(19) 대한민국특허청(KR)
(12) 등록특허공보(B1)

(45) 공고일자 2024년08월22일
(11) 등록번호 10-2697811
(24) 등록일자 2024년08월19일

(51) 국제특허분류(Int. Cl.)
C12N 15/86 (2006.01) *A61K 48/00* (2006.01)
A61P 25/28 (2006.01) *C12N 9/24* (2006.01)
(52) CPC특허분류
C12N 15/86 (2013.01)
A61K 48/005 (2013.01)
(21) 출원번호 10-2020-7012190
(22) 출원일자(국제) 2018년10월03일
심사청구일자 2021년10월01일
(85) 번역문제출일자 2020년04월27일
(65) 공개번호 10-2020-0074132
(43) 공개일자 2020년06월24일
(86) 국제출원번호 PCT/US2018/054227
(87) 국제공개번호 WO 2019/070894
국제공개일자 2019년04월11일
(30) 우선권주장
62/567,311 2017년10월03일 미국(US)
(뒷면에 계속)
(56) 선행기술조사문헌
EP03228690 A1
US20160222325 A1
US20170096626 A1
US20070129516 A1

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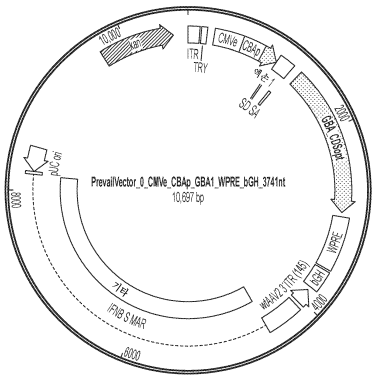
전체 청구항 수 : 총 42 항 심사관 : 손영희

(54) 발명의 명칭 **리소좀 장애를 위한 유전자 요법**

(57) 요 약

일부 측면에서, 본 개시내용은 비정상적인 리소좀 기능과 연관된 질환, 예를 들어, 파킨슨병 (PD) 및 고셰병 치료를 위한 조성물 및 방법에 관한 것이다. 일부 실시양태에서, 본 개시내용은 베타-글루코세레브로시다제 (GBA) 또는 그의 일부를 코딩하는 트랜스진을 단독으로, 또는 하나 이상의 PD 연관 유전자와 함께 조합하여 포함하는 발현 구축물을 제공한다. 일부 실시양태에서, 본 개시내용은 파킨슨병 방법을 필요로 하는 대상체에게 상기 발현 구축물을 투여함으로써 파킨슨병 방법을 제공한다.

대 표 도 - 도1



(52) CPC특허분류
A61P 25/28 (2018.01)
C12N 9/2402 (2013.01)
C12Y 302/01045 (2013.01)
C12N 2750/14143 (2013.01)
C12N 2750/14143 (2013.01)

(30) 우선권주장
62/567,310 2017년10월03일 미국(US)
62/567,301 2017년10월03일 미국(US)
62/567,319 2017년10월03일 미국(US)
62/567,296 2017년10월03일 미국(US)

명 세 서

청구범위

청구항 1

(i) 프로그래뉼린 (PGRN) 단백질을 코딩하는 트랜스진을 포함하는 발현 구축물이며, 여기서 트랜스진은 서열식별번호 68의 뉴클레오티드 서열을 포함하는 것인 발현 구축물; 및

(ii) 상기 발현 구축물을 플랭킹하는 2개의 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR) 서열이며, 여기서 제1 ITR 서열은 5' ITR이고, 제2 ITR 서열은 3' ITR인 ITR 서열

을 포함하는 단리된 핵산.

청구항 2

제1항에 있어서, 트랜스진이 프로모터에 작동가능하게 연결된 것인 단리된 핵산.

청구항 3

제2항에 있어서, 프로모터가 닭-베타 액틴 (CBA) 프로모터인 단리된 핵산.

청구항 4

제3항에 있어서, CMV 인핸서를 추가로 포함하는 단리된 핵산.

청구항 5

제1항 내지 제4항 중 어느 한 항에 있어서, 우드척 간염 바이러스 전사후 조절 요소 (WPRE)를 추가로 포함하는 단리된 핵산.

청구항 6

제1항 내지 제4항 중 어느 한 항에 있어서, 소 성장 호르몬 폴리A 신호 테일을 추가로 포함하는 단리된 핵산.

청구항 7

제1항 내지 제4항 중 어느 한 항에 있어서, 2개의 ITR 서열 각각이 야생형 AAV2 ITR 서열인 단리된 핵산.

청구항 8

제1항 내지 제4항 중 어느 한 항에 있어서, 2개의 ITR 서열 각각이 발현 구축물에 인접한 "D" 영역을 포함하고, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 단리된 핵산.

청구항 9

제1항 내지 제4항 중 어느 한 항에 있어서, ITR 서열 중 하나 이상이 발현 구축물 기준으로 ITR 서열의 외부에 위치한 "D" 영역을 포함하고, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 단리된 핵산.

청구항 10

제1항 내지 제4항 중 어느 한 항에 있어서, 발현 구축물 기준으로 5'에 위치한 ITR 서열이 발현 구축물에 인접한 "D" 영역을 포함하고, 발현 구축물 기준으로 3'에 위치한 ITR 서열이 발현 구축물 기준으로 ITR 서열의 외부에 위치한 "D" 영역을 포함하며, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 단리된 핵산.

청구항 11

제1항에 있어서, 5' ITR의 핵산 서열이 서열식별번호 69의 뉴클레오티드 1 내지 145이고, 3' ITR의 핵산 서열이 서열식별번호 69의 뉴클레오티드 4041 내지 4185인 단리된 핵산.

청구항 12

제1항에 있어서, 5' ITR과 발현 구축물 사이에 TRY 영역을 추가로 포함하며, 여기서 TRY 영역은 서열식별번호 28에 기재된 서열을 갖는 것인 단리된 핵산.

청구항 13

2개의 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR) 서열에 의해 플랭킹된, 프로그래뉼린 (PGRN) 단백질을 코딩하는 트랜스진을 포함하는 발현 구축물을 포함하는 재조합 아데노-연관 바이러스 (rAAV) 벡터이며, 여기서 트랜스진은 서열식별번호 68의 뉴클레오티드 서열을 포함하고, 제1 ITR 서열은 5' ITR이고, 제2 ITR 서열은 3' ITR인 rAAV 벡터.

청구항 14

제13항에 있어서, 트랜스진이 프로모터에 작동가능하게 연결된 것인 rAAV 벡터.

청구항 15

제14항에 있어서, 프로모터가 닭 베타 액틴 (CBA) 프로모터인 rAAV 벡터.

청구항 16

제15항에 있어서, CMV 인핸서를 추가로 포함하는 rAAV 벡터.

청구항 17

제13항 내지 제16항 중 어느 한 항에 있어서, 우드척 간염 바이러스 전사후 조절 요소 (WPRE)를 추가로 포함하는 rAAV 벡터.

청구항 18

제13항 내지 제16항 중 어느 한 항에 있어서, 소 성장 호르몬 폴리A 신호 테일을 추가로 포함하는 rAAV 벡터.

청구항 19

제13항 내지 제16항 중 어느 한 항에 있어서, 2개의 ITR 서열 각각이 야생형 AAV2 ITR 서열인 rAAV 벡터.

청구항 20

제13항 내지 제16항 중 어느 한 항에 있어서, 2개의 ITR 서열 각각이 발현 구축물에 인접한 "D" 영역을 포함하고, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 rAAV 벡터.

청구항 21

제13항 내지 제16항 중 어느 한 항에 있어서, ITR 서열 중 하나 이상이 발현 구축물 기준으로 ITR 서열의 외부에 위치한 "D" 영역을 포함하고, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 rAAV 벡터.

청구항 22

제13항 내지 제16항 중 어느 한 항에 있어서, 발현 구축물 기준으로 5'에 위치한 ITR 서열이 발현 구축물에 인접한 "D" 영역을 포함하고, 발현 구축물 기준으로 3'에 위치한 ITR 서열이 발현 구축물 기준으로 ITR 서열의 외부에 위치한 "D" 영역을 포함하며, 여기서 "D" 영역이 서열식별번호 27에 기재된 서열을 포함하는 것인 rAAV 벡터.

청구항 23

제13항에 있어서, 5' ITR의 핵산 서열이 서열식별번호 69의 뉴클레오티드 1 내지 145이고, 3' ITR의 핵산 서열이 서열식별번호 69의 뉴클레오티드 4041 내지 4185인 rAAV 벡터.

청구항 24

제13항에 있어서, 5' ITR과 발현 구축물 사이에 TRY 영역을 추가로 포함하며, 여기서 TRY 영역은 서열식별번호 28에 기재된 서열을 갖는 것인 rAAV 벡터.

청구항 25

(i) 아데노-연관 바이러스 (AAV) 캡시드 단백질; 및

(ii) 제13항 내지 제16항 중 어느 한 항의 rAAV 벡터

를 포함하는 재조합 아데노-연관 바이러스 (rAAV).

청구항 26

제25항에 있어서, AAV 캡시드 단백질이 AAV9 캡시드 단백질인 rAAV.

청구항 27

5'에서 3' 순서로:

(a) 5' 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR);

(b) CMV 인핸서;

(c) 닭 베타 액틴 (CBA) 프로모터;

(d) 서열식별번호 68의 뉴클레오티드 서열을 포함하는, 프로그래뉼린 (PGRN) 단백질을 코딩하는 트랜스진;

(e) 우드척 간염 바이러스 전사후 조절 요소 (WPRE);

(f) 소 성장 호르몬 폴리A 신호 테일; 및

(g) 3' AAV ITR

을 포함하는 핵산을 포함하는 재조합 아데노-연관 바이러스 (rAAV) 벡터.

청구항 28

(i) AAV 캡시드 단백질; 및

(ii) 제27항의 rAAV 벡터

를 포함하는 재조합 아데노-연관 바이러스 (rAAV).

청구항 29

제28항에 있어서, AAV 캡시드 단백질이 AAV9 캡시드 단백질인 rAAV.

청구항 30

제13항 내지 제16항 및 제27항 중 어느 한 항의 rAAV 벡터를 포함하는 플라스미드.

청구항 31

서열식별번호 68에 기재된 뉴클레오티드 서열을 포함하는 트랜스진을 포함하는 발현 구축물 및 상기 발현 구축물을 플랭킹하는 2개의 AAV ITR 서열을 포함하는 배큘로바이러스 벡터.

청구항 32

(i) 하나 이상의 아데노-연관 바이러스 rep 단백질 및/또는 하나 이상의 아데노-연관 바이러스 cap 단백질을 코딩하는 제1 벡터; 및

(ii) 서열식별번호 68에 기재된 뉴클레오티드 서열을 포함하는 트랜스진을 포함하는 발현 구축물 및 상기 발현 구축물을 플랭킹하는 2개의 AAV ITR 서열을 포함하는 제2 벡터

를 포함하는 단리된 세포.

청구항 33

제32항에 있어서, 제1 벡터가 플라스미드이고 제2 벡터가 플라스미드인 세포.

청구항 34

제32항 또는 제33항에 있어서, 세포가 포유동물 세포 또는 HEK293 세포인 세포.

청구항 35

제32항에 있어서, 제1 벡터가 배큘로바이러스 벡터이고 제2 벡터가 배큘로바이러스 벡터인 세포.

청구항 36

제32항에 있어서, 세포가 곤충 세포인 세포.

청구항 37

제25항의 재조합 아데노-연관 바이러스 (rAAV)를 생산하는 방법이며, 상기 방법은

(i) 하나 이상의 아데노-연관 바이러스 rep 단백질 및/또는 하나 이상의 아데노-연관 바이러스 cap 단백질을 코딩하는 제1 벡터, 및 rAAV 벡터를 인간 체내 세포를 제외한 세포에 전달하는 단계;

(ii) rAAV를 패키징하도록 허용하는 조건 하에 (i)의 세포를 배양하는 단계; 및

(iii) rAAV의 수집을 위해 배양된 숙주 세포 또는 배양 배지를 수확하는 단계

를 포함하는 방법.

청구항 38

파킨슨병을 앓거나 또는 앓을 것으로 의심되는 대상체를 치료하기 위한, 제1항 내지 제4항 중 어느 한 항의 단리된 핵산, 제13항 내지 제16항 및 제27항 중 어느 한 항의 rAAV 벡터, 또는 제28항 또는 제29항의 rAAV를 포함하는 제약 조성물.

청구항 39

제38항에 있어서, 대상체의 CNS에의 직접 주사용으로 제제화된 제약 조성물.

청구항 40

제39항에 있어서, 대상체의 중추 신경계 (CNS)에의 직접 주사가 대뇌내 주사, 실질내 주사, 경막내 주사, 대수조내 주사, 또는 그의 임의의 조합인 제약 조성물.

청구항 41

제39항에 있어서, 대상체의 CNS에의 직접 주사가 컨벡션 강화 전달법 (CED)을 포함하는 것인 제약 조성물.

청구항 42

제38항에 있어서, 말초 주사용 또는 정맥내 주사용으로 제제화되는 제약 조성물.

청구항 43

삭제

청구항 44

삭제

청구항 45

삭제

청구항 46

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발명의 설명

기 술 분 야

[0001] 관련 출원

[0002] 본 출원은 35 U.S.C. 119(e)하에 2017년 10월 3일 출원된 미국 가출원 일련 번호 62/567,296 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"), 2017년 10월 3일 출원된 미국 가출원 일련 번호 62/567,311 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"), 2017년 10월 3일 출원된 미국 가출원 일련 번호 62/567,319 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"), 2018년 10월 3일 출원된 미국 가출원 일련 번호 62/567,301 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"), 및 2017년 10월 3일 출원된 미국 가출원 일련 번호 62/567,310 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS")의 출원일의 이익을 주장하고, 각 출원의 전체 내용이 본원에서 참조로 포함된다.

배 경 기 술

[0003] 고셰병(Gaucher disease)은 리소좀 산 β-글루코세레브로시다제 (Gcase, "GBA")의 결핍에 기인한, 선천성 글리코스핑고리피드 대사 이상 희귀 질환이다. 환자는 간비장비대, 범혈구감소증으로 이어지는 골수 기능 부전, 폐 장애 및 섬유증, 골 결손을 비롯한, 비-CNS 증상 및 소견을 앓는다. 추가로, 상당수의 환자들은 결함성 도약안구 운동 및 시선, 발작, 인지 결손, 발달 지연, 및 파킨슨병을 비롯한 운동 장애를 포함하는, 신경학적 증상을 앓는다.

[0004] 말초 질환 및 조혈 골수 및 내장에서의 주요 임상 증상을 처리하는 여러 치료제들이 존재하며, 이는 하기 기술되는 바와 같은 효소 대체 요법, 결함성 Gcase에 결합하고, 안정성을 개선시키는 샤페론 유사 소분자 약물, 및 증상 및 소견으로 이어지는, 고셰병에서 축적되는 기질의 생산을 차단하는 기질 감소 요법을 포함한다. 그러나, 고셰병의 다른 측면 (특히, 골격 및 뇌에서 발병되는 것)은 치료에 대하여 난치성인 것으로 보인다.

발명의 내용

[0005] (*GBA1* 유전자의 두 염색체 대립유전자 모두에 돌연변이를 갖는) 고셰병 환자 이외에도, GBA1의 단 하나의 대립유전자에만 돌연변이를 갖는 환자는 파킨슨병 (PD)에 걸릴 위험이 매우 높다. 보행 장애, 휴식시 떨림, 경직, 및 흔히 우울증, 수면 장애, 및 인지 저하를 포함하는, PD 증상의 중증도는 효소 활성 감소 정도와 상관관계를 갖는다. 따라서, 고셰병 환자는 가장 중증의 과정을 갖는 반면, *GBA1* 중 단일의 가벼운 돌연변이를 갖는 환자는 전형적으로 더욱 양성의 과정을 갖는다. 돌연변이 보유자는 또한, 실행 기능 장애, 정신병, 및 PD 유사 운동 장애를 특징으로 하는 루이소체 치매, 및 특유의 운동 및 인지 장애를 갖는 다계통 위축을 비롯한, 다른 PD 관련 장애의 위험이 높다. 이러한 장애의 거침없이 계속되는 과정을 변경하는 요법은 현재 없다.

[0006] 예컨대, Gcase (예컨대, *GBA1* 유전자의 유전자 생성물)와 같은 효소 결핍 뿐만 아니라, 리소좀 기능 또는 리소좀으로의 거대분자 수송에 관여하는 다수의 유전자 (예컨대, 리소좀 막 단백질 1 (LIMP), 이는 또한 SCARB2로도 지칭)에서의 일반적인 변이체가 PD 위험 증가와 연관이 있다. 본 개시내용은 부분적으로 하나 이상의 PD 연관 유전자, 예를 들어, Gcase, GBA2, 프로사포신, 프로그래뉼린, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, TMEM106B, 또는 상기 중 임의의 것 (또는 그의 일부)의 조합을 코딩하는 발현 구축물 (예컨대, 벡터)에 기초한다. 일부 실시양태에서, 본원에 기술된 유전자 생성물의 조합은 대상체에서 발현되었을 때, 함께 (예컨대, 시너지적으로) 작용하여 PD의 하나 이상의 징후 및 증상을 감소시킨다.

[0007] 따라서, 일부 측면에서, 본 개시내용은 Gcase (예컨대, *GBA1* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) Gcase 코딩 서열을 포함한다. 일부 실시양태에서, Gcase를 코딩하는 핵산 서열은 서열식별번호(SEQ ID NO:) 14에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_000148.2에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 15에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, Gcase 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0008] 일부 측면에서, 본 개시내용은 프로사포신 (예컨대, *PSAP* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포,

예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) 프로사포신 코딩 서열을 포함한다. 일부 실시양태에서, 프로사포신을 코딩하는 핵산 서열은 서열식별번호 16에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_002769.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 17에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, 프로사포신 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0009] 일부 측면에서, 본 개시내용은 LIMP2/SCARB2 (예컨대, *SCARB2* 유전자의 유전자 생성물)를 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) SCARB2 코딩 서열을 포함한다. 일부 실시양태에서, LIMP2/SCARB2를 코딩하는 핵산 서열은 서열식별번호 18에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_005497.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 29에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, SCARB2 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0010] 일부 측면에서, 본 개시내용은 GBA2 단백질 (예컨대, *GBA2* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) GBA2 코딩 서열을 포함한다. 일부 실시양태에서, GBA2를 코딩하는 핵산 서열은 서열식별번호 30에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_065995.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 31에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, GBA2 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0011] 일부 측면에서, 본 개시내용은 GALC 단백질 (예컨대, *GALC* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) GALC 코딩 서열을 포함한다. 일부 실시양태에서, GALC를 코딩하는 핵산 서열은 서열식별번호 33에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_000144.2에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 34에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, GALC 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0012] 일부 측면에서, 본 개시내용은 CTSB 단백질 (예컨대, *CTSB* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) CTSB 코딩 서열을 포함한다. 일부 실시양태에서, CTSB를 코딩하는 핵산 서열은 서열식별번호 35에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_001899.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 36에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, CTSB 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0013] 일부 측면에서, 본 개시내용은 SMPD1 단백질 (예컨대, *SMPD1* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) *SMPD1* 코딩 서열을 포함한다. 일부 실시양태에서, SMPD1을 코딩하는 핵산 서열은 서열식별번호 37에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_000534.3에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 38에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, SMPD1 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0014] 일부 측면에서, 본 개시내용은 GCH1 단백질 (예컨대, *GCH1* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) GCH1 코딩 서열을 포함한다. 일부 실시양태에서, GCH1을 코딩하는 핵산 서열은 서열식별번호 45에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_000534.3에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번

호 46에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, GCH1 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0015] 일부 측면에서, 본 개시내용은 RAB7L 단백질 (예컨대, *RAB7L* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) RAB7L 코딩 서열을 포함한다. 일부 실시양태에서, RAB7L을 코딩하는 핵산 서열은 서열식별번호 47에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_003920.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 48에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, RAB7L 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0016] 일부 측면에서, 본 개시내용은 VPS35 단백질 (예컨대, *VPS35* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) VPS35 코딩 서열을 포함한다. 일부 실시양태에서, VPS35를 코딩하는 핵산 서열은 서열식별번호 49에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_060676.2에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 50에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, VPS35 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0017] 일부 측면에서, 본 개시내용은 IL-34 단백질 (예컨대, *IL34* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) IL-34 코딩 서열을 포함한다. 일부 실시양태에서, IL-34를 코딩하는 핵산 서열은 서열식별번호 55에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_689669.2에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 56에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, IL_34 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0018] 일부 측면에서, 본 개시내용은 TREM2 단백질 (예컨대, *TREM* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) TREM2 코딩 서열을 포함한다. 일부 실시양태에서, TREM2를 코딩하는 핵산 서열은 서열식별번호 57에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_061838.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 58에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, TREM2 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0019] 일부 측면에서, 본 개시내용은 TMEM106B 단백질 (예컨대, *TMEM106B* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) TMEM106B 코딩 서열을 포함한다. 일부 실시양태에서, TMEM106B를 코딩하는 핵산 서열은 서열식별번호 63에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_060844.2에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 64에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, TMEM106B 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0020] 일부 측면에서, 본 개시내용은 프로그래뉼린 (예컨대, *PGRN* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) 프로사포신 코딩 서열을 포함한다. 일부 실시양태에서, 프로그래뉼린 (PGRN)을 코딩하는 핵산 서열은 서열식별번호 67에 기재된 바와 같은 (예컨대, NCBI 참조 서열 NP_002078.1에 기재된 바와 같은) 아미노산 서열을 포함하는 단백질을 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 68에 기재된 바와 같은 서열을 포함한다. 일부 실시양태에서, 발현 구축물은 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR), 예를 들어, 프로사포신 단백질을 코딩하는 핵산 서열에 플랭킹된 AAV ITR을 포함한다.

