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,70倍，從947,5 ng/ml至

1607,00 ng/ml。

在存在突變W695R (AAV9-17Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到2,32倍，從947,5 ng/ml至2201,67 ng/ml。

在存在突變R296L (AAV9-18Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,23倍，從947,5 ng/ml至1161,83 ng/ml。

在存在突變Q351A (AAV9-19Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,48倍，從947,5 ng/ml至1402,30 ng/ml。

在存在突變Y395F (AAV9-20Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,21倍，從947,5 ng/ml至1145,00 ng/ml。

在存在突變R434L (AAV9-21Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到1,54倍，從947,5 ng/ml至1456,83 ng/ml。

在存在突變N691A (AAV9-22Mut-FIX)的情況下，與具有野生型衣殼蛋白VP1的對照AAV9(AAV9-NullMut-FIX)相比，蛋白產生量增加到2,33倍，從947,5 ng/ml至

2203,33 ng/ml。

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# 【發明申請專利範圍】

【請求項1】一種產生修飾的AAV衣殼的方法，包括：

a)確定修飾的AAV的殼粒蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的修飾的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換。

【請求項2】根據請求項1所述的產生修飾的AAV衣殼的方法，進一步包括檢查步驟f)中產生的具有一個或多個氨基酸置換的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，其中一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

【請求項3】根據請求項1所述的產生修飾的AAV衣殼的方法，進一步包括檢查步驟f)中產生的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，並選擇與不含這些修飾的AAV衣殼相比具有一種或多種改進的性質的修飾的AAV衣殼，其中所述一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的

高效產生(組裝)而提高生成效率。

【請求項4】根據請求項1所述的產生AAV衣殼的方法，其中所述修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

【請求項5】根據請求項1所述的產生AAV衣殼的方法，其中所述修飾的AAV衣殼選自以下AAV血清型：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15或AAV16。

【請求項6】根據請求項1所述的產生AAV衣殼的方法，其中所述結構相似的範本衣殼選自：細小病毒B19、人博卡病毒1(HBoV1)、牛細小病毒(BVP)。

【請求項7】一種用於產生基於重組腺相關病毒的病毒載體的修飾的AAV衣殼，所述修飾的AAV衣殼通過包括以下的方法產生：

a)確定修飾的AAV的殼粒蛋白中的氨基酸，這些氨基酸位於衣殼的相鄰五聚體亞單位的相互作用介面區域；

b)將修飾的AAV衣殼與範本衣殼進行結構比對，以確定修飾AAV的來自相鄰五聚體亞單位之間的相互作用介面區域的每個氨基酸與範本衣殼中與其最接近的氨基酸之間的成對對應性，作為被認為是潛在置換的初始氨基酸的結構類似物，

其中範本衣殼被理解為是指結構相似的病毒衣殼，所述病毒選自除AAV之外的細小病毒科；

c)成對比較步驟b)中確定的AAV衣殼和範本衣殼的氨基酸殘基，以鑒定修飾的AAV衣殼和範本衣殼在衣殼的相鄰五聚體亞單位的相互作用介面區域中的結構差異；

d)選擇誘變位置，其中用於誘變的氨基酸殘基位於修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域，並且在衣殼的相鄰五聚體亞單位的相互作用介面區域中在修飾的AAV衣殼與範本衣殼之間具有結構差異；

e)使用以下原則之一在步驟d)中選擇的位置選擇用於誘變的氨基酸殘基：

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為具有更大體積的除半胱氨酸和甲硫氨酸之外的氨基酸；

-將修飾的AAV的殼粒蛋白中的初始氨基酸置換為在殼粒之間具有增加或減少的極性接觸數量的除半胱氨酸和甲硫氨酸之外的氨基酸；

f)在修飾的AAV衣殼的相鄰五聚體亞單位的相互作用介面區域中引入在步驟d)-e)中選擇的一個或多個氨基酸置換；

並且，如有必要，還包括

g)檢查步驟f)中產生的修飾的AAV衣殼與不含這些修飾的AAV衣殼相比是否存在一種或多種改進的性質，並選擇與不含這些修飾的AAV衣殼相比具有一種或多種改進的性質的修飾的AAV衣殼，其中所述一種或多種改進的性質選自：