[0021] 일부 측면에서, 본 개시내용은 제1 유전자 생성물 및 제2 유전자 생성물을 코딩하는 발현 구축물을 포함하는 단리된 핵산이며, 여기서 각 유전자 생성물은 독립적으로 표 1에 기재된 유전자 생성물 또는 그의 일부로부터 선택되는 것인, 단리된 핵산을 제공한다.

[0022] 일부 실시양태에서, 제1 유전자 생성물 또는 제2 유전자 생성물은 Gcase 단백질, 또는 그의 일부이다. 일부 실시양태에서, 제1 유전자 생성물은 Gcase 단백질이고, 제2 유전자 생성물은 GBA2, 프로사포신, 프로그래뉼린, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, 및 TMEM106B로부터 선택된다.

[0023] 일부 실시양태에서, 발현 구축물은 간섭 핵산 (예컨대, shRNA, miRNA, dsRNA 등)을 추가로 코딩한다. 일부 실시양태에서, 간섭 핵산은 α-시누클레인 (α-시누클레인)의 발현을 억제시킨다. 일부 실시양태에서, α-시누클레인을 표적화하는 간섭 핵산은 서열식별번호 20-25 중 어느 하나에 기재된 서열을 포함한다. 일부 실시양태에서, α-시누클레인을 표적화하는 간섭 핵산은 서열식별번호 20-25 중 어느 하나에 기재된 서열에 결합한다 (예컨대, 그와 하이브리드화한다).

[0024] 일부 실시양태에서, 간섭 핵산은 TMEM106B의 발현을 억제시킨다. 일부 실시양태에서, TMEM106B를 표적화하는 간섭 핵산은 서열식별번호 64 또는 65에 기재된 서열을 포함한다. 일부 실시양태에서, TMEM106B를 표적화하는 간섭 핵산은 서열식별번호 64 또는 65에 기재된 서열에 결합한다 (예컨대, 그와 하이브리드화한다).

[0025] 일부 실시양태에서, 발현 구축물은 하나 이상의 프로모터를 추가로 포함한다. 일부 실시양태에서, 프로모터는 닭 베타 액틴 (CBA) 프로모터, CAG 프로모터, CD68 프로모터, 또는 JeT 프로모터이다. 일부 실시양태에서, 프로모터는 RNA pol II 프로모터 (예컨대, 또는 RNA pol III 프로모터 (예컨대, U6 등)이다.

[0026] 일부 실시양태에서, 발현 구축물은 내부 리보솜 진입 부위 (IRES)를 추가로 포함한다. 일부 실시양태에서, IRES는 제1 유전자 생성물과 제2 유전자 생성물 사이에 위치한다.

[0027] 일부 실시양태에서, 발현 구축물은 자기 절단 펩티드 코딩 서열을 추가로 포함한다. 일부 실시양태에서, 자기 절단 펩티드는 T2A 펩티드이다.

[0028] 일부 실시양태에서, 발현 구축물은 2개의 아데노-연관 바이러스 (AAV) 역전된 말단 반복부 (ITR) 서열을 포함한다. 일부 실시양태에서, ITR 서열은 제1 유전자 생성물 및 제2 유전자 생성물에 플랭킹된다 (예컨대, 5' 단부에서 3' 단부 방향으로 하기와 같이 배열되어 있다: ITR-제1 유전자 생성물-제2 유전자 생성물-ITR). 일부 실시양태에서, 단리된 핵산의 ITR 서열 중 하나에는 기능성 말단 분해 부위 (trs)가 결손되어 있다. 예를 들어, 일부 실시양태에서, ITR 중 하나는 ΔITR이다.

[0029] 일부 측면에서, 본 개시내용은 변형된 "D" 영역 (예컨대, 야생형 AAV2 ITR 기준으로 변형된 D 서열, 서열식별번호 29)을 갖는 ITR을 포함하는 rAAV 벡터에 관한 것이다. 일부 실시양태에서, 변형된 D 영역을 갖는 ITR은 rAAV 벡터의 5' ITR이다. 일부 실시양태에서, 변형된 "D" 영역은 예를 들어, 서열식별번호 26에 기재되어 있는 바와 같은 "S" 서열을 포함한다. 일부 실시양태에서, 변형된 "D" 영역을 갖는 ITR은 rAAV 벡터의 3' ITR이다. 일부 실시양태에서, 일부 실시양태에서, 변형된 "D" 영역은 "D" 영역이 ITR의 3' 단부에 (예컨대, 벡터의 트랜스진 인서트 기준으로 ITR의 외부 또는 말단 단부 상에) 위치하는 3' ITR을 포함한다. 일부 실시양태에서, 변형된 "D" 영역은 서열식별번호 26 또는 27에 기재되어 있는 바와 같은 서열을 포함한다.

[0030] 일부 실시양태에서, 단리된 핵산 (예컨대, rAAV 벡터)는 TRY 영역을 포함한다. 일부 실시양태에서, TRY 영역은 서열식별번호 28에 기재되어 있는 바와 같은 서열을 포함한다.

[0031] 일부 실시양태에서, 본 개시내용에 의해 기술된 단리된 핵산은 서열식별번호 1-78 중 어느 하나에 기재된 서열을 포함하거나 또는 그로 이루어지거나 또는 상기 서열을 갖는 펩티드를 코딩한다.

[0032] 일부 측면에서, 본 개시내용은 본 개시내용에 의해 기술된 바와 같은 단리된 핵산을 포함하는 벡터를 제공한다. 일부 실시양태에서, 벡터는 플라스미드, 또는 바이러스 벡터이다. 일부 실시양태에서, 바이러스 벡터는 재조합 AAV (rAAV) 벡터또는 배큘로바이러스 벡터이다. 일부 실시양태에서, rAAV 벡터는 단일-가닥 (예컨대, 단일-가닥 DNA)이다.

[0033] 일부 측면에서, 본 개시내용은 본 개시내용에 의해 기술된 바와 같은 단리된 핵산, 또는 본 개시내용에 의해 기술된 바와 같은 벡터를 포함하는 숙주 세포를 제공한다.

[0034] 일부 측면에서, 본 개시내용은 본 개시내용에 의해 기술된 바와 같은 캡시드 단백질 및 단리된 핵산 또는 벡터를 포함하는 재조합 아데노-연관 바이러스 (rAAV)를 제공한다.

[0035] 일부 실시양태에서, 캡시드 단백질, 예를 들어, AAV9 캡시드 단백질 또는 AAVrh.10 캡시드 단백질은 혈액-뇌 장벽을 건널 수 있다. 일부 실시양태에서, rAAV는 중추 신경계 (CNS)의 뉴런 세포 및 비-뉴런 세포를 형질도입한다.

[0036] 일부 측면에서, 본 개시내용은 파킨슨병을 앓거나 또는 앓을 것으로 의심되는 대상체에게 본 개시내용에 의해 기술된 바와 같은 조성물 (예컨대, 단리된 핵산 또는 벡터 또는 rAAV를 포함하는 조성물)을 투여하는 단계를 포함하는, 파킨슨병을 앓거나 또는 앓을 것으로 의심되는 대상체를 치료하는 방법을 제공한다.

[0037] 일부 실시양태에서, 투여는 대상체의 CNS에의 직접 주사를 포함한다. 일부 실시양태에서, 직접 주사는 대뇌내 주사, 실질내 주사, 경막내 주사, 대수조내 주사, 또는 그의 임의의 조합이다. 일부 실시양태에서, 대상체의 CNS에의 직접 주사는 컨벡션 강화 전달법 (CED)을 포함한다.

[0038] 일부 실시양태에서, 투여는 말초 주사를 포함한다. 일부 실시양태에서, 말초 주사는 정맥내 주사이다.

도면의 간단한 설명

[0039] 도 1은 Gcase (예컨대, *GBA1* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 2는 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 LIMP2 (SCARB2) 또는 그의 일부를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 LIMP2의 코딩 서열은 내부 리보솜 진입 부위 (IRES)에 의해 이격되어 있다.

도 3은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 LIMP2 (SCARB2) 또는 그의 일부를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 LIMP2의 코딩 서열의 발현은 각각 별개의 프로모터에 의해 구동된다.

도 4는 Gcase (예컨대, *GBA1* 또는 그의 일부), LIMP2 (SCARB2) 또는 그의 일부, 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 5는 Gcase (예컨대, *GBA1* 또는 그의 일부), 프로사포신 (예컨대, *PSAP* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 6은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 프로사포신 (예컨대, *PSAP* 또는 그의 일부)을 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 프로사포신의 코딩 서열은 내부 리보솜 진입 부위 (IRES)에 의해 이격되어 있다.

도 7은 Gcase (예컨대, *GBA1* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. 본 실시양태에서, 벡터는 인간 *GBA1*의 코돈 최적화된 코딩 서열을 구성적으로 발현하기 위해, 4개 파트: CMV 인핸서 (CMVe), CBA 프로모터 (CBAp), 엑손 1, 및 인트론 (int)으로 이루어진, CBA 프로모터 요소 (CBA)를 포함한다. 3' 영역은 또한 WPRE 조절 요소, 이어서, bGH 폴리A 테일을 함유한다. 3개의 전사 조절 활성화 부위가 프로모터 영역의 5' 단부에 포함되어 있다: TATA, RBS, 및 YY1. 플랭킹 ITR을 통해 개재 서열은 정확하게 패키징될 수 있다. 5' ITR 서열의 두 변이체 (삽입 박스)를 평가하였다: 상기 변이체들은 야생형 AAV2 ITR의 20-뉴클레오티드 "D" 영역 내에서 수개의 뉴클레오티드 차이를 보인다. 일부 실시양태에서, rAAV 벡터는 상단 라인에 제시된 "D" 도메인 뉴클레오티드 서열을 함유한다. 일부 실시양태에서, rAAV 벡터 돌연변이체 "D" 도메인 (예컨대, "S" 도메인, 여기서 뉴클레오티드 변이는 하단 라인에 제시)을 보유한다.

도 8은 도 6에 기술된 벡터의 한 실시양태를 도시한 개략도이다.

도 9는 파킨슨병을 앓는 CBE 마우스 모델에서의 Gcase (예컨대, *GBA1* 또는 그의 일부)를 코딩하는 트랜스진을 포함하는 rAAV의 전달에 대한 대표적인 데이터를 보여주는 것이다. P8에 PBS 비히클, 25 mg/kg CBE, 37.5 mg/kg CBE, 또는 50 mg/kg CBE (좌측부터 우측으로)의 매일 IP 전달을 개시하였다. 매일 2회에 걸쳐 생존 (좌측 상단)을 체크하였고, 체중 (우측 상단)은 매일 체크하였다. 모든 군은 n = 8으로 시작하였다. P23에 오픈 필드에서의 총 이동 거리 (좌측 하단) 및 P24에 로타로드에서 떨어질 때까지의 시간 (하단의 중간)에 의해 행동을 사정하였다. CBE를 중단한 조건 (3일째) 및 CBE를 중단하지 않은 조건 (1일째), 둘 모두의 PBS 및 25 mg/kg CBE 처리군 중의 마우스의 피질에서 GCase 기질의 수준을 분석하였다. 응집체 GluSph 및 GalSph 수준 (우측 하단)은 조직 습식 중량 1 mg당 pmol로 제시되어 있다. 평균이 제시되어 있다. 오차 막대는 SEM이다. *p<0.05; **p<0.01; ***p<0.001, 선형 회귀에 의한 처리군에 대한 명목 p 값.

도 10은 CBE 마우스 모델에서의 최대 rAAV 용량에 대한 연구 디자인의 한 실시양태를 도시한 개략도이다. 간략하면, rAAV를 P3에 ICV 주사에 의해 전달하고, P8에 매일 CBE 처리를 개시하였다. P24-25에 오픈 필드 및 로타로드 검정법으로 행동을 사정하고, P36 및 P38에 기질 수준을 측정하였다.

도 11은 CBE 마우스 모델에서의 최대 rAAV 용량의 생전 사정에 대한 대표적인 데이터를 보여주는 것이다. P3에, 마우스를 ICV 전달을 통해 부형제 또는 8.8e9 vg rAAV-GBA1로 처리하였다. P8에 PBS 또는 25 mg/kg CBE의 매일 IP 전달을 개시하였다. 연구 종료시, 마우스 중 절반을 P36 (1일째)에 그의 최종 CBE 투약 후 다음날 희생시켰고, 나머지 절단은 P38 (3일째)에 희생시키기 전에 3일 동안 CBE를 중단하였다. 모든 처리군 (부형제 + PBS의 경우, n = 8, rAAV-GBA1+ PBS의 경우, n = 7, 부형제 + CBE의 경우, n = 8, 및 변이체 + CBE의 경우, n = 9)의 체중을 매일 측정하였고 (좌측 상단), P36의 체중을 분석하였다 (우측 상단). P23에 오픈 필드에서의 총 이동 거리 (좌측 하단) 및 P24에 로타로드에서 떨어질 때까지의 시간 (우측 하단)에 의해 행동을 사정하고, 각 동물에 대하여 3회 시도에 대한 중간값으로서 평가하였다. 치사에 기인하여, 행동 검정법에서 부형제 + CBE 군의 경우, n = 7인 반면, 다른 군은 모두 n=8이었다. 동물들 간의 평균이 제시되어 있다. 오차 막대는 SEM이다. *p<0.05; ***p<0.001, CBE 처리된 동물에서 선형 회귀에 의한 처리군에 대한 명목 p 값.

도 12는 CBE 마우스 모델에서의 최대 rAAV 용량의 생화학적 사정에 대한 대표적인 데이터를 보여주는 것이다. CBE 중단 전 (1일째) 또는 CBE 중단 후 (3일째), 모든 처리군 (부형제 + PBS의 경우, n = 8, 변이체 + PBS의 경우, n = 7, 부형제 + CBE의 경우, n = 7, 및 변이체 + CBE의 경우, n = 9)의 피질을 사용하여 군에서의 GCase 활성 (좌측 상단), GluSph 수준 (우측 상단), GluCer 수준 (좌측 하단), 및 벡터 게놈 (우측 하단)을 측정하였다. 생체분포는 게놈 DNA 1 μg당 벡터 게놈으로 제시되어 있다. 평균이 제시되어 있다. 오차 막대는 SEM이다. (*)p<0.1; **p<0.01; ***p<0.001, CBE 처리된 동물에서 선형 회귀에 의한 처리군에 대한 명목 p 값, 여기서 수집일 및 성별은 공변량으로서 보정.

도 13은 부형제 + PBS, 부형제 + CBE, 및 변이체 + CBE 처리군의 투여 이후의 CBE 마우스 모델에서의 행동 및 생화학적 상관관계에 대한 대표적인 데이터를 보여주는 것이다. 처리군 간에, 로타로드에서의 수행능은 GluCer 축적과 음의 상관관계를 가졌고 (A, 선형 회귀에 의해 p=0.0012), GluSph 축적은 증가된 GCase 활성과 음의 상관관계를 가졌다 (B, 선형 회귀에 의해 p=0.0086).

도 14는 CBE 마우스 모델에서의 변이체의 생체분포에 대한 대표적인 데이터를 보여주는 것이다. 모든 처리군 (부형제 + PBS의 경우, n = 8, 변이체+ PBS의 경우, n = 7, 부형제 + CBE의 경우, n = 7, 및 변이체+ CBE의 경우, n = 9)에 대해 간, 비장, 신장, 및 생식선에서 벡터 게놈의 존재를 사정하였다. 생체분포는 게놈 DNA 1 μg당 벡터 게놈으로 제시되어 있다. 벡터 참조 표준 곡선을 이용하여 정량적 PCR에 의해 벡터 게놈 존재를 정량화하였고; A260 광학 밀도 측정에 의해 게놈 DNA 농도를 평가하였다. 평균이 제시되어 있다. 오차 막대는 SEM이다. *p<0.05; **p<0.01; ***p<0.001, CBE 처리된 동물에서 선형 회귀에 의한 처리군에 대한 명목 p 값, 여기서 수집일 및 성별은 공변량으로서 보정.

도 15는 CBE 마우스 모델에서의 rAAV 용량 범위의 생전 사정에 대한 대표적인 데이터를 보여주는 것이다. 마우스는 P3에 ICV 전달에 의해 부형제 또는 3개의 상이한 용량의 rAAV-GBA1: 3.2e9 vg, 1.0e10 vg, 또는 3.2e10 vg 중 하나를 받았다. P8에, 25 mg/kg CBE의 매일 IP 처리를 개시하였다. 부형제 및 CBE 또는 부형제 및 PBS를 받은 마우스는 대조군으로서의 역할을 하였다. 모든 처리군은 군당 n = 10 (5M/5F)으로 시작하였다. 모든 마우스를 그의 최종 CBE 투약 후 다음날 (P38-P40) 희생시켰다. 모든 처리군을 매일 체중을 측정하고, P36에 그의 체중을 분석하였다. P24에 로타로드에서 떨어질 때까지의 시간, 및 P30에 테이퍼 빔(Tapered Beam)을 건너는 데 소요되는 시간에 의해 운동 수행능을 사정하였다. 조기 치사에 기인하여, 행동 검정법에 참여한 마우스의 마리수는 부형제 + PBS의 경우, n = 10, 부형제 + CBE의 경우, n = 9, 및 3.2e9 vg rAAV-GBA1+ CBE의 경우, n = 6, 1.0e10 vg rAAV-GBA1+ CBE의 경우, n = 10, 3.2e10 vg rAAV-GBA1+ CBE의 경우, n = 7이었다. 평균이 제시되어 있다. 오차 막대는 SEM이고; * p<0.05; **p<0.01, CBE 처리군에서 선형 회귀에 의한 명목 p 값, 여기서 성별은 공변량으로서 보정.

도 16은 CBE 마우스 모델에서의 rAAV 용량 범위의 생화학적 사정에 대한 대표적인 데이터를 보여주는 것이다. 모든 처리군 (부형제 + PBS의 경우, n = 10, 부형제 + CBE의 경우, n = 9, 및 3.2e9 vg rAAV-GBA1+ CBE의 경우, n = 6, 1.0e10 vg rAAV-GBA1+ CBE의 경우, n = 10, 3.2e10 vg rAAV-GBA1+ CBE의 경우, n = 7)의 피질을 이용하여 GCase 활성, GluSph 수준, GluCer 수준, 및 벡터 게놈을 측정하였다. GCase 활성은 총 단백질 1 mg당 GCase ng로 제시되어 있다. GluSph 및 GluCer 수준은 조직 습식 중량 1 mg당 pmol로 제시되어 있다. 생체분포는 게놈 DNA 1 μg당 벡터 게놈으로 제시되어 있다. 벡터 참조 표준 곡선을 이용하여 정량적 PCR에 의해 벡

터 게놈 존재를 정량화하였고; A260 광학 밀도 측정에 의해 게놈 DNA 농도를 평가하였다. 벡터 게놈 존재를 또한 간에서도 측정하였다 (E). 평균이 제시되어 있다. 오차 막대는 SEM이다. **p<0.01; ***p<0.001, CBE 처리군에서 선형 회귀에 의한 명목 p 값, 여기서 성별은 공변량으로서 보정.

도 17은 유전적 마우스 모델에서 최대 용량 rAAV-GBA1에서의 테이퍼 빔 분석에 대한 대표적인 데이터를 보여주는 것이다. rAAV-GBA1 투여 후 4주째에 빔 워크(Beam Walk)에 의해 처리군 (WT + 부형제 (n = 5), 4L/PS-NA + 부형제 (n = 6), 및 4L/PS-NA + rAAV-GBA1 (n = 5))의 운동 수행능을 검정하였다. 총 슬립수 및 활동 시간은 상이한 빔에서의 5회 시도에 대한 총합으로 제시되어 있다. 속도 및 속도당 슬립수는 상이한 빔에서의 5회 시도에 대한 평균으로 제시되어 있다. 평균이 제시되어 있다. 오차 막대는 SEM이다.

도 18은 프로그래뉼린 (PGRN) 단백질을 코딩하는 rAAV 구축물의 시험관내 발현에 대한 대표적인 데이터를 보여주는 것이다. 좌측 패널은 프로그래뉼린 (PGRN) ELISA 검정법의 표준 곡선을 보여주는 것이다. 하단 패널은 rAAV가 형질도입된 HEK293T 세포의 세포 용해물 중에서 ELISA 검정법에 의해 측정된 PGRN 발현의 용량-반응을 보여주는 것이다. MOI = 감염 다중도 (세포당 벡터 게놈).

도 19는 프로사포신 (*PSAP*), *SCARB2*, 및/또는 하나 이상의 억제성 핵산과 함께 조합된, *GBA1*을 코딩하는 rAAV 구축물의 시험관내 발현에 대한 대표적인 데이터를 보여주는 것이다. 데이터는, HEK293 세포를 각 구축물로 형질감염시켰을 때, 모의 형질감염된 세포와 비교하여 관심 트랜스진이 과다발현되었다는 것을 나타낸다.

도 20은 ITR의 "외부"에 (예컨대, 트랜스진 인서트 또는 발현 구축물 기준으로 ITR의 말단에 인접하게) 위치하는 "D" 영역을 포함하는 rAAV 벡터 (상단), 및 벡터의 "내부"에 (예컨대, 벡터의 트랜스진 인서트에 인접한 위치에) ITR을 갖는 야생형 rAAV 벡터를 도시한 개략도이다.

도 21은 GBA2 또는 그의 일부, 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 22는 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 갈락토실세라미다제 (예컨대, *GALC* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 갈락토실세라미다제의 코딩 서열의 발현은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 23은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 갈락토실세라미다제 (예컨대, *GALC* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 갈락토실세라미다제의 코딩 서열의 발현은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 24는 Gcase (예컨대, *GBA1* 또는 그의 일부), 카텝신 B (예컨대, *CTSB* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 카텝신 B의 코딩 서열의 발현은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 25는 Gcase (예컨대, *GBA1* 또는 그의 일부), 스핑고마이엘린 포스포디에스테라제 1 (예컨대, *SMPD1* 그의 일부, 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 26은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 갈락토실세라미다제 (예컨대, *GALC* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 갈락토실세라미다제의 코딩 서열은 내부 리보솜 진입 부위 (IRES)에 의해 이격되어 있다.

도 27은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 카텝신 B (예컨대, *CTSB* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 카텝신 B의 코딩 서열의 발현은 각각 별개의 프로모터에 의해 구동된다.

도 28은 Gcase (예컨대, *GBA1* 또는 그의 일부), GCH1 (예컨대, *GCH1* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 GCH1의 코딩 서열은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 29는 Gcase (예컨대, *GBA1* 또는 그의 일부), RAB7L1 (예컨대, *RAB7L1* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 RAB7L1의 코딩 서열은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 30은 Gcase (예컨대, *GBA1* 또는 그의 일부), GCH1 (예컨대, *GCH1* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 GCH1의 코딩 서열

의 발현은 내부 리보솜 진입 부위 (IRES)이다.

도 31은 VPS35 (예컨대, *VPS35* 또는 그의 일부) 및 α-Syn 및 TMEM106B에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 32는 Gcase (예컨대, *GBA1* 또는 그의 일부), IL-34 (예컨대, *IL34* 또는 그의 일부), 및 α-Syn에 대한 간섭 RNA를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 IL-34의 코딩 서열은 T2A 자기 절단 펩티드 서열에 의해 이격되어 있다.

도 33은 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 IL-34 (예컨대, *IL34* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 IL-34의 코딩 서열은 내부 리보솜 진입 부위 (IRES)에 의해 이격되어 있다.

도 34는 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 TREM2 (예컨대, *TREM2* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 TREM2의 코딩 서열의 발현은 각각 별개의 프로모터에 의해 구동된다.

도 35는 Gcase (예컨대, *GBA1* 또는 그의 일부) 및 IL-34 (예컨대, *IL34* 또는 그의 일부)를 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. Gcase 및 IL-34의 코딩 서열의 발현은 각각 별개의 프로모터에 의해 구동된다.

도 36a-36b는 qPCR 및 ELISA에 의해 측정된 바, 대조군 형질도입된 세포 대비 HEK293 세포에서의 TREM2 및 GBA1의 과다발현에 대한 대표적인 데이터를 보여주는 것이다. 도 36a는 TREM2의 과다발현에 대한 데이터를 보여주는 것이다. 도 36b는 동일 구축물로부터의 GBA1의 과다발현에 대한 데이터를 보여주는 것이다.

도 37은 GFP 리포터 검정법 (상단) 및 α-Syn 검정법 (하단)에 의해, 시험관내에서 *SCNA*의 침묵화가 성공적으로 이루어졌다는 것을 나타내는 대표적인 데이터를 보여주는 것이다.

도 38은 GFP 리포터 검정법 (상단) 및 α-Syn 검정법 (하단)에 의해, 시험관내에서 *TMEM106B*의 침묵화가 성공적으로 이루어졌다는 것을 나타내는 대표적인 데이터를 보여주는 것이다.

도 39는 PGRN을 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다.

도 40은 "D" 서열이 야생형으로 (동그라미 표시) 또는 대안적으로 (예컨대, "외부에"; 사각형 표시) 배치되어 있는 ITR을 갖는 rAAV를 이용한 HEK293 세포의 형질도입에 대한 데이터를 보여주는 것이다. "외부에" 배치된 ITR을 갖는 rAAV는 야생형 ITR을 갖는 rAAV만큼 효율적으로 세포를 형질도입시킬 수 있었다.

발명을 실시하기 위한 구체적인 내용

[0040] 본 개시내용은 부분적으로 대상체에서 PD 연관 유전자 생성물의 조합의 발현을 위한 조성물 및 방법에 기초한다. 유전자 생성물은 단백질, 단백질의 단편 (예컨대, 일부), PD 연관 유전자를 억제시키는 간섭 핵산 등일 수 있다. 일부 실시양태에서, 유전자 생성물은 PD 연관 유전자에 의해 코딩되는 단백질 또는 단백질 단편이다. 일부 실시양태에서, 유전자 생성물은 PD 연관 유전자를 억제시키는 간섭 핵산 (예컨대, shRNA, siRNA, miRNA, amiRNA 등)이다.

[0041] PD 연관 유전자란, PD와 유전적으로, 생화학적으로 또는 기능적으로 연관이 있는 유전자 생성물을 코딩하는 유전자를 지칭한다. 예를 들어, (단백질 Gcase를 코딩하는) *GBA1* 유전자 중 돌연변이를 갖는 개체는 *GBA1* 중에 돌연변이를 가지지 않는 것과 비교하였을 때, PD가 발생할 위험이 증가되어 있는 것으로 관찰되었다. 또 다른 예에서, PD는 α-시누클레인 (α-Syn)을 포함하는 단백질 응집체의 축적과 연관이 있고; 따라서, (α-Syn을 코딩하는) *SCNA*가 PD 연관 유전자이다. 일부 실시양태에서, 본원에 기술된 발현 카세트는 야생형 또는 비-돌연변이체 형태의 PD 연관 유전자 (또는 그의 코딩 서열)이다. PD 연관 유전자의 예는 표 1에 열거되어 있다.

[0042] <표 1>

[0043] PD 연관 유전자의 예

명칭	유전자	기능	NCBI 수탁 번호
리소좀 막 단백질 2	*SCARB2/LIMP2*	글루코실세라미다제에 대한 리소좀 수용체 (GBA 표적화)	NP_005497.1 (이소형 1), NP_001191184.1 (이소형 2)
프로사포신	*PSAP*	리소좀 구획으로 국재화되고, 짧은 올리고당 기를 갖는 글리코스핑고리피드의 이화작용을 촉진시키는 사포신 A, B, C, 및 D에 대한 전구체	AAH01503.1, AAH07612.1, AAH04275.1, AAA60303.1
베타-글루코세레브로시다제	*GBA1*	글루코세레브로시드의 베타-글리코시드 결합을 절단한다	NP_001005742.1 (이소형 1), NP_001165282.1 (이소형 2), NP_001165283.1 (이소형 3)
비-리소좀 글루코실세라미다제	*GBA2*	글루코실세라미드의 유리 글루코스 및 세라미드로의 전환을 촉진시킨다	NP_065995.1 (이소형 1), NP_001317589.1 (이소형 2)
갈락토실세라미다제	*GALC*	세라미드 유도체로부터 갈락토스를 제거한다	EAW81359.1 (이소형 CRA_a), EAW81360.1 (이소형 CRA_b), EAW81362.1 (이소형 CRA_c)
스핑고마이엘린 포스포디에스테라제 1	*SMPD1*	스핑고마이엘린을 세라미드로 전환시킨다	EAW68726.1 (이소형 CRA_a), EAW68727.1 (이소형 CRA_b), EAW68728.1 (이소형 CRA_c), EAW68729.1 (이소형 CRA_d)

[0044]

카텝신 B	*CTSB*	단백질의 세포내 분해 및 전환에 관여하는 것으로 간주되는 티올 프로테아제; 이는 또한 종양 침윤 및 전이에도 연루	AAC37547.1, AAH95408.1, AAH10240.1
RAB7, 구성원 RAS 온코진 패밀리-유사 1	*RAB7L1*	소포 수송을 조절한다	AAH02585.1
액포 단백질 분류 연관 단백질 35	*VPS35*	레트로머 카그로-선택적 복합체의 성분	NP_060676.2
GTP 시클로히드롤라제 1	*GCH1*	구아노신 트리포스페이트를 가수분해하여 7,8-디히드로네오프테린 트리포스페이트를 형성하는 것을 담당	AAH25415.1
인터류킨 34	*IL34*	단핵구의 성장 또는 생존을 증가시키고; 콜로니 자극 인자 1 수용체에 결합함으로써 활성을 유도한다	AAH29804.1
골수 세포 상에서 발현되는 트리거링(triggering) 수용체 2	*TREM2*	TYRO 단백질 티로신 키아제 결합 단백질과 수용체 신호전달 복합체를 형성하고; 면역 반응에서 작용하고, 만성 염증에 관여할 수 있다	AAF69824.1
프로그래뉼린	*PGRN*	발생, 염증, 세포 증식 및 단백질 항상성에서 중요한 역할을 한다	NP_002087.1

[0045]

[0046] *단리된 핵산 및 벡터*

[0047] 단리된 핵산은 DNA 또는 RNA일 수 있다. 일부 측면에서, 본 개시내용은 하나 이상의 PD 연관 유전자, 예를 들어, Gcase (예컨대, *GBA1* 유전자의 유전자 생성물) 또는 그의 일부를 코딩하는 발현 구축물을 포함하는 단리된 핵산 (예컨대, rAAV 벡터)을 제공한다. β-글루코세레브로시다제 또는 GBA로도 지칭되는 Gcase란, 당지질 대사에서 중간체인 화학물질 글루코세레브로시드의 베타-글리코시드 결합을 절단하는 리소좀 단백질을 지칭한다. 인간에서, Gcase는 염색체 1에 위치하는, *GBA1* 유전자에 의해 코딩된다. 일부 실시양태에서, *GBA1*은 NCBI 참조 서열 NCBI 참조 서열 NP_000148.2 (서열식별번호 14)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대, 포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) Gcase 코딩 서열, 예컨대, 서열식별번호 15에 기재된 서열을 포함한다.

[0048] 일부 측면에서, 본 개시내용은 프로사포신 (예컨대, *PSAP* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산을 제공한다. 프로사포신은 짧은 올리고당 기를 갖는 글리코스핑고리피드의 이화작용을 촉진시키는 스핑고리피드 활성인자 단백질 (사포신) A, B, C, 및 D에 대한 전구체 당단백질이다. 인간에서, *PSAP* 유전자는 염색체 10에 위치한다. 일부 실시양태에서, *PSAP*는 NCBI 참조 서열 NP_002769.1 (예컨대, 서열식별번호 16)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 (예컨대,

포유동물 세포, 예를 들어, 인간 세포에서의 발현을 위해 코돈 최적화된) 프로사포신 코딩 서열, 예컨대, 서열식별번호 17에 기재된 서열을 포함한다.

[0049] 본 개시내용의 측면은 LIMP2/SCARB2 (예컨대, *SCARB2* 유전자의 유전자 생성물)를 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. SCARB2는 세포 내에서의 리소좀 및 엔도솜 수송을 조절하는 막 단백질을 지칭한다. 인간에서, *SCARB2* 유전자는 염색체 4에 위치한다. 일부 실시양태에서, *SCARB2* 유전자는 NCBI 참조 서열 NP_005497.1 (서열식별번호 18)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 19에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 SCARB2 코딩 서열을 포함한다.

[0050] 본 개시내용의 측면은 GBA2 단백질 (예컨대, *GBA2* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. GBA2 단백질은 비-리소좀 글루코실세라미다제를 지칭한다. 인간에서, *GBA2* 유전자는 염색체 9에 위치한다. 일부 실시양태에서, *GBA2* 유전자는 NCBI 참조 서열 NP_065995.1 (서열식별번호 30)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 31에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 GBA2 코딩 서열을 포함한다.

[0051] 본 개시내용의 측면은 GALC 단백질 (예컨대, *GALC* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. GALC 단백질은, 갈락토세레브로시드, 갈락토실스핑고신, 락토실세라미드, 및 모노갈락토실디글리세리드의 갈락토스 에스테르 결합을 가수분해하는 효소인 갈락토실세라미다제 (또는 갈락토세레브로시다제)를 지칭한다. 인간에서, *GALC* 유전자는 염색체 14에 위치한다. 일부 실시양태에서, *GALC* 유전자는 NCBI 참조 서열 NP_000144.2 (서열식별번호 33)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 34에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 GALC 코딩 서열을 포함한다.

[0052] 본 개시내용의 측면은 CTSB 단백질 (예컨대, *CTSB* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. CTSB 단백질은, 세포내 단백질분해에서 중요한 역할을 하는 리소좀 시스테인 프로테아제인 카텝신 B를 지칭한다. 인간에서, *CTSB* 유전자는 염색체 8에 위치한다. 일부 실시양태에서, *CTSB* 유전자는 NCBI 참조 서열 NP_001899.1 (서열식별번호 35)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 36에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 CTSB 코딩 서열을 포함한다.

[0053] 본 개시내용의 측면은 SMPD1 단백질 (예컨대, *SMPD1* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. SMPD1 단백질은, 스핑고리피드 대사에 관여하는 히드롤라제 효소인 스핑고마이엘린 포스포디에스테라제 1을 지칭한다. 인간에서, *SMPD1* 유전자는 염색체 11에 위치한다. 일부 실시양태에서, *SMPD1* 유전자는 NCBI 참조 서열 NP_000534.3 (서열식별번호 37)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 38에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 SMPD1 코딩 서열을 포함한다.

[0054] 본 개시내용의 측면은 GCH1 단백질 (예컨대, *GCH1* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. GCH1 단백질은, 폴레이트 및 바이오프테린 생합성 경로의 일부인 히드롤라제 효소인 GTP 시클로히드롤라제 I을 지칭한다. 인간에서, *GCH1* 유전자는 염색체 14에 위치한다. 일부 실시양태에서, *GCH1* 유전자는 NCBI 참조 서열 NP_000152.1 (서열식별번호 45)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 46에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 GCH1 코딩 서열을 포함한다.

[0055] 본 개시내용의 측면은 RAB7L 단백질 (예컨대, *RAB7L* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. RAB7L 단백질은, GTP 결합 단백질인, RAB7, 구성원 RAS 온코진 패밀리 유사 1을 지칭한다. 인간에서, *RAB7L* 유전자는 염색체 1에 위치한다. 일부 실시양태에서, *RAB7L* 유전자는 NCBI 참조 서열 NP_003920.1 (서열식별번호 47)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 48에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 RAB7L 코딩 서열을 포함한다.

[0056] 본 개시내용의 측면은 VPS35 단백질 (예컨대, *VPS35* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. VPS35 단백질은, 단백질의 엔도솜에서 트랜스-골지 네트워크로의 역행 수송에 관여하는 단백질 복합체의 일부인, 액포 단백질 분류 연관 단백질 35를 지칭한다. 인간에서, *VPS35* 유전자는

염색체 16에 위치한다. 일부 실시양태에서, *VPS35* 유전자는 NCBI 참조 서열 NP_060676.2 (서열식별번호 49)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 50에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 VPS35 코딩 서열을 포함한다.

[0057] 본 개시내용의 측면은 IL-34 단백질 (예컨대, *IL34* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. IL-34 단백질은, 단핵구의 성장 및 생존을 증가시키는 시토카인인 인터류킨 34를 지칭한다. 인간에서, *IL34* 유전자는 염색체 16에 위치한다. 일부 실시양태에서, *IL34* 유전자는 NCBI 참조 서열 NP_689669.2 (서열식별번호 55)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 56에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 IL-34 코딩 서열을 포함한다.

[0058] 본 개시내용의 측면은 TREM2 단백질 (예컨대, *TREM2* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. TREM2 단백질은, 골수 세포 상에서 발견되는 면역글로불린 슈퍼패밀리 수용체인, 골수 세포 상세어 발현되는 트리거링(triggering) 수용체 2를 지칭한다. 인간에서, *TREM2* 유전자는 염색체 6에 위치한다. 일부 실시양태에서, *TREM2* 유전자는 NCBI 참조 서열 NP_061838.1 (서열식별번호 57)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 58에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 TREM2 코딩 서열을 포함한다.

[0059] 본 개시내용의 측면은 TMEM106B 단백질 (예컨대, *TMEM106B* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. TMEM106B 단백질은, 수상돌기 형태발생 및 리소좀 수송 조절에 관여하는 단백질인, 막횡단 단백질 106B를 지칭한다. 인간에서, *TMEM106B* 유전자는 염색체 7에 위치한다. 일부 실시양태에서, *TMEM106B* 유전자는 NCBI 참조 서열 NP_060844.2 (서열식별번호 62)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 63에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 TMEM106B 코딩 서열을 포함한다.

[0060] 본 개시내용의 측면은 프로그래뉼린 단백질 (예컨대, *PGRN* 유전자의 유전자 생성물)을 코딩하는 발현 구축물을 포함하는 단리된 핵산에 관한 것이다. PGRN 단백질은, 발생, 염증, 세포 증식 및 단백질 항상성에 관여하는 단백질인 프로그래뉼린을 지칭한다. 인간에서, *PGRN* 유전자는 염색체 17에 위치한다. 일부 실시양태에서, *PGRN* 유전자는 NCBI 참조 서열 NP_002078.1 (서열식별번호 67)에 의해 제시되는 펩티드를 코딩한다. 일부 실시양태에서, 단리된 핵산은 서열식별번호 68에 기재된 서열을 포함한다. 일부 실시양태에서, 단리된 핵산은 코돈 최적화된 PGRN 코딩 서열을 포함한다.

[0061] 일부 측면에서, 본 개시내용은 제1 유전자 생성물 및 제2 유전자 생성물을 코딩하는 발현 구축물을 포함하는 단리된 핵산이며, 여기서 각 유전자 생성물은 독립적으로 표 1에 기재된 유전자 생성물 또는 그의 일부로부터 선택되는 것인, 단리된 핵산을 제공한다.

[0062] 일부 실시양태에서, 유전자 생성물은 자연적으로 발생된 유전자의 코딩 부분 (예컨대, cDNA)에 의해 코딩된다. 일부 실시양태에서, 제1 유전자 생성물은 *GBA1* 유전자에 의해 코딩된 단백질 (또는 그의 단편)이다. 일부 실시양태에서, 유전자 생성물은 표 1에 열거된 또 다른 유전자, 예를 들어, *SCARB2/LIMP2* 유전자 또는 *PSAP* 유전자에 의해 코딩된 단백질 (또는 그의 단편)이다. 그러나, 통상의 기술자는 제1 유전자 생성물 (예컨대, Gcase) 및 제2 유전자 생성물 (예컨대, LIMP2 등)의 발현 순서가 일반적으로 역전될 수 있다는 것 (예컨대, LIMP2가 제1 유전자 생성물이고, Gcase가 제2 유전자 생성물이다)을 이해한다. 일부 실시양태에서, 유전자 생성물은 표 1에 열거된 유전자의 단편 (또는 일부)이다. 단백질 단편은 표 1에 열거된 유전자에 의해 코딩된 단백질의 약 50%, 약 60%, 약 70%, 약 80%, 약 90% 또는 약 99%를 포함할 수 있다. 일부 실시양태에서, 단백질 단편은 표 1에 열거된 유전자에 의해 코딩된 단백질의 50% 내지 99.9% (예컨대, 50% 내지 99.9% 사이의 임의의 값)를 포함한다.

[0063] 일부 실시양태에서, 발현 구축물은 모노시스트론성이다 (예컨대, 발현 구축물은 제1 유전자 생성물 및 제2 유전자 생성물을 포함하는 단일 융합 단백질을 코딩한다). 일부 실시양태에서, 발현 구축물은 폴리시스트론성이다 (예컨대, 발현 구축물은 2개의 전혀 다른 유전자 생성물, 예를 들어, 2개의 상이한 단백질 또는 단백질 단편을 코딩한다).

[0064] 폴리시스트론성 발현 벡터는 하나 이상의 (예컨대, 1, 2, 3, 4, 5개 또는 그 초과의) 프로모터를 포함할 수 있다. 임의의 적합한 프로모터, 예를 들어, 구성적 프로모터, 유도성 프로모터, 내인성 프로모터, 조직 특이적 프로모터 (예컨대, CNS 특이적 프로모터) 등이 사용될 수 있다. 일부 실시양태에서, 프로모터는 닭 베타-액틴

프로모터 (CBA 프로모터), CAG 프로모터 (예를 들어, 문헌 [Alexopoulou et al. (2008) *BMC Cell Biol.* 9:2; doi: 10.1186/1471-2121-9-2]에 의해 기술된 것과 같은 것), CD68 프로모터, 또는 JeT 프로모터 (예를 들어, 문헌 [Tornøe et al. (2002) *Gene* 297(1-2):21-32]에 의해 기술된 것과 같은 것)이다. 일부 실시양태에서, 프로모터는 제1 유전자 생성물, 제2 유전자 생성물, 또는 제1 유전자 생성물 및 제2 유전자 생성물을 코딩하는 핵산 서열에 작동가능하게 연결된다. 일부 실시양태에서, 발현 카세트는 전사 인자 결합 서열, 인트론 스플라이스 부위, 폴리(A) 부가 부위, 인핸서 서열, 리프레서 결합 부위, 또는 상기의 임의의 조합을 포함하나, 이에 제한되지 않는, 하나 이상의 추가의 조절 서열을 포함한다.