-提高細胞轉導效率，

-增加目標蛋白的產量，

-由於基於重組腺相關病毒(rAAV)的包殼病毒載體的高效產生(組裝)而提高生成效率。

【請求項8】根據請求項7所述的修飾的AAV衣殼，其中所述修飾的AAV衣殼選自：人AAV、猿AAV或禽AAV。

【請求項9】根據請求項7所述的修飾的AAV衣殼，其中所述修飾的AAV衣殼選自以下AAV血清型：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV13、AAV14、AAV15或AAV16。

【請求項10】一種用於產生基於重組腺相關病毒的病毒載體的修飾的AAV衣殼，其包含具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368。

【請求項11】根據請求項10所述的修飾的AAV衣殼，其包含：

a)具有選自下組的氨基酸序列的修飾的AAV衣殼蛋白VP1：SEQ ID No: 14、20、26、32、38、44、50、56、62、68、74、80、86、92、98、104、110、116、122、

128、134、140、146、152、158、164、170、176、182、188、194、200、206、212、218、224、230、236、242、248、254、260、266、272、278、284、290、296、302、308、314、320、326、332、338、344、350、356、362或368；

b)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP2，所述修飾的AAV衣殼蛋白VP2具有選自下組的氨基酸序列：SEQ ID No: 16、22、28、34、40、46、52，58、64、70、76、82、88、94、100、106、112、118、124、130、136、142、148、154、160、166、172、178、184、190、196、202、208、214、220、226、232、238、244、250、256、262、268、274、280、286、292、298、304、310、316、322、328、334、340、346、352、358、364或370；

c)對應於其蛋白VP1的修飾的AAV衣殼蛋白VP3，所述修飾的AAV衣殼蛋白VP3具有選自下組的氨基酸序列：SEQ ID No: 18、24、30、36、42、48、54，60、66、72、78、84、90、96、102、108、114、120、126、132、138、144、150、156、162、168、174、180、186、192、198、204、210、216、222、228、234、240、246、252、258、264、270、276、282、288、294、300、306、312、318、324、330、336、342、348、354、360、366或372。