[0065] 일부 실시양태에서, 제1 유전자 생성물을 코딩하는 핵산 서열 및 제2 유전자 생성물을 코딩하는 핵산 서열은 내부 리보솜 진입 부위 (IRES)를 코딩하는 핵산 서열에 의해 이격된다. IRES 부위의 예는 예를 들어, 문헌 [Mokrejs et al. (2006) *Nucleic Acids Res.* 34(Database issue):D125-30]에 기술되어 있다. 일부 실시양태에서, 제1 유전자 생성물을 코딩하는 핵산 서열 및 제2 유전자 생성물을 코딩하는 핵산 서열은 자기 절단 펩티드를 코딩하는 핵산 서열에 의해 이격된다. 자기 절단 펩티드의 예는 T2A, P2A, E2A, F2A, BmCPV 2A, 및 BmIFV 2A, 및 문헌 [Liu et al. (2017) *Sci Rep.* 7: 2193]에 의해 기술된 것을 포함하나, 이에 제한되지 않는다. 일부 실시양태에서, 자기 절단 펩티드는 T2A 펩티드이다.

[0066] 병리학상, 장애, 예컨대, PD 및 고셰병은 대개는 α-시누클레인 (α-Syn) 단백질로 구성된 단백질 응집체의 축적과 연관이 있다. 따라서, 일부 실시양태에서, 본원에 기술된 단리된 핵산은 α-Syn 단백질의 발현을 감소시키거나, 또는 방해하는 억제성 핵산을 포함한다. 억제성 핵산을 코딩하는 서열은 발현 벡터의 비번역 영역 (예컨대, 인트론, 5'UTR, 3'UTR 등)에 배치될 수 있다.

[0067] 일부 실시양태에서, 억제성 핵산은 발현 구축물의 인트론에, 예를 들어, 제1 유전자 생성물을 코딩하는 서열의 상류쪽 인트론에 위치한다. 억제성 핵산은 이중 가닥 RNA (dsRNA), siRNA, 마이크로RNA (miRNA), 인공 miRNA (amiRNA), 또는 RNA 압타머일 수 있다. 일반적으로, 억제성 핵산은 표적 RNA (예컨대, mRNA)의 약 6개 내지 약 30개의 (예컨대, 6 내지 30 사이의 임의의 정수 (6 및 30 포함)) 연속적인 뉴클레오티드에 결합한다 (예컨대, 그와 하이브리드화한다). 일부 실시양태에서, 억제성 핵산 분자는 miRNA 또는 amiRNA, 예를 들어, *SNCA* (α-Syn 단백질을 코딩하는 유전자) 또는 *TMEM106B* (예컨대, TMEM106B 단백질을 코딩하는 유전자)를 표적화하는 miRNA이다. 일부 실시양태에서, miRNA는 하이브리드화 대상인 *SNCA* mRNA의 영역과의 미스매치는 포함하지 않는다 (예컨대, miRNA는 "완벽한 것이다"). 일부 실시양태에서, 억제성 핵산은 shRNA (예컨대, *SNCA* 또는 *TMEM106B*를 표적화하는 shRNA)이다. 일부 실시양태에서, 억제성 핵산은 miR-155 스캐폴드 및 *SCNA* 또는 *TMEM106B* 표적화 서열을 포함하는 인공 miRNA (amiRNA)이다.

[0068] 본원에 기술된 단리된 핵산은 그 자체 단독으로, 또는 벡터의 일부로서 존재할 수 있다. 일반적으로, 벡터는 플라스미드, 코스미드, 파지미드, 박테리아 인공 염색체 (BAC), 또는 바이러스 벡터 (예컨대, 아데노바이러스 벡터, 아데노-연관 바이러스 (AAV) 벡터, 레트로바이러스 벡터, 배큘로바이러스 벡터 등)일 수 있다. 일부 실시양태에서, 벡터는 플라스미드 (예컨대, 본원에 기술된 바와 같은 단리된 핵산을 포함하는 플라스미드)이다. 일부 실시양태에서, rAAV 벡터는 단일-가닥 (예컨대, 단일-가닥 DNA)이다. 일부 실시양태에서, 벡터는 재조합 AAV (rAAV) 벡터이다. 일부 실시양태에서, 벡터는 배큘로바이러스 벡터 (예컨대, 오토그라파 칼리포르니카 핵다각체병 (AcNPV) 벡터)이다.

[0069] 전형적으로, rAAV 벡터 (예컨대, rAAV 게놈)는 두 AAV 역전된 말단 반복부 (ITR) 서열에 의해 플랭킹된 트랜스진 (예컨대, 하기 각각의 것: 프로모터, 인트론, 인핸서 서열, 단백질 코딩 서열, 억제성 RNA 코딩 서열, 폴리A 테일 서열 등 중 하나 이상의 것을 포함하는 발현 구축물)을 포함한다. 일부 실시양태에서, rAAV 벡터의 트랜스진은 본 개시내용에 의해 기술된 바와 같은 단리된 핵산을 포함한다. 일부 실시양태에서, rAAV 벡터의 두 ITR 서열은 각각 전장의 ITR (예컨대, 길이 대략 145 bp, 및 기능성 *Rep* 결합 부위 (RBS) 및 말단 분해 부위 (trs)를 함유하는 것)이다. 일부 실시양태에서, rAAV 벡터의 ITR들 중 하나는 말단절단된 것이다 (예컨대, 단축된 것이거나, 또는 전장의 것이 아니다). 일부 실시양태에서, 말단절단된 ITR에는 기능성 말단 분해 부위 (trs)가 결여되어 있고, 자기 상보성 AAV 벡터 (scAAV 벡터) 제조에 사용된다. 일부 실시양태에서, 말단절단된 ITR은 예를 들어, 문헌 [McCarty et al. (2003) *Gene Ther.* 10(26):2112-8]에 기술된 바와 같이, ΔITR이다.

[0070] 본 개시내용의 측면은 야생형 AAV ITR 기준으로, 예를 들어, 야생형 AAV2 ITR (예컨대, 서열식별번호 29) 기준으로 하나 이상의 변형 (예컨대, 핵산 부가, 결실, 치환 등)을 갖는 ITR을 포함하는 단리된 핵산 (예컨대, rAAV 벡터)에 관한 것이다. 야생형 AAV2 ITR의 구조는 도 20에 제시되어 있다. 일반적으로, 야생형 ITR은, 자기 어닐링하여, 각각 B/B' 및 C/C'로 지칭되는 서열에 의해 형성된) 2개의 크로스 아암, (서열 A/A'에 의해 형성된)

더욱 긴 스템 영역, 및 "D" 영역으로 지칭되는 단일-가닥 말단 영역으로 이루어진 회문 이중 가닥 T 형상의 헤어핀 구조를 형성하는 125개의 뉴클레오티드 영역을 포함한다 (도 20). 일반적으로, ITR의 "D" 영역은 A/A' 서열에 의해 형성된 스템 영역과 rAAV 벡터의 트랜스진을 함유하는 인서트 사이에 위치한다 (예컨대, ITR의 말단 기준으로 ITR의 "내부"에 또는 rAAV 벡터의 트랜스진 인서트 또는 발현 구축물에 인접하게 위치한다). 일부 실시양태에서, "D" 영역은 서열식별번호 27에 기재된 서열을 포함한다. "D" 영역은 예를 들어, 문헌 [Ling et al. (2015) *J Mol Genet Med* 9(3)]에 개시되어 있는 바와 같이, 캡시드 단백질에 의한 rAAV 벡터의 캡시드화에서 중요한 역할을 하는 것으로 관찰되었다.

[0071] 본 개시내용은 부분적으로 ITR의 "외부"에 (예컨대, 트랜스진 인서트 또는 발현 구축물 기준으로 ITR의 말단에 인접하게) 위치하는 "D" 영역을 포함하는 rAAV 벡터가 비변형된 (예컨대, 야생형) ITR로 ITR을 갖는 rAAV 벡터보다 AAV 캡시드 단백질에 의해 효율적으로 캡시드화된다는 놀라운 발견에 기초한다. 일부 실시양태에서, 변형된 "D" 서열 (예컨대, "외부 위치에 있는 "D" 서열")을 갖는 rAAV 벡터가 야생형 ITR 서열을 갖는 rAAV 벡터 기준으로 감소된 독성을 갖는다.

[0072] 일부 실시양태에서, 변형된 "D" 서열은 야생형 "D" 서열 (예컨대, 서열식별번호 27) 기준으로 적어도 하나의 뉴클레오티드를 포함한다. 변형된 "D" 서열은 야생형 "D" 서열 (예컨대, 서열식별번호 27) 기준으로 적어도 1, 2, 3, 4, 5, 6, 7, 8, 9, 10개, 또는 10개 초과의 뉴클레오티드 치환을 가질 수 있다. 일부 실시양태에서, 변형된 "D" 서열은 야생형 "D" 서열 (예컨대, 서열식별번호 27) 기준으로 적어도 10, 11, 12, 13, 14, 15, 16, 17, 18, 또는 19개의 핵산 치환을 포함한다. 일부 실시양태에서, 변형된 "D" 서열은 야생형 "D" 서열 (예컨대, 서열식별번호 27)과 약 10% 내지 약 99% (예컨대, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 또는 99%) 동일하다. 일부 실시양태에서, 변형된 "D" 서열은 문헌 [Wang et al. (1995) *J Mol Biol* 250(5):573-80]에 기술된 바와 같이 "S" 서열로도 지칭되는, 서열식별번호 26에 기재된 서열을 포함한다.

[0073] 본 개시내용에 의해 기술된 바와 같은 단리된 핵산 또는 rAAV 벡터는 예를 들어, 서열식별번호 28에 기재된 바와 같은, 또는 문헌 [Francois, et al. 2005. The Cellular TATA Binding Protein Is Required for Rep-Dependent Replication of a Minimal Adeno-Associated Virus Type 2 p5 Element. J Virol]에 기술된 바와 같은 "TRY" 서열을 추가로 포함할 수 있다. 일부 실시양태에서, TRY 서열은 ITR (예컨대, 5' ITR)과 단리된 핵산 또는 rAAV 벡터의 발현 구축물 (예컨대, 트랜스진 코딩 인서트) 사이에 위치한다.

[0074] 일부 측면에서, 본 개시내용은 본 개시내용에 의해 기술된 바와 같은 단리된 핵산 또는 rAAV 벡터를 포함하는 배큘로바이러스 벡터에 관한 것이다. 일부 실시양태에서, 배큘로바이러스 벡터는 예를 들어, 문헌 [Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43] 및 [Smith et al. (2009) *Mol Ther* 17(11):1888-1896]에 의해 기술된 바와 같은, 오토그라파 칼리포르니카 핵 다각체병 (AcNPV) 벡터이다.

[0075] 일부 측면에서, 본 개시내용은 본원에 기술된 바와 같은 단리된 핵산 또는 벡터를 포함하는 숙주 세포를 제공한다. 숙주 세포는 원핵 세포 또는 진핵 세포일 수 있다. 예를 들어, 숙주 세포는 포유동물 세포, 박테리아 세포, 효모 세포, 곤충 세포 등일 수 있다. 일부 실시양태에서, 숙주 세포는 포유동물 세포, 예를 들어, HEK293T 세포이다. 일부 실시양태에서, 숙주 세포는 박테리아 세포, 예를 들어, E. 콜라이(*E. coli*) 세포이다.

[0076] *rAAV*

[0077] 일부 측면에서, 본 개시내용은 본원에 기술된 바와 같은 핵산을 코딩하는 트랜스진을 포함하는 재조합 AAV (rAAV) (예컨대, 본원에 기술된 바와 같은 rAAV 벡터)에 관한 것이다. "rAAV"라는 용어는 일반적으로 하나 이상의 AAV 캡시드 단백질에 의해 캡시드화된 rAAV 벡터를 포함하는 바이러스 입자를 지칭한다. 본 개시내용에 의해 기술된 rAAV는 AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, 및 AAV10으로부터 선택되는 혈청형을 갖는 캡시드 단백질을 포함할 수 있다. 일부 실시양태에서, rAAV는 비-인간 숙주로부터의 캡시드 단백질, 예를 들어, 레수스 AAV 캡시드 단백질, 예컨대, AAVrh.10, AAVrh.39 등을 포함한다. 일부 실시양태에서, 본 개시내용에 의해 기술된 rAAV는 야생형 캡시드 단백질의 변이체인 캡시드 단백질, 예컨대, 유래 기점이 되는 야생형 AAV 캡시드 단백질 기준으로 적어도 1, 2, 3, 4, 5, 6, 7, 8, 9, 10개, 또는 10개 초과의 (예컨대, 15, 20, 25, 50, 100개 등) 아미노산 치환 (예컨대, 돌연변이)을 포함하는 캡시드 단백질 변이체를 포함한다.

[0078] 일부 실시양태에서, 본 개시내용에 의해 기술된 rAAV는 특히 CSF 공간 내로, 또는 뇌 실질 내로 직접 도입되었을 때, CNS를 통해 쉽게 확산된다. 따라서, 일부 실시양태에서, 본 개시내용에 의해 기술된 rAAV는 혈액-뇌 장벽 (BBB)을 건널 수 있는 캡시드 단백질을 포함한다. 예를 들어, 일부 실시양태에서, rAAV는 AAV9 또는

AAVrh.10 혈청형을 갖는 캡시드 단백질 포함한다. rAAV 제조는 예를 들어, 문헌 [Samulski et al. (1989) *J Virol.* 63(9):3822-8] 및 [Wright (2009) *Hum Gene Ther.* 20(7): 698-706]에 의해 기술되어 있다.

[0079] 일부 실시양태에서, 본 개시내용에 의해 기술된 바와 같은 rAAV (예컨대, AAV 캡시드 단백질에 의해 캡시드화되어 rAAV 캡시드 입자를 형성하는 재조합 rAAV 게놈을 포함하는 것)는 배큘로바이러스 벡터 발현 시스템 (BEVS)에서 제조된다. BEVS를 이용하여 rAAV를 제조하는 것은 예를 들어, 문헌 [Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43], [Smith et al. (2009) *Mol Ther* 17(11):1888-1896], 미국 특허 번호 8,945,918, 미국 특허 번호 9,879,282, 및 국제 PCT 공개 WO 2017/184879에 의해 기술되어 있다. 그러나, rAAV는 임의의 적합한 방법 (예컨대, 재조합 rep 및 cap 유전자를 이용하는 방법)을 이용하여 제조될 수 있다.

[0080] *제약 조성물*

[0081] 일부 측면에서, 본 개시내용은 본원에 기술된 바와 같은 단리된 핵산 또는 rAAV 및 제약상 허용되는 담체를 포함하는 제약 조성물을 제공한다. 본원에서 사용되는 바, "제약상 허용되는"이라는 용어는 물질, 예컨대, 담체 또는 희석제가 화합물의 생물학적 활성 또는 특성을 폐기하지 않고, 비교적 비독성이라는 것, 예컨대, 물질이 비바람직한 생물학적 효과를 유발하지 않거나, 또는 그와 함께 함유되어 있는 조성물의 성분 중 어느 것과도 유해한 방식으로 상호작용하지 않으면서, 개체에게 투여될 수 있다는 것을 지칭한다.

[0082] 본원에서 사용되는 바, "제약상 허용되는 담체"라는 용어는 본 발명 내에서 유용한 화합물이 그의 의도된 기능을 수행할 수 있도록 상기 화합물을 환자 내에 또는 환자에게 운반 또는 수송하는 데 관여하는, 제약상 허용되는 물질, 조성물 또는 담체, 예컨대, 액체 또는 고체 충전제, 안정제, 분산화제, 현탁화제, 희석제, 부형제, 증점제, 용매 또는 캡슐화 물질을 의미한다. 본 발명의 실시에서 사용되는 제약 조성물에 포함될 수 있는 추가 성분은 관련 기술분야에 공지되어 있고, 예를 들어, 문헌 [Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA)] (상기 문헌은 본원에서 참조로 포함된다)에 기술되어 있다.

[0083] 본원에서 제공된 조성물 (예컨대, 제약 조성물)은 장관 (예컨대, 경구적), 비경구적, 정맥내, 근육내, 동맥내, 골수내, 경막내, 피하, 뇌실내, 경피, 피부간(interdermal), 직장, 질내, 복강내, 국소 (분제, 연고, 크림제, 및/또는 점적제에 의한 경우), 점막, 비강, 협착, 설하를 비롯한, 임의의 경로에 의해; 기관내 주입, 기관지 주입, 및/또는 흡입에 의해; 및/또는 경구용 스프레이, 비강용 스프레이, 및/또는 에어로졸로서 투여될 수 있다. 경구 투여, 정맥내 투여 (예컨대, 전신 정맥내 주사), 혈액 및/또는 림프 공급을 통한 국부 투여, 및/또는 환부로의 직접 투여가 구체적으로 고려되는 경로이다. 일반적으로, 가장 적절한 투여 경로는 작용제의 성질 (예컨대, 위장관 환경에서의 그의 안정성), 및/또는 대상체의 상태 (예컨대, 대상체가 경구 투여를 허용할 수 있는지 여부)를 비롯한, 다양한 인자에 의존할 것이다. 특정 실시양태에서, 본원에 기술된 화합물 또는 제약 조성물은 대상체의 안구로의 국소 투여에 적합하다.

[0084] *방법*

[0085] 본 개시내용은 부분적으로 함께 (예컨대, 시너지적으로) 작용하여 파킨슨병을 치료하는, 대상체에서의 PD 연관 유전자 생성물의 조합의 발현을 위한 조성물에 기초한다. 본원에서 사용되는 바, "치료하다" 또는 "치료하는"이라는 것은 (a) 파킨슨병의 발병을 예방하거나, 또는 지연시키고/거나; (b) 파킨슨병의 중증도를 감소시키고/거나; (c) 파킨슨병의 특징적인 증상의 발생을 감소시키거나, 또는 예방하고/거나; (d) 파킨슨병의 특징적인 증상의 악화를 예방하는 것을 지칭한다. 파킨슨병의 증상으로는 예를 들어, 운동 기능장애 (예컨대, 떨림, 경직, 몸동작이 느려지는 서동, 보행 곤란), 인지 기능장애 (예컨대, 치매, 우울증, 불안), 감정 및 행동 기능장애를 포함한다.

[0086] 따라서, 일부 측면에서, 본 개시내용은 파킨슨병을 앓거나 또는 앓을 것으로 의심되는 대상체에게 본 개시내용에 의해 기술된 바와 같은 조성물 (예컨대, 단리된 핵산 또는 벡터 또는 rAAV를 포함하는 조성물)을 투여하는 단계를 포함하는, 파킨슨병을 앓거나 또는 앓을 것으로 의심되는 대상체를 치료하는 방법을 제공한다.

[0087] 일부 실시양태에서, 조성물은 대상체의 CNS로 직접, 예를 들어, 대상체의 뇌 및/또는 척수 내로의 직접 주사에 의해 투여된다. CNS 직접 투여 방식의 예로는 대뇌내 주사, 뇌실내 주사, 수조내 주사, 실질내 주사, 경막내 주사, 및 상기의 임의의 조합을 포함하나, 이에 제한되지 않는다. 일부 실시양태에서, 대상체의 CNS 내로 직접 주사하면, 그 결과로 트랜스진은 대상체의 중뇌, 선조체 및/또는 대뇌 피질에서 발현된다 (예컨대, 제1 유전자 생성물, 제2 유전자 생성물, 및 적용가능할 경우, 제3 유전자 생성물은 발현된다). 일부 실시양태에서, CNS 내로 직접 주사하면, 그 결과로 트랜스진은 대상체의 척수 및/또는 CSF에서 발현된다 (예컨대, 제1 유전자 생성물, 제2 유전자 생성물, 및 적용가능할 경우, 제3 유전자 생성물은 발현된다).

[0088] 일부 실시양태에서, 대상체의 CNS로의 직접 주사는 컨벡션 강화 전달법 (CED)을 포함한다. 컨벡션 강화 전달법은 뇌를 외과적으로 노출시키고, 뇌의 표적 부위 내로 직접 소경 카테터를 배치한 후, 이어서, 치료제 (예컨대, 본원에 기술된 바와 같은 조성물 또는 rAAV)를 대상체의 뇌로 직접 주입하는 것을 포함하는 치료 전략법이다. CED는 예를 들어, 문헌 [Debinski et al. (2009) *Expert Rev Neurother.* 9(10):1519-27]에 의해 기술되어 있다.

[0089] 일부 실시양태에서, 조성물은 대상체에게 예를 들어, 말초 주사에 의해 말초적으로 투여된다. 말초 주사의 예로는 피하 주사, 정맥내 주사, 동맥내 주사, 복강내 주사, 또는 상기의 임의의 조합을 포함한다. 일부 실시양태에서, 말초 주사는 동맥내 주사, 예를 들어, 대상체의 경동맥 내로의 주사이다.

[0090] 일부 실시양태에서, 본 개시내용에 의해 기술된 바와 같은 조성물 (예컨대, 단리된 핵산 또는 벡터 또는 rAAV를 포함하는 조성물)은 말초적으로 및 대상체의 CNS로 직접, 상기 두 방식 모두에 의해 투여된다. 예를 들어, 일부 실시양태에서, 대상체는 동맥내 주사에 의해 (예컨대, 경동맥 내로의 주사에 의해), 및 실질내 주사에 의해 (예컨대, CED에 의한 실질내 주사에 의해) 조성물을 투여받는다. 일부 실시양태에서, CNS로의 직접 주사 및 말초 주사는 동시에 이루어진다 (예컨대, 같은 시간에 수행된다). 일부 실시양태에서, 직접 주사는 말초 주사 이전에 (예컨대, 말초 주사 전 1분 내지 1주 사이에, 또는 그를 초과하는 시간에) 이루어진다. 일부 실시양태에서, 직접 주사는 말초 주사 이후에 (예컨대, 말초 주사 후 1분 내지 1주 사이에, 또는 그를 초과하는 시간에) 이루어진다.

[0091] 대상체에게 투여되는 본 개시내용에 의해 기술된 바와 같은 조성물 (예컨대, 단리된 핵산 또는 벡터 또는 rAAV를 포함하는 조성물)의 양은 투여 방법에 의존하여 달라질 것이다. 예를 들어, 일부 실시양태에서, 본원에 기술된 바와 같은 rAAV는 약 10^{9} 게놈 카피 (GC)/kg 내지 약 10^{14} GC/kg (예컨대, 약 10^{9} GC/kg, 약 10^{10} GC/kg, 약 10^{11} GC/kg, 약 10^{12} GC/kg, 약 10^{12} GC/kg, 또는 약 10^{14} GC/kg)의 역가로 대상체에게 투여된다. 일부 실시양태에서, 대상체는 CSF 공간으로의 주사에 의해, 또는 실질내 주사에 의해 고역가로 (예컨대, >10^{12} 게놈 카피 GC/kg (rAAV)) 투여받는다.

[0092] 본 개시내용에 의해 기술된 바와 같은 조성물 (예컨대, 단리된 핵산 또는 벡터 또는 rAAV를 포함하는 조성물)은 1회 또는 다회에 걸쳐 (예컨대, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20회 또는 그 초과) 대상체에게 투여될 수 있다. 일부 실시양태에서, 조성물은 대상체에게 연속하여 (예컨대, 만성적으로), 예를 들어, 주입 펌프를 통해 투여된다.

[0093] 실시예

[0094] *실시예 1: rAAV 벡터*

[0095] 삼중-플라스미드 형질감염을 위해 세포, 예컨대, HEK293 세포를 사용하여 AAV 벡터를 생성한다. ITR 서열은 각 관심 트랜스진에 대한 프로모터/인핸서 요소, 3' 폴리A 신호, 및 번역 후 신호, 예컨대, WPRE 요소를 포함하는 발현 구축물에 플랭킹된다. 단백질 서열의 융합에 의해; 또는 2A 펩티드 링커, 예컨대, T2A 또는 P2A 사용으로 펩티드 결합이 생성되지 못하게 방해됨에 따라 아미노산이 부가된 2개의 펩티드 단편이 유도됨으로써; IRES 요소를 사용하여; 또는 2개의 별개의 발현 카세트를 이용하는 발현에 의해 예컨대, *GBA1* 및 *LIMP2* 및/또는 프로사포신과 같은 다중 유전자 생성물이 동시에 발현될 수 있다. 발현 유전자 상류쪽의 효율적으로 스플라이싱된 짧은 인트론 서열의 존재가 발현 수준을 개선시킬 수 있다. shRNA 및 다른 조절 RNA가 잠재적으로 상기 서열 내에 포함될 수 있다. 본 개시내용에 의해 기술된 발현 구축물의 예는 도 1-8 및 21-35 및 하기 표 2에 제시되어 있다.

[0096] <표 2>

명칭	프로모터 1	shRNA	CDS1	폴리A 1	비시스트론성 요소	프로모터 2	CDS2	폴리A2	ITR 사이의 길이
CMVe_CBAp_GBA1_WPRE_bGH	CBA		GBA1	WPRE-bGH					3741
LT1s_JetLong_mRNAiaSYn_SCARB2-T2A-GBA1_bGH	JetLong	aSyn	SCARB2	bGH	T2A		GBA1		4215
LI1_JetLong_SCARB2-IRES-GBA1_bGH	JetLong		SCARB2	bGH	IRES		GBA1		4399
FP1_JetLong_GBA1_bGH_JetLong_SCARB2_SV40L	JetLong		GBA1	bGH		JetLong	SCARB2	SV40L	4464
PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-T2A-GBA1_bGH_4353nt	JetLong	aSyn	PSAP	bGH	T2A	-	GBA1	-	4353
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1_SymtheticpolyA_4337nt	JetLong	-	PSAP	합성 pA	IRES	-	GBA1	-	4337
PrevailVector_10s_JetLong_mRNAiaSy_GBA2_WPRE_bGH_4308nt	JetLong	aSyn	GBA2	WPRE_bGH	-	-	-	-	4308
PrevailVector_FT4_JetLong_GBA1_T2A_GALC_SyntheticpolyA_4373nt	JetLong	-	GBA1	합성 pA	T2A	-	GALC	-	4373
PrevailVector_LT4_JetLong_GALC_T2A_GBA1_SyntheticpolyA_4373nt	JetLong	-	GALC	합성 pA	T2A	-	GBA1	-	4373
PrevailVector_LT5s_JetLong_mRNAiaSyn_CTSB-T2A-	JetLong	aSyn	CTSB	WPRE_bGH	T2A	-	GBA1	-	4392

[0097]

[0098]

GBA1_WPRE_bGH_4392nt									
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_4477nt	JetLong	aSyn	GBA1	합성 pA	T2A	-	SMPD1	-	4477
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SymtheticpolyA_4820nt	JetLong	-	GALC	합성 pA	IRES	-	GBA1	-	4820
PrevailVector_FP5_JetLong_GBA1_bGH_JetLong_CTSB_SV40l_4108nt	JetLong	-	GBA1	bGH	-	JetLong	CTSB	SV40L	4108
PrevailVector_FT6s_JetLong_mRNAiaSyn_GBA1-T2A-GCH1_WPRE_bGH_4125nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	GCH1	-	4125
PrevailVector_LT7s_JetLong_mRNAiaSyn_RAB7L1-T2A-GBA1_WPRE_bGH_3984nt	JetLong	aSyn	RAB7L1	WPRE_bGH	T2A	-	GBA1	-	3984
PrevailVector_FI6s_JetLong_mRNAiaSYn_GBA1-IRES-GCH1_bGH_3978nt	JetLong	aSyn	GBA1	bGH	IRES	-	GCH1	-	3978
PrevailVector_9st_JetLong_mRNAiaSyn_mRNAiTMEM106B_VPS35_WPRE_bGH_4182nt	JetLong	aSyn & TMEM106B	VPS35	WPRE_bGH	-	-	-	-	4182
PrevailVector_FT12s_JetLong_mRNAiaSyn_GBA1-T2A-IL34_WPRE_bGH_4104nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	IL34	-	4104

[0099]

PrevailVector_FI12s_JetLong_mRNAiaSYn_GBA1-IRES-IL34_bGH_3957nt	JetLong	aSyn	GBA1	bGH	IRES	-	IL34	-	3957
PrevailVector_FP8_JetLong_GBA1_bGH_CD68_TREM2_SV40l_4253nt	JetLong	-	GBA1	bGH	-	CD68	TREM2	SV40L	4253
PrevailVector_FP12_CMVe_CBA_GBA1_bGH_JetLong_IL34_SV40l_4503nt	CBA		GBA1	bGH		JetLong	IL34	SV40L	4503

[0100] *실시예 2: GBA 결핍 세포로의 바이러스 형질도입에 관한 세포 기반 검정법*

[0101] 예를 들어, GD 환자 단핵구, 또는 hES 세포로부터의 섬유모세포, 또는 환자 유래의 유도 만능 줄기 세포 (iPSC)로서 *GBA1* 결핍 세포를 수득한다. 이들 세포는 기질, 예컨대, 글루코실세라미드 및 글루코실스핑고신 (GlcCer 및 GlcSph)을 축적한다. 야생형 또는 돌연변이체 배양 세포주를 Gcase 억제제, 예컨대, CBE로 처리하는 것 또한 GBA 결핍 세포를 수득하는 데 사용된다.

[0102] 상기 세포 모델을 이용하여, 해당 단백질에 대한 항체 또는 포스포-αSyn을 이용한 후, 형광 현미경법을 이용하여 영상화함으로써 단백질 응집체, 예컨대, α-시누클레인의 축적에 의해 리소좀 결함을 정량화한다. 단백질 마커, 예컨대, LAMP1, LAMP2, LIMP1, LIMP2에 대한 ICC에 의해, 또는 염료, 예컨대, 리소트래커(Lysotracker)

를 사용하여, 또는 형광성 덱스트란 또는 다른 마커의 세포내이입 구획을 통한 흡수에 의해 리소좀 이상에 대한 영상화 또한 수행한다. 리소좀과의 결함성 융합에 기인하는 자가포식 마커 축적에 대한, 예컨대 LC3에 대한 영상화 또한 수행될 수 있다. 웨스턴 블롯 및/또는 ELISA는 상기 마커의 비정상적인 축적을 정량화하는 데 사용된다. 또한, 당지질 기질 및 GBA1의 생성물의 축적은 표준 접근법을 이용하여 측정된다.

[0103] 치료 종점 (예컨대, PD 연관 병상의 감소)은 활성 및 기능을 확인하고, 정량화하기 위해 AAV 벡터의 형질도임의 발현과 관련하여 측정된다. Gcase는 또한 단백질 ELISA 측정을 사용하여, 또는 표준 Gcase 활성 검정법에 의해 정량화될 수 있다.

[0104] *실시예 3: 돌연변이체 마우스를 이용한 생체내 검정법*

[0105] 본 실시예는 돌연변이체 마우스를 이용한 AAV 벡터에 관한 생체내 검정법을 기술한다. 돌연변이체 마우스에서의 상기와 같은 AAV 벡터에 관한 생체내 연구는 예를 들어, 문헌 [Liou *et al.* (2006) *J. Biol. Chem.* 281(7): 4242-4253], [Sun *et al.* (2005) *J. Lipid Res.* 46:2102-2113], 및 [Farfel-Becker *et al.* (2011) *Dis. Model Mech.* 4(6):746-752]에 의해 기술된 검정법을 이용하여 수행된다.

[0106] 예를 들어, 5-10 ㎕의 주사 부피로 진한 AAV 스톡을 사용하여 (예컨대, $2x10^{11}$ vg/마우스 용량의) 비히클 대조군 및 AAV 벡터의 경막내 또는 뇌실내 전달을 수행한다. 컨벡션 강화 전달법에 의한 실질내 전달을 수행한다.

[0107] 처리는 증상 발병 이전 또는 발병 이후에 개시한다. 측정되는 종점은 CNS 및 CSF 중 기질 축적, ELISA에 의한 Gcase 효소 축적 및 효소 활성의 축적, 운동 및 인지 종점, 리소좀 기능장애, 및 α-시누클레인 단량체, 원시섬유 또는 원섬유의 축적이다.

[0108] *실시예 4: 질환의 화학적 모델*

[0109] 본 실시예는 화학적으로 유도된 고세병을 앓는 마우스 모델 (예컨대, CBE 마우스 모델)을 이용하는 AAV 벡터에 관한 생체내 검정법을 기술한다. 예를 들어, 문헌 [Vardi *et al.* (2016) *J Pathol.* 239(4):496-509]에 기술된 바와 같이, 화학적으로 유도된 고세병을 앓는 마우스 모델에서 상기 AAV 벡터에 관한 생체내 연구를 수행한다.

[0110] 예를 들어, 5-10 ㎕의 주사 부피로 진한 AAV 스톡을 사용하여 (예컨대, $2x10^{11}$ vg/마우스의) 비히클 대조군 및 AAV 벡터의 경막내 또는 뇌실내 전달을 수행한다. 컨벡션 강화 전달법에 의한 실질내 전달을 수행한다. 꼬리 정맥 주사에 의해 말초 전달을 달성한다.

[0111] 처리는 증상 발병 이전 또는 발병 이후에 개시한다. 측정되는 종점은 CNS 및 CSF 중 기질 축적, ELISA에 의한 Gcase 효소 축적 및 효소 활성의 축적, 운동 및 인지 종점, 리소좀 기능장애, 및 α-시누클레인 단량체, 원시섬유 또는 원섬유의 축적이다.

[0112] *실시예 5: PD, LBD, 고세병 환자에서의 임상 시험*

[0113] 일부 실시양태에서, 특정 형태의 고세병 (예컨대, GD1)을 앓는 환자는 파킨슨병 (PD) 또는 루이소체 치매 (LBD)가 발생할 위험이 증가되어 있다. 본 실시예는 고세병, PD 및/또는 LBD를 앓는 환자에서 본 개시내용에 의해 기술된 바와 같은 rAAV의 안전성 및 효능을 사정하는 임상 시험을 기술한다.

[0114] 문헌 [Grabowski et al. (1995) *Ann. Intern. Med.* 122(1):33-39]에 기술된 것과 유사한 연구 디자인을 사용하여 고세병, PD 및/또는 LBD 치료를 위한 상기 벡터의 임상 시험을 수행한다.

[0115] *실시예 6: 말초 질환 치료*

[0116] 일부 실시양태에서, 예를 들어, 문헌 [Biegstraaten *et al.* (2010) *Brain* 133(10):2909-2919]에 기술된 바와 같이, 특정 형태의 고세병을 앓는 환자는 말초 신경병증의 증상을 보인다.

[0117] 본 실시예는 고세병 (예컨대, 타입 1 고세병)과 연관된 말초 신경병증의 치료를 위한 본원에 기술된 바와 같은 AAV 벡터에 관한 생체내 검정법을 기술한다. 간략하면, 말초 신경병증의 징후 또는 증상을 앓는 것으로 확인된 타입 1 고세병 환자는 본 개시내용에 의해 기술된 바와 같은 rAAV를 투여받는다. 일부 실시양태에서, rAAV의 투여 후에, 예를 들어, 문헌 [Biegstraaten *et al.*]에 기술된 방법을 사용하여 대상체의 말초 신경병증 징후 및 증상을 모니터링한다.

[0118] 환자에 (예컨대, 환자의 혈청에, 환자의 말초 조직에 (예컨대, 간 조직, 비장 조직 등에) 존재하는 본 개시내용에 의해 기술된 바와 같은 형질도입된 유전자 생성물의 수준을 예를 들어, 웨스턴 블롯 분석, 효소 기능성 검정

법, 또는 영상화 연구에 의해 검정한다.

[0119] *실시예 7: CNS 형태의 치료*

[0120] 본 실시예는 CNS 형태의 고셰병의 치료를 위한 본원에 기술된 바와 같은 rAAV 벡터에 관한 생체내 검정법을 기술한다. 간략하면, CNS 형태의 고셰병 (예컨대, 타입 2 또는 타입 3 고셰병)을 앓는 것으로 확인된 고셰병 환자는 본 개시내용에 의해 기술된 바와 같은 rAAV를 투여받는다. 환자의 CNS에 (예컨대, 환자의 CNS의 혈청에, 환자의 뇌척수액 (CSF)에, 또는 환자의 CNS 조직에) 존재하는 본 개시내용에 의해 기술된 바와 같은 형질도입된 유전자 생성물의 수준을 예를 들어, 웨스턴 블롯 분석, 효소 기능성 검정법, 또는 영상화 연구에 의해 검정한다.

[0121] *실시예 8: GBA1에 돌연변이를 갖는 대상체에서 파킨슨병의 유전자 요법*

[0122] 본 실시예는 *GBA1* 중의 돌연변이를 특징으로 하는 파킨슨병을 앓는 대상체에게 *GBA1*을 코딩하는 재조합 아데노-연관 바이러스 (rAAV)를 투여하는 것을 기술한다.

[0123] rAAV-GBA1 벡터 인서트는, 인간 GBA1 (마룬)의 코돈 최적화된 코딩 서열 (CDS)을 구성적으로 발현하기 위해, 4개 파트: CMV 인핸서 (CMVe), CBA 프로모터 (CBAp), 엑손 1, 및 인트론 (int)으로 이루어진, CBA 프로모터 요소 (CBA)를 함유한다. 3' 영역은 또한 우드척 간염 바이러스 전사후 조절 요소(Woodchuck hepatitis virus Posttranscriptional Regulatory Element: WPRE), 전사후 조절 요소, 이어서, 소 성장 호르몬 폴리A 신호 (bGH 폴리A) 테일을 함유한다. 플랭킹 ITR을 통해 개재 서열은 정확하게 패키징될 수 있다. 5' ITR 서열의 두 변이체 (도 7, 삽입 박스, 하단 서열)를 평가하였다: 상기 변이체들은 ITR의 20-뉴클레오티드 "D" 영역 내에서 수개의 뉴클레오티드 차이를 보이며, 이는 패키징 및 발현 효율에 영향을 미치는 것으로 간주된다. rAAV-GBA1 벡터 생성물은 도 7 (삽입 박스, 상단 서열)에 제시된 "D" 도메인 뉴클레오티드 서열을 함유한다. 변이체 벡터는 임상전 연구에서 유사하게 수행된, 돌연변이체 "D" 도메인 (본원에서 "S" 도메인으로 명명, 여기서 뉴클레오티드 변이는 음영 표시로 제시)을 보유한다. 백본은 카나마이신에 대한 내성을 부여하는 유전자 뿐만 아니라, 역 패키징을 막는 스터퍼(stuffer) 서열을 함유한다. rAAV-GBA1 벡터를 도시하는 개략도는 도 8에 제시되어 있다. rAAV-GBA1 벡터는 AAV9 혈청형 캡시드 단백질을 이용하여 rAAV로 패키징된다.

[0124] 대수조 내로의 (대수조내; ICM) 투시 조영 유도하의 후두하 주사를 통해 rAAV-GBA1을 단일 용량으로 대상체에게 투여한다. rAAV-GBA1 투약 요법 연구의 한 실시양태는 하기와 같다:

[0125] 단일 용량의 rAAV-GBA1을, 비임상 약리학 및 독성 연구의 결과에 기초하여 결정된 두 용량 수준 (3e13 vg (저용량); 1e14 vg (고용량) 등) 중 하나로 환자 (N=12)에게 투여한다.

[0126] 초기 연구는 rAAV-GBA1 벡터 및 rAAV-GBA1 S-변이체 구축물 (하기에서 추가로 기술)의 효능 및 안전성을 사정하기 위해, GCase의 억제제인 콘두리톨-b-에폭시드 (CBE)를 매일 전달하는 것을 포함하는 화학적 마우스 모델에서 수행하였다. 추가로, 동형접합성 *GBA1* 돌연변이를 보유하고, 사포신 (4L/PS-NA)이 부분적으로 결핍되어 있는 유전적 마우스 모델에서도 초기 연구를 수행하였다. 벡터 안전성 및 효능을 추가로 평가하기 위해 마우스 및 비인간 영장류 (NHP)에서 추가의 용량 범위 연구를 수행한다.

[0127] AAV 백본 중의 약간 다른 두 버전의 5' 역전된 말단 반복부 (ITR)를 시험하여 제조가능성 및 트랜스진 발현을 사정하였다 (도 7). 145 bp 5' ITR 내의 20 bp "D" 도메인은 최적의 바이러스 벡터 제조를 위해 필요한 것으로 간주되지만, "D" 도메인 내의 돌연변이 또한 일부 경우에 트랜스진 발현을 증가시키는 것으로 보고된 바 있다. 따라서, 무손상 "D" 도메인을 보유하는 바이러스 벡터 rAAV-GBA1 이외에, 돌연변이체 D 도메인 (본원에서 "S" 도메인으로 명명)을 포함하는 제2 벡터 형태 또한 평가하였다. rAAV-GBA1 및 변이체, 둘 모두 동일한 트랜스진을 발현한다. 하기에서 상세하게 설명되는 바와 같이, 상기 두 벡터 모두 생체내에서 효과적인 바이러스를 생산한 반면, 야생형 "D" 도메인을 함유하는 rAAV-GBA1은 추가 개발을 위해 선택하였다.

[0128] GCase 결핍인 CBE 모델을 확립하기 위해, 어린 마우스에 GCase의 특정 억제제인 CBE를 투약하였다. 생후 8일째 (P8)를 시작으로 매일 IP 주사에 의해 CBE를 마우스에 제공하였다. 행동 표현형을 보이는 모델을 확립하기 위해 3개의 상이한 CBE 용량 (25 mg/kg, 37.5 mg/kg, 50 mg/kg) 및 PBS를 시험하였다 (도 9). 더 높은 고용량의 CBE는 용량에 의존하는 방식으로 치사에 이르게 하였다. 50 mg/kg CBE로 처리된 마우스는 모두 P23까지 사망하였고, 37.5 mg/kg CBE로 처리된 8마리의 마우스 중 5마리는 P27까지 사망하였다. 25 mg/kg CBE로 처리된 마우스 중에서 치사는 없었다. CBE 주사맞은 마우스는 오픈 필드 검정법에서 일반적인 운동 장애를 보이지 않은 반면 (PBS를 받은 마우스와 동일한 속도로 동일한 거리를 이동), CBE로 처리된 마우스는 로타로드 검정법에 의해

측정된 바, 운동 협조 및 균형 장애를 보였다.

[0129] 연구 종료시까지 살아남은 마우스를 그의 최종 CBE 투약 후 그 다음날 (P27, "1일째"), 또는 CBE 중단 3일 후 (P29, "3일째") 희생시켰다. 1일째 및 3일째 코호트, 둘 모두에서 GCase 기질의 축적을 평가하기 위해 25 mg/kg CBE를 받은 마우스의 피질에서 지질 분석을 수행하였다. (본 실시예에서 응집체로 측정된) GluSph 및 GalSph 수준은, GCase 부전증과 일치하는 PBS로 처리된 대조군과 비교하였을 때, CBE로 처리된 마우스에서 유의적으로 축적되었다.