【請求項12】一種分離的核酸，其編碼根據請求項7至11所述的修飾的衣殼。

【請求項13】一種用於向受試者遞送異源核酸序列的

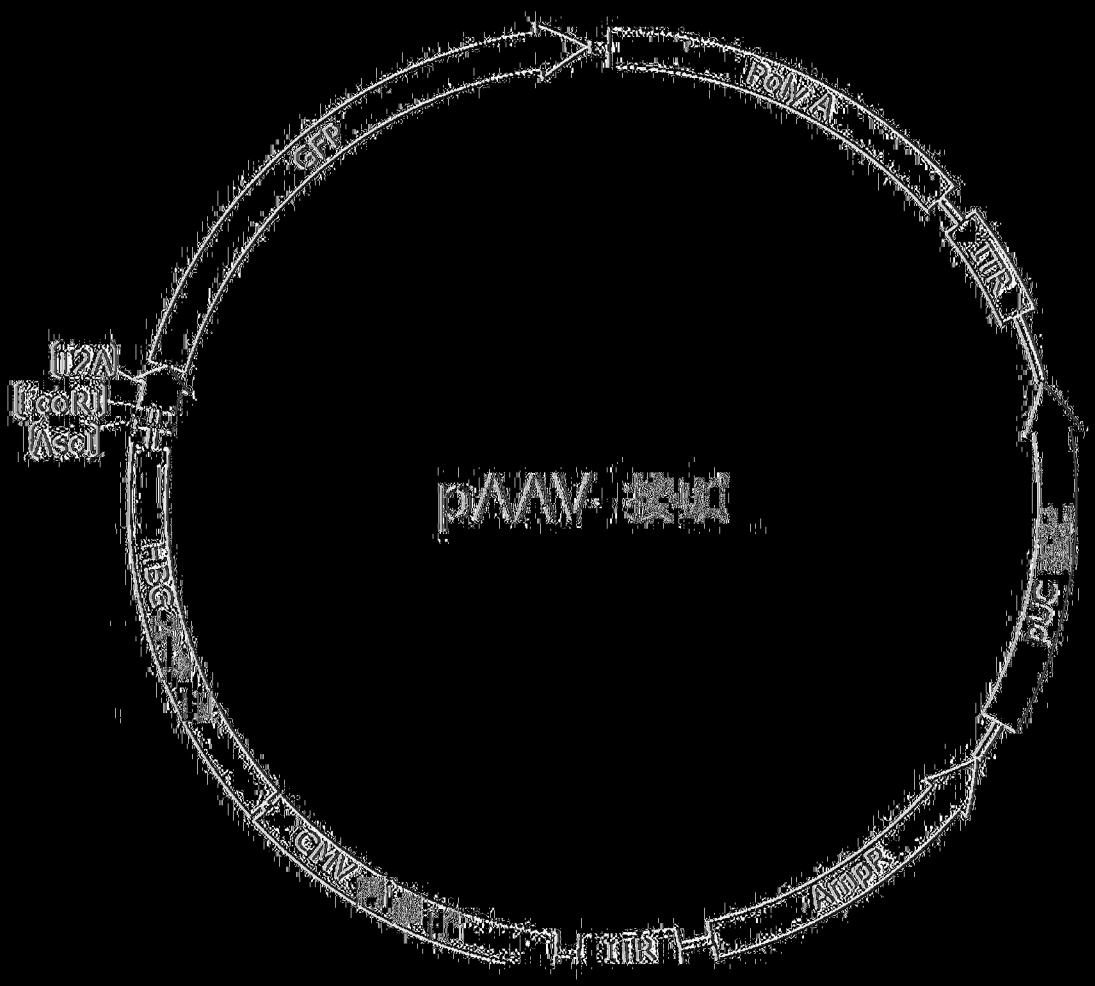
基於重組腺相關病毒的載體，所述載體包含：

1)根據請求項7至11中任一項所述的修飾的AAV衣殼，和

2)異源核酸序列，其包含促進由異源核酸序列編碼的產物在靶細胞中表達的調節序列。

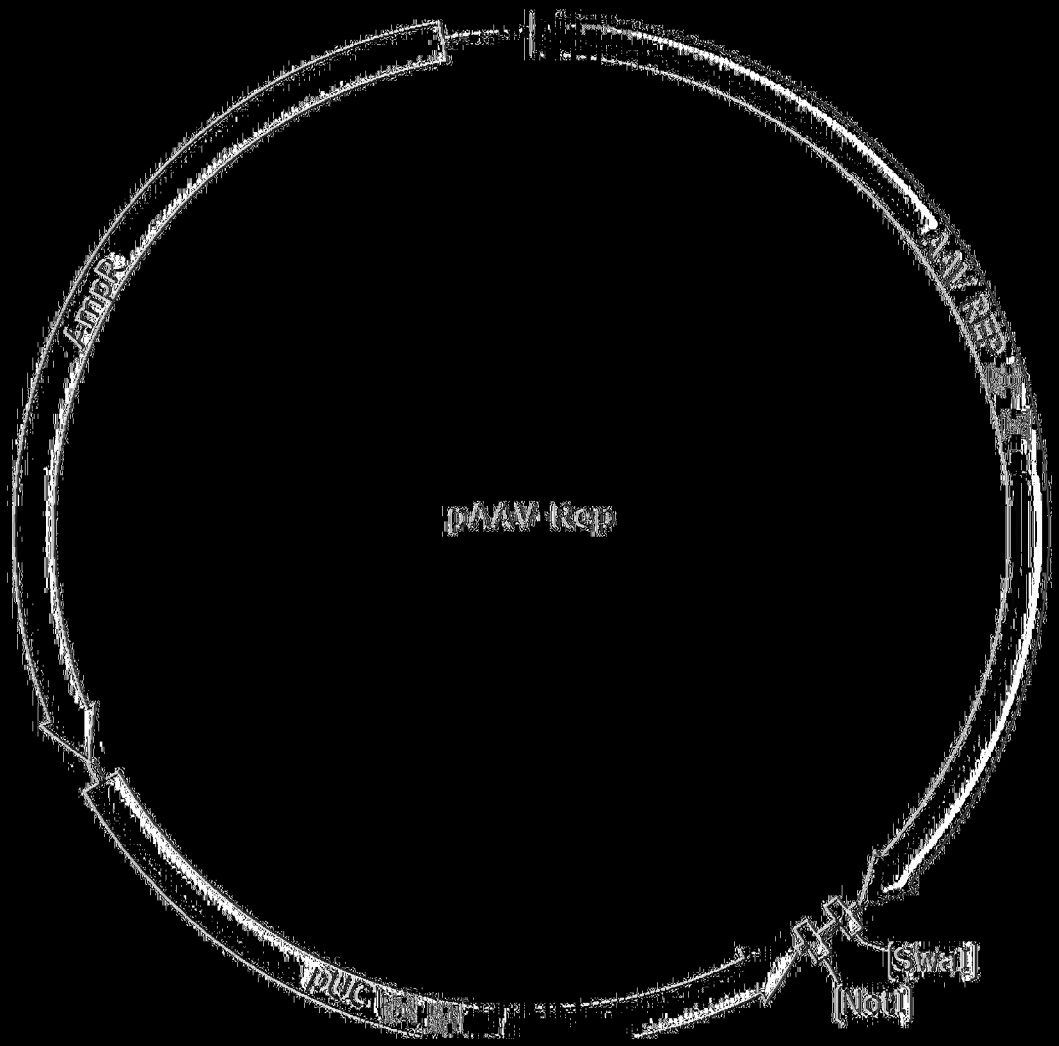
【請求項14】根據請求項13所述的基於重組腺相關病毒的載體，其中所述異源核酸序列的表達產物是治療性多肽或報告多肽。