[0130] 상기 기술된 연구에 기초하여, 25 mg/kg CBE 용량은 생존에는 영향을 주지 않으면서, 행동 장애를 일으켰는 바, 이에, 상기 용량을 선택하였다. CBE 처리 동안 뇌 전역에 걸쳐 GBA1을 광범위하게 분포시키고, 트랜스진을 발현시키기 위해, 생후 3일째 (P3) 뇌실내 (ICV) 주사에 의해 rAAV-GBA1 또는 부형제를 전달한 후, 이어서, P8에 IP CBE 또는 PBS의 매일 처리를 개시하였다 (도 10).

[0131] rAAV-GBA1을 받은, CBE로 처리된 마우스는 로타로드에서 부형제를 받은 것보다 통계학상 유의적으로 더욱 우수한 수행능을 보였다 (도 11). 변이체 처리군의 마우스는 다른 행동 척도, 예컨대, 검사 동안 이동한 총 거리에 있어서는 부형제로 처리된 마우스와 다르지 않았다 (도 11).

[0132] 생전 연구 완료시, 생화학적 분석을 위해, 마우스 중 절반을 최종 CBE 투약 후 그 다음날 (P36, "1일째"), 또는 CBE 중단 3일 후 (P38, "3일째") 희생시켰다 (도 12). 생물학적으로 삼중으로 수행된 형광분석 효소 검정법을 이용하여, 피질에서 GCase 활성을 사정하였다. rAAV-GBA1로 처리된 마우스에서는 GCase 활성이 증가한 반면, CBE 처리는 GCase 활성을 감소시켰다. 추가로, CBE 및 rAAV-GBA1, 둘 모두를 받은 마우스는 PBS 처리군과 유사한 GCase 활성 수준을 보였는데, 이는 rAAV-GBA1 전달이 CBE 처리에 의해 유도된 GCase 활성 억제를 극복할 수 있다는 것을 시사하는 것이다. 기질 GluCer 및 GluSph의 수준을 조사하기 위해 마우스의 운동 피질에서 지질 분석을 수행하였다. 두 지질 모두 CBE를 받은 마우스의 뇌에 축적되었고, rAAV-GBA1 처리는 기질 축적을 유의적으로 감소시켰다.

[0133] 지질 수준은 처리군 간에 GCase 활성 및 로타로드에서의 수행능, 둘 모두와 음의 상관관계를 가졌다. rAAV-GBA1 투여 후 GCase 활성 증가는 기질 감소 및 운동 기능 증진과 연관이 있었다 (도 13). 도 14에 제시된 바와 같이, qPCR에 의해 측정된 바, 벡터 게놈 존재에 의해 예비 생체분포를 사정하였다 (여기서 게놈 DNA 1 μg당 >100 벡터 게놈인 것을 양성인 것으로 정의한다). CBE로 처리 및 비처리된, rAAV-GBA1을 받은 마우스, 둘 모두 피질에서 rAAV-GBA1 벡터 게놈에 대해 양성이었고, 이는 ICV 전달을 통해 rAAV-GBA1이 피질로 전달된다는 것을 시사하는 것이다. 추가로, 벡터 게놈이 간에서 검출되었고, 비장에서는 거의 검출되지 않았고, 심장, 신장 또는 생식선에서는 전혀 검출되지 않았다. 모든 척도에서 1일째 군과 3일째 군 사이에 통계학상 유의적인 차이는 없었다.

[0134] CBE 모델에서의 좀더 큰 대규모 연구를 통해 CBE 모델에서 rAAV-GBA1의 유효 용량을 추가로 조사하였다. 25 mg/kg CBE 용량 모델을 이용하여, 부형제 또는 rAAV-GBA1을 P3에 ICV를 통해 전달하고, P8에 IP PBS 또는 CBE의 매일 처리를 개시하였다. 이전 연구에서 CBE를 중단한 군과 CBE를 중단하지 않은 군 사이에 유사성이 관찰되었다는 점을 고려하여, 모든 마우스를 최종 CBE 투약 (P38-40) 후 다음날 희생시켰다. 군당 마우스 10마리씩 (5M/5F), 하기 5개 군으로 하여, 3개의 상이한 rAAV-GBA1 용량의 효과를 사정하였다:

[0135] 부형제 ICV + PBS IP

[0136] 부형제 ICV + 25 mg/kg CBE IP

[0137] 3.2e9 vg (2.13e10 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP

[0138] 1.0e10 vg (6.67e10 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP

[0139] 3.2e10 vg (2.13e11 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP.

[0140] 최고 용량의 rAAV-GBA1은 P37에 CBE 처리와 관련된 체중 증가 불능을 구제하였다. 추가로, 부형제 + CBE 처리군과 비교하였을 때, 상기 용량은 로타로드 및 테이퍼 빔에서의 수행능을 통계학상 유의적으로 증가시켰다 (도 15). 부형제 처리군 및 rAAV-GBA1 처리군, 둘 모두를 포함하는, 여러 군에서 치사가 관찰되었다 (부형제 + PBS: 0; 부형제 + 25 mg/kg CBE: 1; 3.2e9 vg rAAV-GBA1+ 25 mg/kg CBE: 4; 1.0e10 vg rAAV-GBA1+ 25 mg/kg CBE: 0; 3.2e10 vg rAAV-GBA1+ 25 mg/kg CBE: 3).

[0141] 생전 연구 완료시, 생화학적 분석을 위해, 마우스를 희생시켰다 (도 16). 형광분석 검정법에 의해 생물학적으

로 삼중으로 피질에서 GCase 활성을 사정하였다. CBE로 처리된 마우스는 감소된 GCase 활성을 보인 반면, 고용량의 rAAV-GBA1을 받은 마우스는 CBE 처리군과 비교하였을 때, GCase 활성의 통계학상 유의적인 증가를 보였다. CBE로 처리된 마우스는 또한 GluCer 및 GluSph의 축적을 보였으며, 이 둘 모두는 고용량의 rAAV-GBA1 투여에 의해 구제되었다.

[0142] 확립된 화학적 CBE 모델에 추가로, Gba1에서의 V394L GD 돌연변이에 대해 동형접합성이고, 또한 GCase 국재화 및 활성에 영향을 주는 사포신이 부분적으로 결핍되어 있는 4L/PS-NA 유전적 모델에서도 또한 rAAV-GBA1을 평가한다. 상기 마우스는 빔 워크, 로타로드, 및 와이어 행(wire hang) 검정법에서의 그의 수행능에 의해 입증되는 바와 같이, 운동 강도, 협조, 및 균형 장애를 보인다. 전형적으로, 이들 마우스의 수명은 22주 미만이다. 초기 연구에서, P23에 3 ㎕의 최대 역가 바이러스를 ICV에 의해 전달하였고, 여기서 최종 용량은 2.4e10 vg (6.0e10 vg/g 뇌)였다. 군당 마우스 6마리씩이며, 처리군은 하기와 같았다:

[0143] WT + 부형제 ICV

[0144] 4L/PS-NA + 부형제 ICV

[0145] 4L/PS-NA + 2.4e10 vg (6.0e10 vg/g 뇌) rAAV-GBA1 ICV.

[0146] rAAV-GBA1 전달 후 4주째에 빔 워크 검사에 의한 운동 수행능을 사정하였다. rAAV-GBA1을 받은 돌연변이체 마우스 군은, 운동 기능은 거의 WT 수준으로까지 회복되면서, 부형제로 처리된 돌연변이체 마우스와 비교하였을 때, 총 슬립수는 더 적고, 속도당 슬립수도 더 적은 경향을 보였다 (도 17). 운동 표현형은 상기 마우스가 노화됨에 따라 점점 더 심각해지기 때문에, 상기 행동 및 다른 행동 검사에서의 그의 수행능은 더 늦은 후속 시점에 사정한다. 생전 연구 완료시, 상기 마우스에서 지질 수준, GCase 활성, 및 생체분포를 사정한다.

[0147] 0.03x, 0.1x, 및 1x 제안된 1상의 임상 고용량에 상응하는, 추가의 더 낮은 용량의 rAAV-GBA1을 CBE 모델을 이용하여 현재 시험하고 있다. 각 군은 군당 마우스 10마리씩 (5M/5F) 포함한다:

[0148] 부형제 ICV

[0149] 부형제 ICV + 25 mg/kg CBE IP

[0150] 3.2e8 vg (2.13e9 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP

[0151] 1.0e9 vg (6.67e9 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP

[0152] 1.0e10 vg (6.67e10 vg/g 뇌) rAAV-GBA1 ICV + 25 mg/kg CBE IP.

[0153] 운동 표현형 이외에도, 피질에서 지질 수준 및 GCase 활성을 사정한다. 시간 경과에 따른 처리 및 분석 또한 수행한다.

[0154] 효능 및 안전성 데이터를 평가하기 위해 더 큰 용량 범위의 연구를 개시하였다. 10마리의 4L/PS-NA 마우스 (군당 5M/5F)에 10 ㎕의 rAAV-GBA1을 주사하였다. 상대 성장 측정 뇌 중량 계산을 이용하면, 용량은 0.15x, 1.5x, 4.4x, 및 14.5x 제안된 1상의 임상 고용량과 상관관계가 있다. 주사군은 하기 군으로 이루어진다:

[0155] WT + 부형제 ICV

[0156] 4L/PS-NA + 부형제 ICV

[0157] 4L/PS-NA + 4.3e9 vg (1.1e10 vg/g 뇌) rAAV-GBA1 ICV

[0158] 4L/PS-NA + 4.3e10 vg (1.1e11 vg/g/ 뇌) rAAV-GBA1 ICV

[0159] 4L/PS-NA + 1.3e11 vg (3.2e11 vg/g 뇌) rAAV-GBA1 ICV

[0160] 4L/PS-NA + 4.3e11 vg (1.1e12 vg/g 뇌) rAAV-GBA1 ICV.

[0161] CBE 모델에서의 비임상 연구에 관한 요약은 하기 표 3에 제시되어 있다.

[0162] <표 3>

[0163] CBE 마우스 모델에서의 결과 요약

시험 물질	연구 번호	용량 코호트	행동 변화			지질	효소	BD	
			로타로드	테이퍼 빔	오픈 필드			뇌	간
rAAV-GBA1	CBE 모델에서의 PRV-2018-005 용량 범위 rAAV-GBA1	3.2e9 vg (2.13e10 vg/g 뇌)	NS	NS	NS	NS	NS	+	-
		1.10e10 vg (6.67e10 vg/g 뇌)	T	NS	NS	T/S	NS	+	+
		2.3e10vg (2.13e11 vg/g 뇌)	S	S	NS	S	S	+	+
변이체	CBE 모델에서의 PRV-2018-005 용량 범위 변이체	8.8e9 vg (5.9e10 vg/g 뇌)	S	N/A	NS	S	S	+	+

[0164]

[0165] 양성 생체분포는 >100 vg/1 μg 게놈 DNA인 것으로 정의된다는 점에 주의한다.

[0166] 약어: BD = 생체분포; NS = 비유의적; T = 경향; S = 유의적; N/A = 적용불가능; + = 양성; - = 음성.

[0167] *실시예 9: rAAV 벡터의 시험관내 분석*

[0168] rAAV 구축물을 시험관내 및 생체내에서 시험하였다. 도 18은 프로그래뉼린 (PGRN) 단백질을 코딩하는 rAAV 구축물의 시험관내 발현에 대한 대표적인 데이터를 보여주는 것이다. 좌측 패널은 프로그래뉼린 (PGRN) ELISA 검정법의 표준 곡선을 보여주는 것이다. 하단 패널은 rAAV가 형질도입된 HEK293T 세포의 세포 용해물 중에서 ELISA 검정법에 의해 측정된 PGRN 발현의 용량-반응을 보여주는 것이다. MOI = 감염 다중도 (세포당 벡터 게놈).

[0169] 프로사포신 (*PSAP*) 및 *SCARB2*를 단독으로, 또는 *GBA1* 및/또는 하나 이상의 억제성 RNA와 함께 조합하여 코딩하는 rAAV 벡터의 시험관내 활성을 사정하는 예비 연구를 수행하였다. PSAP 및 프로그래뉼린 (PGRN)을 코딩하는 한 구축물도 시험하였다. 시험된 벡터는 표 4에 제시된 것을 포함한다. "Opt"는 포유동물 세포 (예컨대, 인간 세포)에서의 발현을 위해 코돈 최적화된 핵산 서열을 지칭한다. 도 19는 HEK293 세포를 각 구축물로 형질감염시켰을 때, 모의 형질감염된 세포와 비교하여 상응한 유전자 생성물이 과다발현되었다는 것을 나타내는 대표적인 데이터를 보여주는 것이다.

[0170] TREM2를 단독으로, 또는 하나 이상의 억제성 RNA와 함께 조합하여 코딩하는 rAAV 벡터의 시험관내 활성을 사정하는 예비 연구를 수행하였다. 시험된 벡터는 표 4에 제시된 것을 포함한다. "Opt"는 포유동물 세포 (예컨대, 인간 세포)에서의 발현을 위해 코돈 최적화된 핵산 서열을 지칭한다. 도 36a-36b는 HEK293 세포를 각 구축물로 형질감염시켰을 때, 모의 형질감염된 세포와 비교하여 상응한 유전자 생성물이 과다발현되었다는 것을 나타내는 대표적인 데이터를 보여주는 것이다.

[0171] <표 4>

ID	프로모터	억제성 RNA	프로모터	트랜스진
I00015	JL_인트론	*SCNA*	JetLong	Opt-PSAP_GBA1
I00039	-	-	JetLong	Opt-PSAP-GRN
I00046	-	-	CBA	Opt-PSAP
I00014	JetLong	*SCNA*	JetLong	Opt-SCARB2_GBA1
I00040			JL, CD68	opt-GBA1, TREM2

[0172]

[0173] *실시예 10: SCNA 및 TMEM106B shRNA 구축물 시험*

[0174] *HEK293 세포*

[0175] 본 연구에서는 인간 배아 신장 293 세포주 (HEK293) (#85120602, 시그마-알드리치(Sigma-Aldrich)를 사용하였다. HEK293 세포를, 100 유닛/ml 페니실린 및 100 ㎍/ml 스트렙토마이신 (#15140122, 써모 피셔 사이언티픽(Thermo Fisher Scientific))을 함유하는, 10% 우태아 혈청 [FBS] [#10082147, 써모 피셔 사이언티픽])으로 보충된 배양 배지 (D-MEM [#11995065, 써모 피셔 사이언티픽] 중에서 유지시켰다.

[0176] *플라스미드 형질감염*

[0177] 제조사의 설명서에 따라 리포펙타민(Lipofectamine) 2000 형질감염 시약 (#11668019, 써모 피셔 사이언티픽)을 이용하여 플라스미드 형질감염을 수행하였다. 간략하면, HEK293 세포 (#12022001, 시그마-알드리치)를 항생제가 없는 배양 배지 중에 $3x10^5$개의 세포/ml인 밀도로 플레이팅하였다. 다음날, 플라스미드 및 리포펙타민 2000 시약을 옵티-MEM(Opti-MEM) 용액 (#31985062, 써모 피셔 사이언티픽) 중에서 조합하였다. 5분 후, 혼합물을 HEK293 배양물에 첨가하였다. 72시간 후, RNA 또는 단백질 추출을 위해 세포를 수거하거나, 또는 세포에 대해 영상화 분석을 수행하였다. 영상화 분석을 위해, 세포 플레이팅 이전에 플레이트를 0.01% 폴리-L-리신 용액 (P8920, 시그마-알드리치)으로 미리 코팅하였다.

[0178] *정량적 실시간 PCR (qRT-PCR)에 의한 유전자 발현 분석*

[0179] 제조사의 설명서에 따라 파워 SYBR 그린 셀-투-CT 키트(Power SYBR Green Cells-to-CT Kit) (#4402955, 써모 피셔 사이언티픽)를 이용하여 정량적 실시간 PCR (qRT-PCR)에 의해 상대적인 유전자 발현 수준을 측정하였다. 리포펙타민 2000 형질감염 시약을 이용하여 48 웰 플레이트 상에 플레이팅된 HEK293 세포 ($7.5x10^4$개의 세포/웰) 내로 후보 플라스미드를 일시적으로 형질감염시켰다 (50 ㎕ 옵티-MEM 용액 중 0.5 ㎍ 플라스미드 및 1.5 ㎕ 시약). 72시간 후, 세포로부터 RNA를 추출하고, 역전사를 위해 사용하여 제조사의 설명서에 따라 cDNA를 합성하였다. 정량적 PCR 분석을 위해, 파워 SYBR 그린 PCR 마스터 믹스(Power SYBR Green PCR Master Mix) (#4367659, 써모 피셔 사이언티픽)와 함께 유전자 특이적 프라이머 쌍 (최종 농도 250 nM)을 이용하여 2~5 ㎕의 cDNA 생성물을 이중으로 증폭시켰다. *SNCA*, *TMEM106B*, 및 *GAPDH* 유전자에 대한 프라이머 서열은 5'- AAG AGG GTG TTC TCT ATG TAG GC -3' (서열식별번호 71), 5'- GCT CCT CCA ACA TTT GTC ACT T -3' (서열식별번호 72) (*SNCA*에 대한 것), 5'-ACA CAG TAC CTA CCG TTA TAG CA-3' (서열식별번호 73), 5'-TGT TGT CAC AGT AAC TTG CAT CA-3' (서열식별번호 74) (*TMEM106B*에 대한 것), 및 5'- CTG GGC TAC ACT GAG CAC C -3' (서열식별번호 75), 5'- AAG TGG TCG TTG AGG GCA ATG -3' (서열식별번호 76) (*GAPDH*에 대한 것)이었다. 콴트스튜디오 3 리얼-타임 PCR 시스템(QuantStudio 3 Real-Time PCR system) (써모 피셔 사이언티픽)에서 정량적 PCR을 수행하였다. 하우스키핑 유전자 *GAPDH*에 의해 발현 수준을 정규화하였고, 비교 CT 방법을 이용하여 계산하였다.

[0180] *형광 영상화 분석*

[0181] *SNCA* 및 *TMEM106B* 넉다운 플라스미드를 확인하는 데 EGFP 코딩 영역 하류의 인간 *SNCA* 유전자의 3'-UTR을 함유하는 EGFP 리포터 플라스미드를 사용하였다. EGFP 리포터 플라스미드 및 후보 넉다운 플라스미드를 리포펙타민 2000 형질감염 시약을 이용하여, 폴리-L-리신으로 코팅된 96 웰 플레이트 상에 플레이팅된 HEK293 세포 ($3.0 x10^4$개의 세포/웰) 내로 동시에 형질감염시켰다 (10 ㎕ 옵티-MEM 용액 중 0.04 ㎍ 리포터 플라스미드, 0.06 ㎍

넉다운 플라스미드 및 0.3 ㎕ 시약). 72시간 후, 배리오스칸 LUX(Varioskan LUX) 다중모드 판독기 (써모 피셔 사이언티픽)를 이용하여 여기 488 nm/방출 512 nm에서 EGFP 신호의 형광 강도를 측정하였다. 세포를 RT에서 10분 동안 4% PFA로 고정시키고, 40 ㎍/ml 7-아미노액티노마이신 D (7-AAD)를 함유하는 D-PBS와 함께 RT에서 30 min 동안 인큐베이션시켰다. D-PBS로 세척한 후, 배리오스칸 판독기를 이용하여 여기 546 nm/방출 647 nm에서 7-AAD 신호의 형광 강도를 측정하여 세포 개수를 정량화하였다. 7-AAD 신호 수준에 대해 정규화된 EGFP 신호를 대조군 넉다운 샘플과 비교하였다.

[0182] *효소-결합된 면역흡착 검정법 (ELISA)*

[0183] 단백질 수준으로 넉다운 플라스미드를 확인하는 데 *SNCA* 코딩 영역 하류의 인간 *SNCA* 유전자 또는 *TMEM106B* 유전자의 3'-UTR을 함유하는 α-시누클레인 리포터 플라스미드를 사용하였다. HEK293 세포로부터 추출된 용해물을 이용하여 ELISA (#KHB0061, 써모 피셔 사이언티픽)에 의해 α-시누클레인 단백질의 수준을 측정하였다. 후보 플라스미드를 리포펙타민 2000 형질감염 시약을 이용하여, 48 웰 플레이트 상에 플레이팅된 HEK293 세포 (7.5×10^4개의 세포/웰) 내로 일시적으로 형질감염시켰다 (25 ㎕ 옵티-MEM 용액 중 0.1 ㎍ 리포터 플라스미드, 0.15 ㎍ 넉다운 플라스미드 및 0.75 ㎕ 시약). 72시간 후, 프로테아제 억제제 칵테일 (#P8340, 시그마-알드리치)로 보충된 방사성면역침전 검정법 (RIPA) 완충제 (#89900, 써모 피셔 사이언티픽) 중에서 세포를 용해시키고, 몇 초 동안 초음파 처리하였다. 얼음 상에서 30 min 동안 인큐베이션시킨 후, 용해물을 4℃에서 15 min 동안 20,000 x g로 원심분리하고, 상청액을 수집하였다. 단백질 수준을 정량화하였다. 배리오스칸 플레이트 판독기 중 450 nm에서 플레이트를 판독하고, 소프트맥스 프로 5(SoftMax Pro 5) 소프트웨어를 이용하여 농도를 계산하였다. 측정된 단백질 농도를 비신코닌산 검정법 (#23225, 써모 피셔 사이언티픽)을 이용하여 측정된 전체 단백질 농도로 정규화하였다.

[0184] 도 37 및 하기 표 5는 GFP 리포터 검정법 (상단) 및 α-Syn 검정법 (하단)에 의해, 시험관내에서 *SCNA*의 침묵화가 성공적으로 이루어졌다는 것을 나타내는 대표적인 데이터를 보여주는 것이다. 도 38 및 하기 표 6은 GFP 리포터 검정법 (상단) 및 α-Syn 검정법 (하단)에 의해, 시험관내에서 *TMEM106B*의 침묵화가 성공적으로 이루어졌다는 것을 나타내는 대표적인 데이터를 보여주는 것이다.

[0185] <표 5>

ID	프로모터	넉다운	프로모터	과다발현
I00007	CMV_인트론	SNCA_mi	CMV	opt-GBA1
I00008	H1	SNCA_sh	CMV	opt-GBA1
I00009	H1	SNCA_Pubsh4	CMV	opt-GBA1
I00014	JL_인트론	SNCA_mi	JetLong	opt-SCARB2_GBA
I00015	JL_인트론	SNCA_mi	JetLong	opt-PSAP_GBA
I00016	JL_인트론	SNCA_mi	JetLong	opt-CTSB_GBA
I00019	JL_인트론	SNCA_TMEM_mi	JetLong	opt-VPS35
I00023	JL_인트론	SNCA_mi	JetLong	opt-GBA1_IL34
I00024	JL_인트론	SNCA_mi	JetLong	opt-GBA2
I00028	인트론	SNCA_Broadsh	CMV	opt-GBA1
I00029	인트론	SNCA_Pubsh4	CMV	opt-GBA1

[0186]

[0187] <표 6>

ID	프로모터	넉다운	프로모터	과다발현
I00010	H1	TMEM_Pubsh	CMV	opt-GRN
I00011	JL_인트론	TMEM_mi	JetLong	opt-GBA1_GRN
I00012	H1	TMEM_sh	CMV	opt-GRN
I00019	JL_인트론	SNCA_TMEM_mi	JetLong	opt-VPS35

[0188]

[0189] *실시예 11: ITR "D" 서열 배치 및 세포 형질도입*

[0190] ITR "D" 서열의 배치가 rAAV 벡터의 세포 형질도입에 미치는 효과를 조사하였다. 도 20에 제시된 바와 같은, 1) 야생형 ITR (예컨대, 트랜스진 인서트에 인접하게 위치하고, ITR의 말단에는 원위에 위치하는 "D" 서열) 또는 2) "D" 서열이 벡터의 "외부"에 위치하는 ITR (예컨대, ITR의 말단에 인접하게 위치하고, 트랜스진 인서트에는 원위에 위치하는 "D" 서열)을 갖는 Gcase 코딩 rAAV를 HEK293 세포에 형질도입시켰다. 놀랍게도, 데이터는 "외부" 위치에 위치하는 "D" 서열을 갖는 rAAV가 효율적으로 패키징될 수 있고, 세포를 형질도입시킬 수 있는 능력을 보유한다는 것을 나타낸다 (도 40).

[0191] *실시예 12: 프로그래뉼린 rAAV의 시험관내 시험*

[0192] 도 39는 PGRN을 코딩하는 발현 구축물을 포함하는 벡터의 한 실시양태를 도시한 개략도이다. 프로그래뉼린은 예컨대, 대수조 내로의 주사와 같은 실질내 또는 경막내 주사에 의한 PGRN (예컨대, 코돈 최적화된 PGRN)을 코딩하는 rAAV 벡터의 주사에 의해 GRN 결실에 대해 이형접합성 또는 동형접합성인, *GRN* 결핍인 설치류의 CNS에서 과다발현된다.

[0193] 마우스가 2개월 또는 6개월되었을 때, 그에 주사하고, 6개월 또는 12개월까지 노화시키고, 하기 중 하나 이상의 것에 대하여 분석하였다: RNA 및 단백질 수준에서의 GRN의 발현 수준, 행동 검정법 (예컨대, 운동 개선), 생존 검정법 (예컨대, 생존 개선), 소교세포 및 염증성 마커, 신경아교증, 뉴런 손실, 지방갈색소증, 및/또는 리소좀 마커 축적 구제, 예컨대, LAMP1. PGRN 결핍 마우스에 대한 검정법은 예를 들어, 문헌 [Arrant et al. (2017) *Brain* 140: 1477-1465]; [Arrant et al. (2018) *J. Neuroscience* 38(9):2341-2358]; 및 [Amado et al. (2018) doi:https://doi.org/10.1101/30869] (상기 문헌의 전체 내용이 본원에서 참조로 포함된다)에 의해 기술되어 있다.

[0194] 등가물

[0195] 본 출원은 하기 문헌의 내용, 그 전문을 참조로 포함한다: 2018년 10월 3일 출원된, 대리인 사건 번호 P1094.70002WO00으로 언급되는 국제 PCT 출원; 2018년 10월 3일 출원된, 대리인 사건 번호 P1094.70004WO00으로 언급되는 국제 PCT 출원; 2017년 10월 3일 출원된 가출원 일련 번호 62/567,296 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 2017년 10월 3일 출원된 가출원 일련 번호 62/567,311 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 2017년 10월 3일 출원된 가출원 일련 번호 62/567,319 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 2017년 10월 3일 출원된 가출원 일련 번호 62/567,301 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 2017년 10월 3일 출원된 가출원 일련 번호 62/567,310 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 2017년 10월 3일 출원된 가출원 일련 번호 가출원 일련 번호 62/567,303 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS"); 및 2017년 10월 3일 출원된 가출원 일련 번호 62/567,305 (발명의 명칭: "GENE THERAPIES FOR LYSOSOMAL DISORDERS").

[0196] 따라서, 본 발명의 적어도 하나의 실시양태의 여러 측면을 기술하였는 바, 관련 기술분야의 통상의 기술자는 다양한 변경, 변형 및 개선을 쉽게 착안해 낼 수 있음을 이해하여야 한다. 상기 변경, 변형 및 개선은 본 개시내용의 일부가 되는 것으로 의도되고, 본 발명의 정신 및 범주 내에 포함되는 것으로 의도된다. 따라서, 상기 설명 및 도면은 단지 예일 뿐이다.

[0197] 본원에 본 발명의 여러 실시양태가 기술되고, 예시되었지만, 관련 기술분야의 통상의 기술자는 기능을 수행하고/거나, 본원에 기술된 결과 및/또는 이점들 중 하나 이상의 것을 수득하기 위해 각종의 다른 수단 및/또는 구조

를 쉽게 구상해 낼 수 있을 것이며, 상기 변이 및/또는 변형은 각각 본 발명의 범주 내에 포함되는 것으로 간주된다. 더욱 일반적으로, 관련 기술분야의 통상의 기술자는 본원에 기술된 모든 파라미터, 치수, 물질 및 구성은 예시적인 것임을 의미하며, 실제 파라미터, 치수, 물질 및/또는 구성은 본 발명의 교시(들)가 사용되는 구체적인 적용 또는 적용들에 의존하게 된다는 것을 쉽게 이해할 것이다. 관련 기술분야의 통상의 기술자는 단지 통상의 실험만을 사용하고도, 본원에 기술된 본 발명의 구체적인 실시양태에 대한 다수의 등가물을 쉽게 이해하거나, 또는 확인할 수 있을 것이다. 그러므로, 상기 실시양태는 단지 예시로서 제공되는 것이며, 첨부된 청구범위 및 그의 등가물의 범주 내에서 본 발명은 구체적으로 기술되고, 청구된 것과 다르게 실시될 수 있다는 것을 이해하여야 한다. 본 발명은 본원에 기술된 각각의 개별 특징, 시스템, 물품, 물질, 및/또는 방법에 관한 것이다. 추가로, 상기 특징, 시스템, 물품, 물질, 및/또는 방법이 서로 모순되지 않는다면, 둘 이상의 상기 특징, 시스템, 물품, 물질, 및/또는 방법의 임의의 조합이 본 발명의 범주 내에 포함된다.

[0198] 명세서 중 및 청구범위 중 본원에서 사용되는 바, "단수형태"는 달리 명확하게 그 반대로 명시되지 않는 한, "적어도 하나"를 의미하는 것으로 이해되어야 한다.

[0199] 명세서 중 및 청구범위 중 본원에서 사용되는 바, "및/또는"이라는 어구는 그렇게 결합되어 있는 요소 중 "어느 하나 또는 그 둘 모두," 즉, 일부 경우에서는 공동으로 존재하고, 다른 경우에는 분리되어 존재하는 요소를 의미하는 것으로 이해되어야 한다. 달리 명확하게 그 반대로 명시되지 않는 한, 구체적으로 확인된 요소와 관련이 있든 또는 관련이 없든 상관없이, "및/또는" 절에 의해 구체적으로 확인된 요소 이외의 다른 요소가 임의적으로 존재할 수 있다. 따라서, 비제한적인 예로서, 예컨대, "포함하는"이라는 제한을 두지 않은 개방형의 표현과 함께 결합하여 사용될 때, "A 및/또는 B"라고 언급하는 것은 한 실시양태에서는 (임의적으로 B 이외의 다른 요소를 포함하면서) B 없이 A를 지칭할 수 있고; 또 다른 실시양태에서는 (임의적으로 A 이외의 다른 요소를 포함하면서) A 없이 B를 지칭할 수 있고; 추가의 또 다른 실시양태에서는 (임의적으로 다른 요소를 포함하면서) A 및 B, 둘 모두를 지칭할 수 있으며; 기타의 경우도 존재할 수 있다.

[0200] 명세서 중 및 청구범위 중 본원에서 사용되는 바, "또는"은 상기 정의된 "및/또는"과 동일한 의미를 갖는 것으로 이해되어야 한다. 예를 들어, 목록에서 항목을 분리할 때, "또는" 또는 "및/또는"은 포괄적인 의미로, 즉, 요소의 개수 또는 요소의 목록 중 적어도 하나를 포함할 뿐만 아니라, 그 중 1개 초과의 것을 포함하고, 열거되지 않은 추가 항목을 포함하는 것으로 해석되어야 한다. 그 반대로 명확하게 명시된 용어, 예컨대, "~중 단 하나만" 또는 "~중 정확하게 하나," 또는 청구범위에서 사용될 때, "~으로 이루어진"이라는 용어만이 요소의 개수 또는 요소의 목록 중 정확히 하나의 요소를 포함하는 것을 의미할 것이다. 일반적으로, 본원에서 사용되는 바, "또는"이라는 용어는 오직, 배타적인 용어, 예컨대, "둘 중 어느 하나," "~중 하나," "~중 단 하나만" 또는 "~중 정확하게 하나"라는 용어가 선행될 때 배타적인 대안 (즉, "하나 또는 나머지 다른 하나이지만, 그 둘 모두는 아닌 것")을 나타내는 것으로 해석되어야한다. 청구범위에서 사용될 때, "본질적으로 ~으로 이루어진"이라는 것은 특허법 분야에서 사용되는 바와 같이, 그의 일반적인 의미를 가져야 한다.

[0201] 본원, 본 명세서 및 청구범위에서 사용되는 바, 하나 이상의 요소의 목록과 관련하여, "적어도 하나의"라는 어구는, 반드시 요소의 목록 내에 구체적으로 열거된 각각의 모든 요소 중 적어도 하나를 포함하여야 하는 것이 아니라, 요소의 목록에서 요소의 임의의 조합을 배제하는 것이 아니라, 적어도 하나의 요소가 요소의 목록에서 요소 중 임의의 하나 이상의 것으로부터 선택된다는 것을 의미하는 것으로 이해되어야 한다. 상기 정의는 또한, 구체적으로 확인된 요소와 관련이 있든 또는 없든 그에 상관없이, "적어도 하나의"라는 어구가 지칭하는 요소의 목록 내에 구체적으로 확인된 요소 이외의 요소가 임의적으로 존재할 수 있다는 것도 허용한다. 따라서, 비제한적인 예로서, "A 및 B 중 적어도 하나의" (또는 동일하게, "A 또는 B 중 적어도 하나의," 또는 동일하게, "A 및/또는 B 중 적어도 하나의")라는 것은 한 실시양태에서는 (임의적으로 B 이외의 다른 요소를 포함하면서) B는 존재하지 않고, 임의적으로 1개 초과의 A를 포함하는 적어도 하나의 A를 지칭할 수 있고; 또 다른 실시양태에서는, (임의적으로 A 이외의 다른 요소를 포함하면서) A는 존재하지 않고, 임의적으로 1개 초과의 B를 포함하는 적어도 하나의 B를 지칭할 수 있고; 추가의 또 다른 실시양태에서, 임의적으로 1개 초과의 A를 포함하는 적어도 하나의 A, 및 임의적으로 1개 초과의 B를 포함하는 적어도 하나의 B (및 다른 요소를 포함하는 것) 등을 칭할 수 있다.

[0202] 상기 명세서 뿐만 아니라, 청구항에서, 예컨대, "포함하는," "비롯한," "보유하는," "갖는," "함유하는," "수반하는," "보유하는" 등의 모든 전환 어구는 제한을 두지 않은 개방형인 것으로, 즉, ~을 포함하나, 이에 제한되지 않는다는 것을 의미하는 것으로 이해되어야 한다. 미국 특허청의 심사 절차에 관한 매뉴얼, 섹션 2111.03(United States Patent Office Manual of Patent Examining Procedures, Section 2111.03)에 기재되어 있는 바와 같이, 오직 "~으로 이루어진" 및 "본질적으로 ~으로 이루어진"이라는 전환 어구만이 각각 폐쇄형 또

는 반-폐쇄형 전환 어구여야 한다.

[0203] 청구항 중의 청구항을 수식하는 서수 용어, 예컨대, "제1," "제2," "제3" 등의 사용은 그 단독으로 한 청구항의 또 다른 청구항 대비의 임의의 우선함, 우위임, 또는 순서, 또는 방법의 조치가 수행되는 시간적 순서를 내포하는 것이 아니라, 이는 단지 청구항을 구별하기 위해 특정명을 갖는 한 청구항을 동일명을 갖는 또 다른 항을 구별하는 (서수 용어의 사용을 위한) 라벨로서 사용된다.

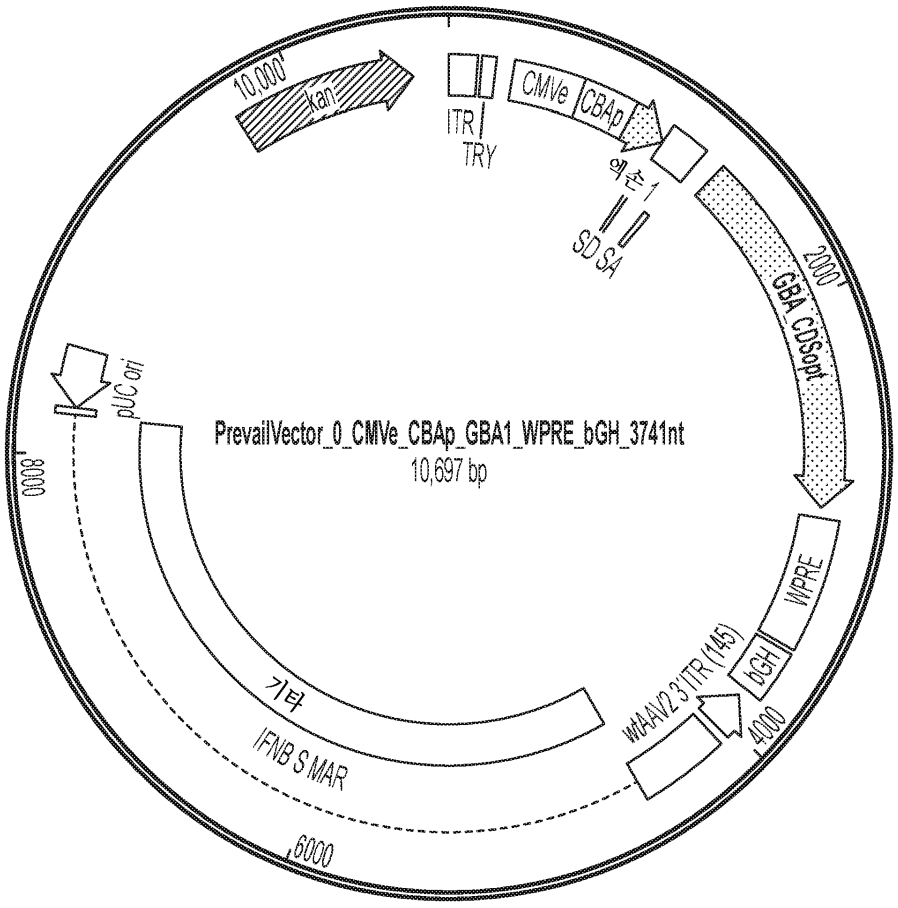
[0204] 명백하게 반대로 명시되지 않는 한, 1개 초과의 단계 또는 조치를 포함하는 본원에서 청구되는 임의의 방법에서, 본 방법의 단계 또는 조치의 순서가 반드시 본 방법의 단계 또는 조치가 언급된 순서로 제한되는 것은 아니라는 것도 또한 이해하여야 한다.

[0205] 서열

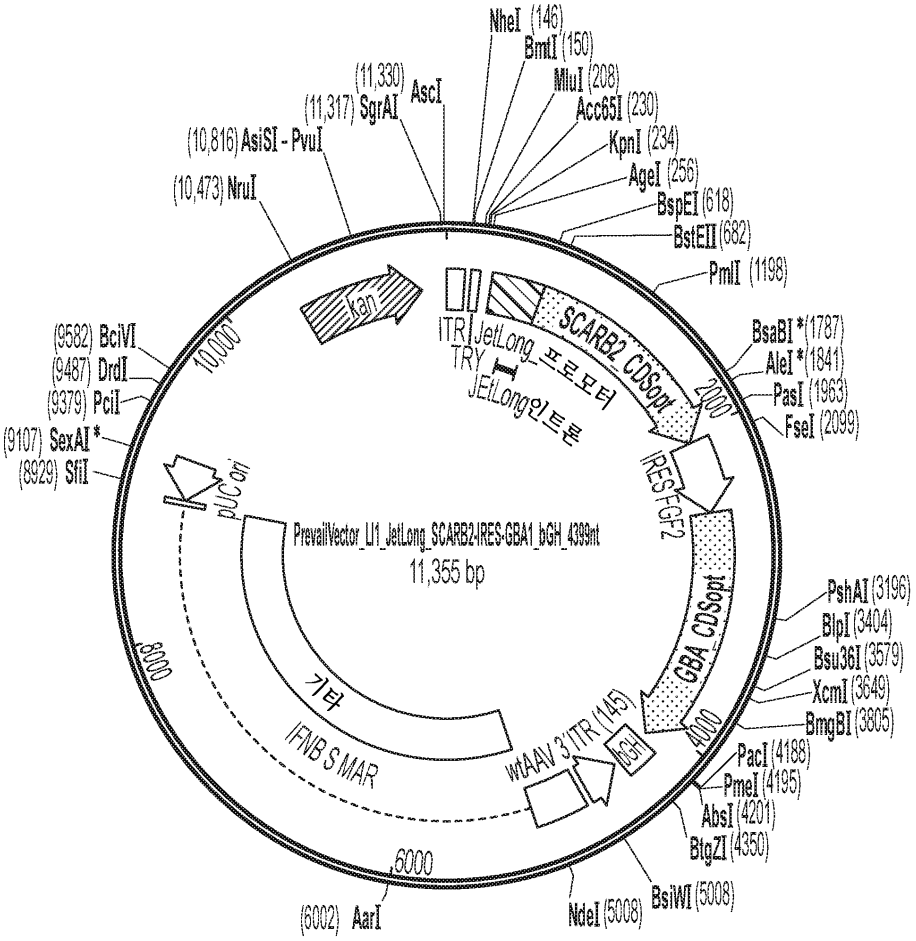
[0206] 일부 실시양태에서, 하나 이상의 유전자 생성물 (예컨대, 제1, 제2 및/또는 제3 유전자 생성물)을 코딩하는 발현 카세트는 서열식별번호 1-78 중 어느 하나에 기재된 서열을 포함하거나 또는 그로 이루어진다 (또는 상기 서열을 갖는 펩티드를 코딩한다). 일부 실시양태에서, 유전자 생성물은 서열식별번호 1-78 중 어느 하나의 일부 (예컨대, 단편)에 의해 코딩된다.