# [(發明圖式)]

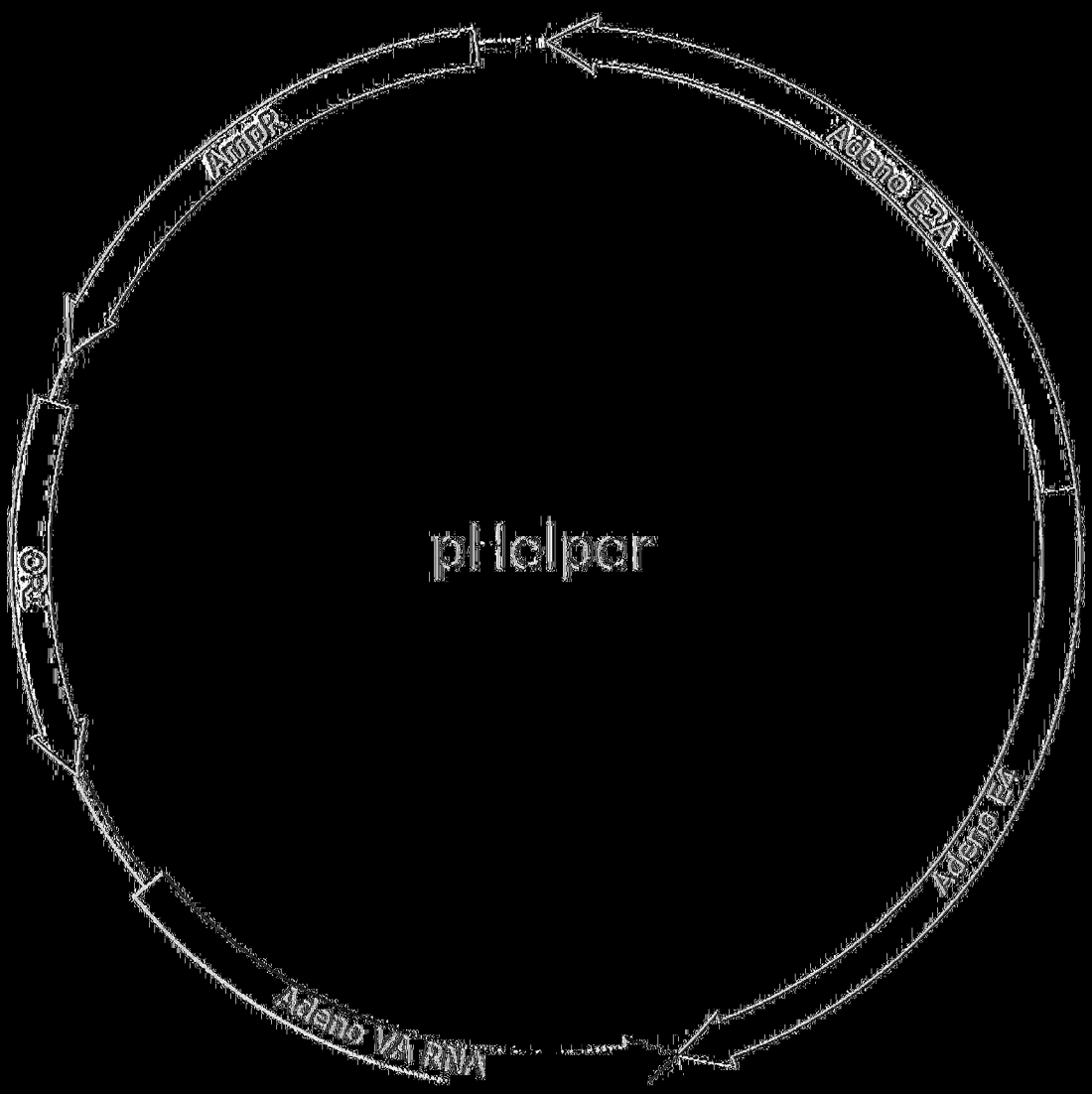



[(圖 1)]

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圖(圖 2)




|(圖 3)|




圖 4




圖 5

圖 6

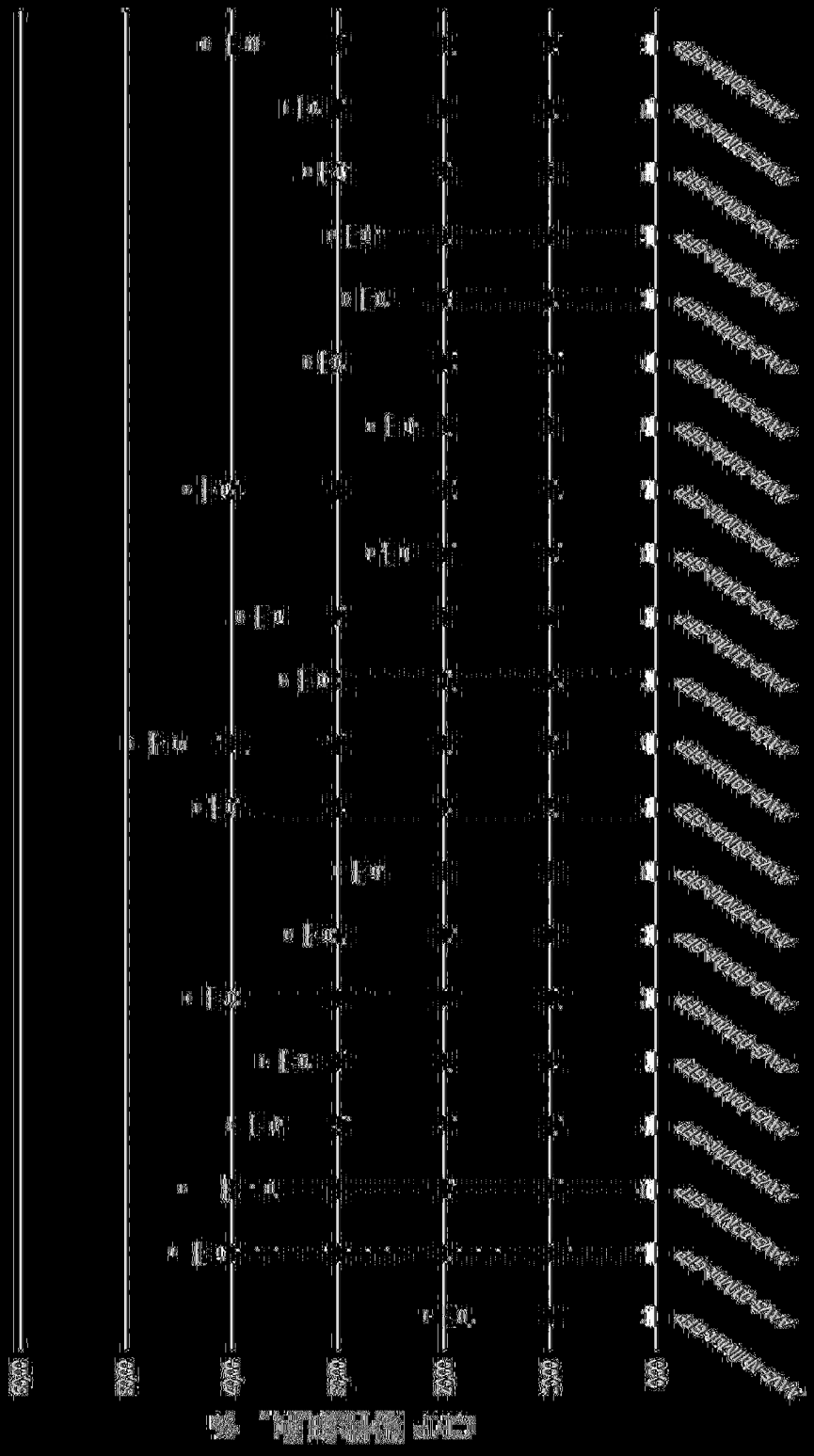
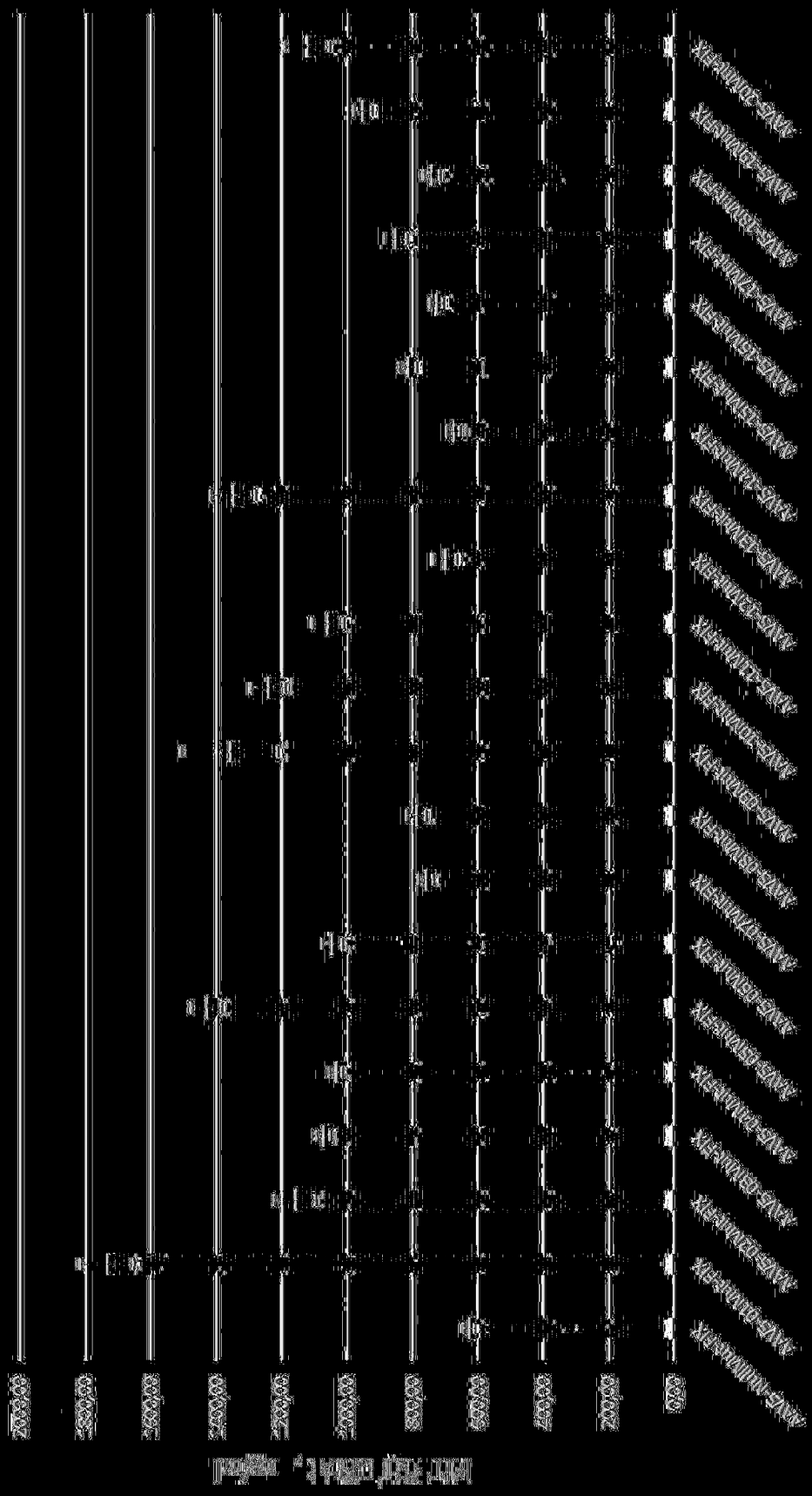


圖 6

圖 3



(圖2)