도면

도면1



도면2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BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
BstEII (682)
PmlI (1198)
BsaBI *(1787)
AleI *(1841)
PasI (1963)
FseI (2099)
PshAI (3196)
BlpI (3404)
Bsu36I (3579)
XcmI (3649)
BmgBI (3805)
PacI (4188)
PmeI (4195)
AbsI (4201)
BtgZI (4350)
BsiWI (5008)
NdeI (5008)
(6002) AarI
(9582) BciVI
(9487) DrdI
(9379) PciI
(9107) SexAI *
(8929) SfiI
(10,816) AsiSI - PvuI
(10,473) NruI
(11,330) AscI
(11,317) SgrAI
Kan
ITR
JetLong_프로모터
TRY
JEtLong인트론
SCARB2 CDSopt
IRES
FGF2
GBA CDSopt
pUC ori
PrevailVector_LI1_JetLong_SCARB2IRES-GBA1_bGH_4399nt
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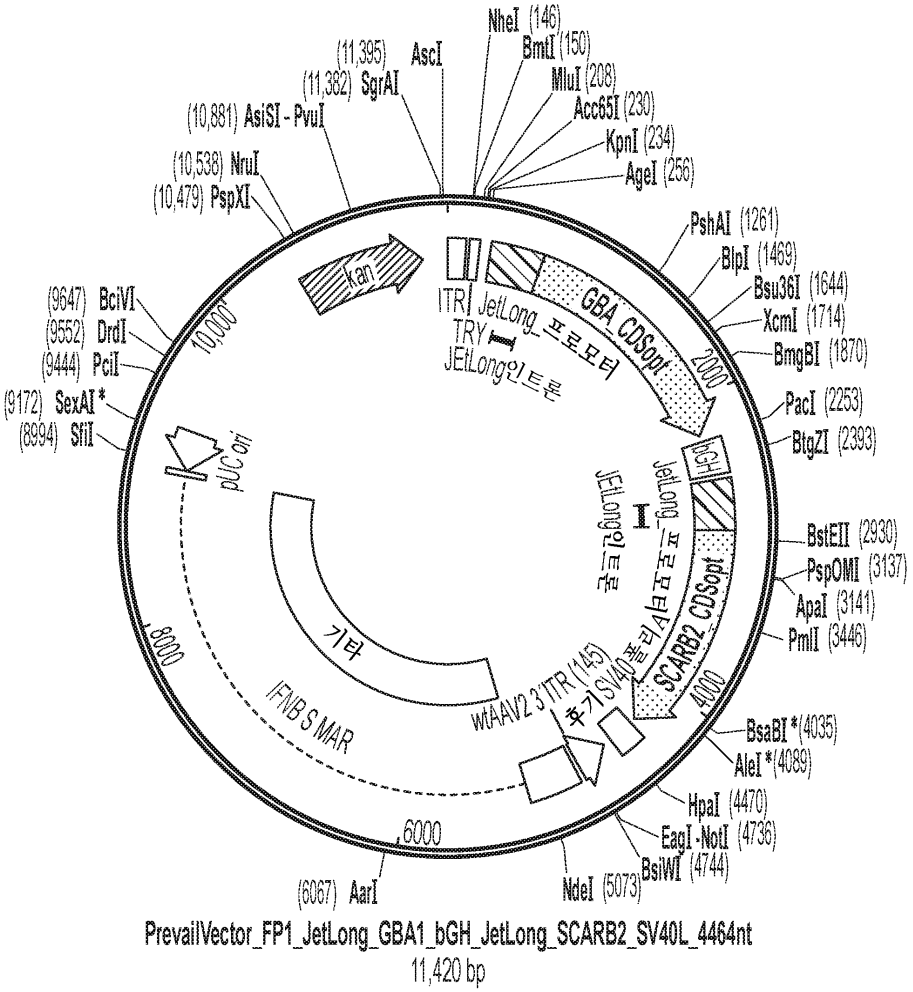
기타
IFNB S MAR
wAAV 3'ITR (145)
bGH
2000
4000
6000
8000
10000

도면3



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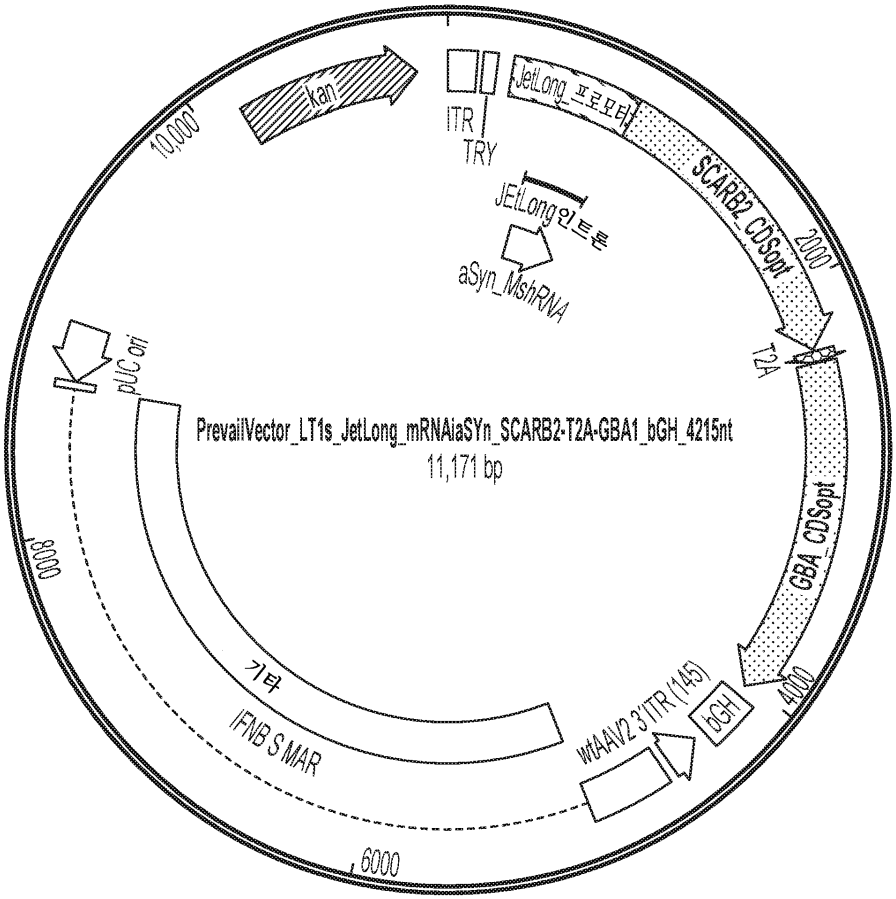
도면4


10,000
Kan
ITR
TRY
JetLong_프로모터
SCARB2_CDSopt
2000
JetLong인트론
aSyn_MshRNA
T2A
pUC ori
PrevailVector_LT1s_JetLong_mRNAiaSYn_SCARB2-T2A-GBA1_bGH_4215nt
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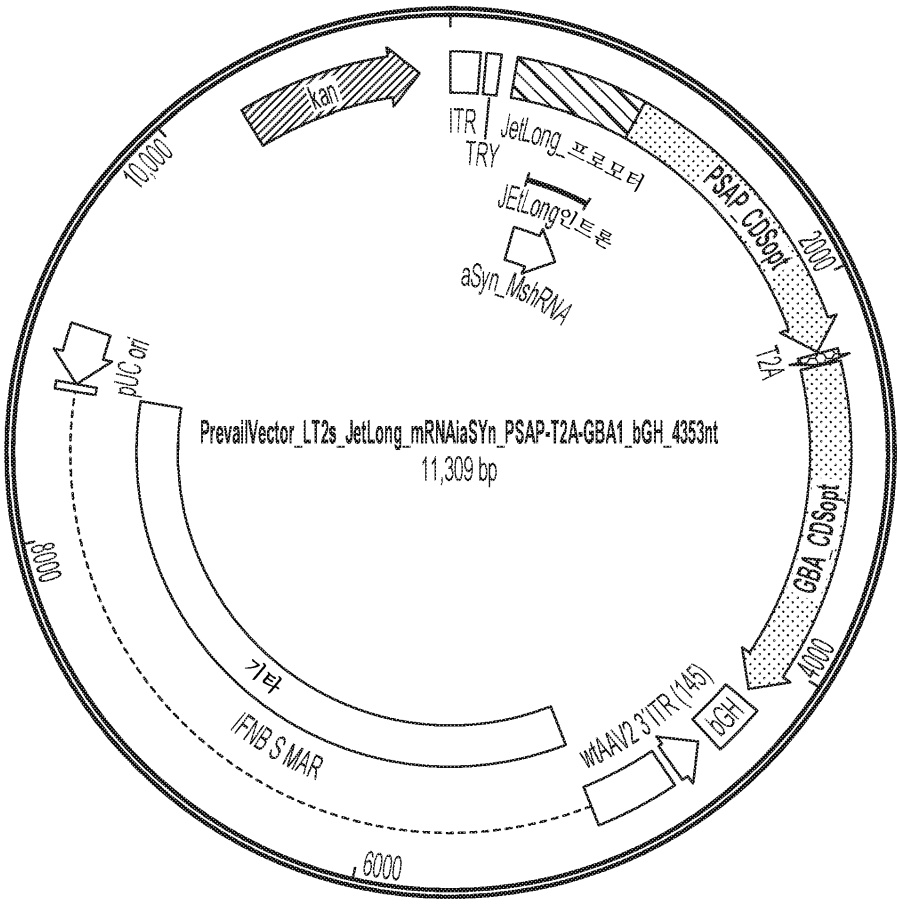
GBA_CDSopt
8000
4000
기타
IFNB S MAR
wtAAV2 3'ITR (145)
bGH
6000

도면5


Kan
ITR
TRY
JetLong_프로모터
JETLong인트론
aSyn_MshRNA
PSAP CDSopt
T2A
GBA CDSopt
bGH
wtAAV2 3'ITR(145)
IFNB S MAR
기타
pUC ori
PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-T2A-GBA1_bGH_4353nt
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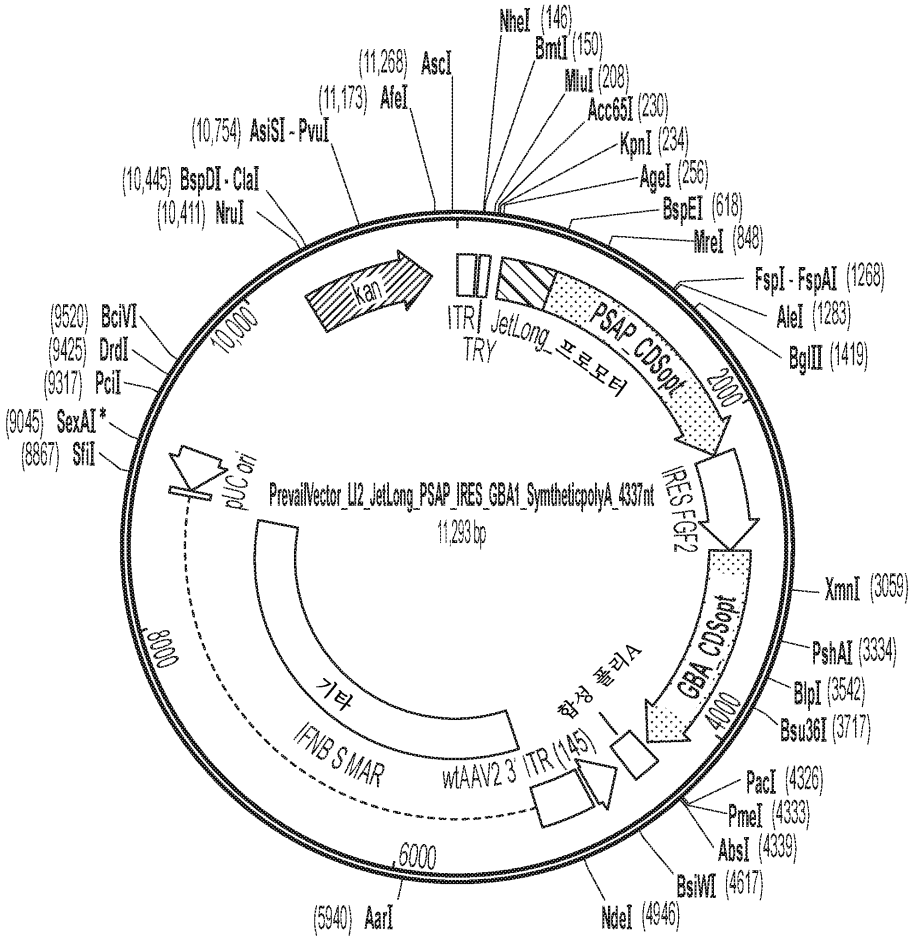
2000
4000
6000
8000

도면6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BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
MreI (848)
FspI - FspAI (1268)
AleI (1283)
BglII (1419)
XmnI (3059)
PshAI (3334)
BlpI (3542)
Bsu36I (3717)
PacI (4326)
PmeI (4333)
AbsI (4339)
BsiWI (4617)
NdeI (4946)
(5940) AarI
(6000)
(8000)
(9045) SexAI*
(8867) SfiI
(9520) BciVI
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(9317) PciI
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(10,754) AsiSI - PvuI
(11,173) AfeI
(11,268) AscI
(10,000)
(2000)
(4000)
Kan
ITR
TRY
JetLong_프로모터
PSAP CDSopt
IRES FGF2
GBA_CDSopt
pUC ori
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1_SyntheticpolyA_4337nt
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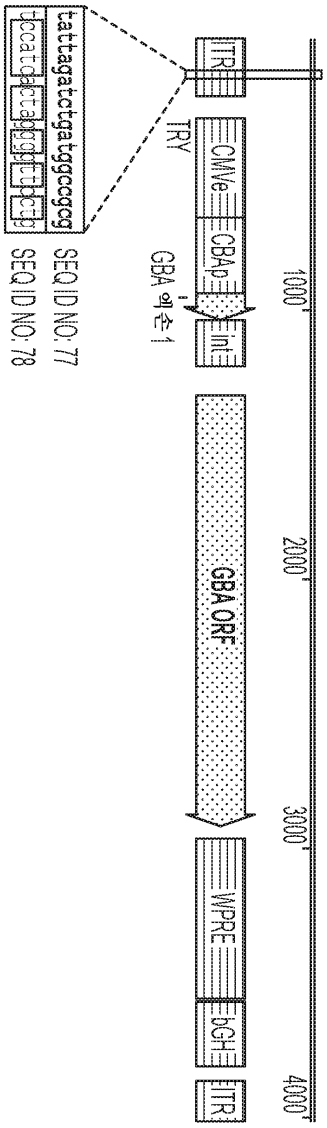
기타
IFNB S/MAR
wtAAV2 3' ITR (145)
합성 폴리A

도면7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CMVe
CBAp
Int
GBA ORF
WPRE
bGH
ITR
1000
2000
3000
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TRY
GBA 엑손1
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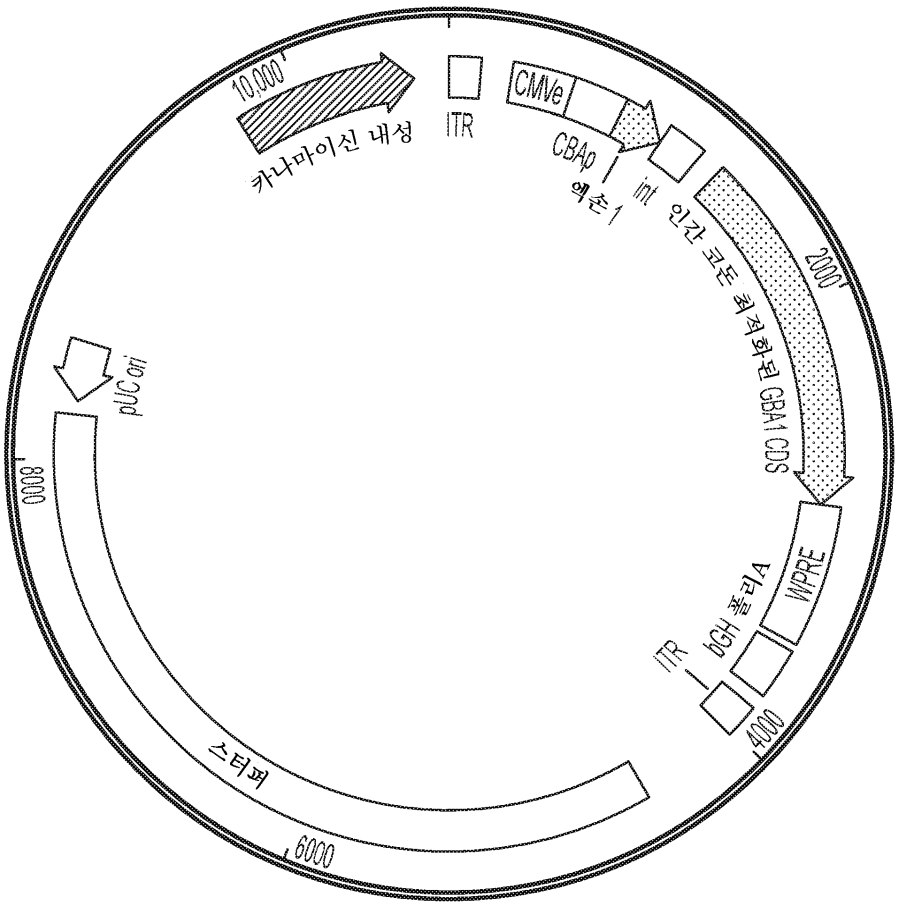
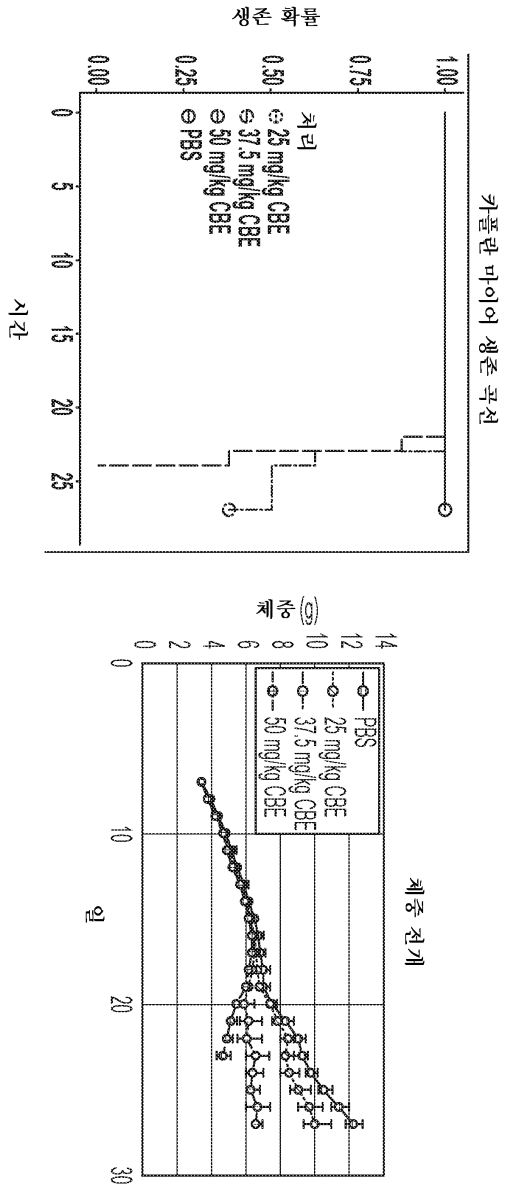
SEQ ID NO: 77
SEQ ID NO: 78

도면8

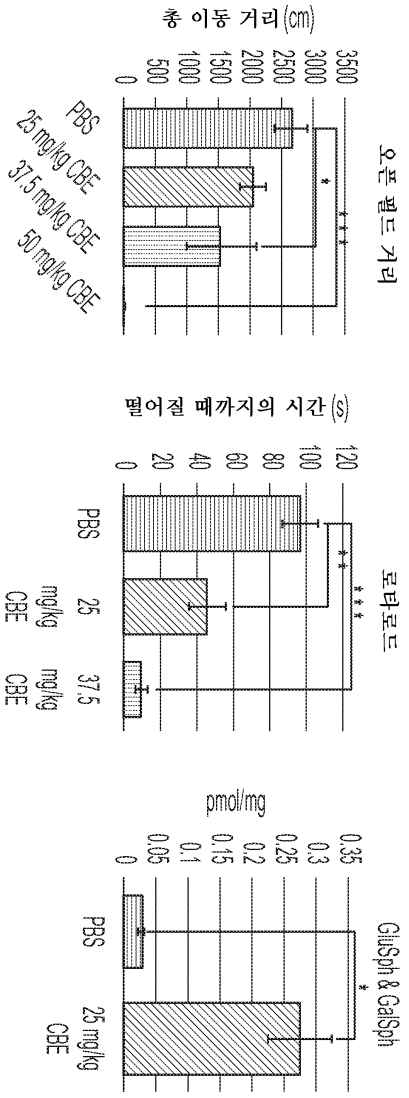

10,000
카나마이신 내성
ITR
CMVe
CBAp
엑손1
int
인간 코돈 최적화된 GBA1 CDS
2000
WPRE
bGH 폴리A
ITR
4000
6000
스터퍼
8000
pUC ori

도면9i

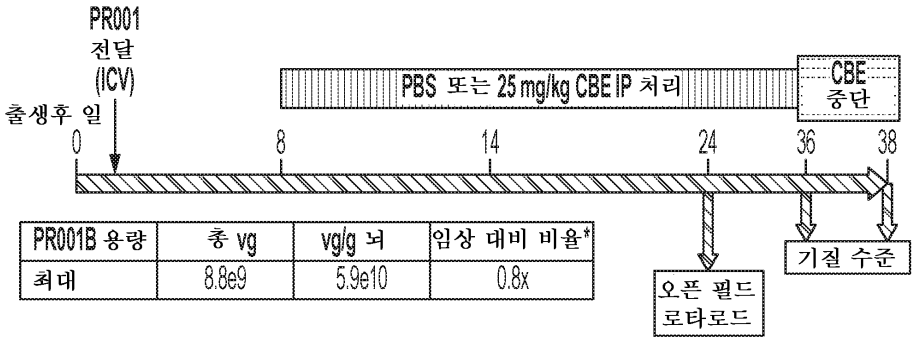

생존 확률
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
시간
처리
25 mg/kg CBE
37.5 mg/kg CBE
50 mg/kg CBE
PBS
카플란 마이어 생존 곡선

체중 (g)
0
2
4
6
8
10
12
14
0
10
20
30
일
PBS
25 mg/kg CBE
37.5 mg/kg CBE
50 mg/kg CBE
체중 진개

도면9ii

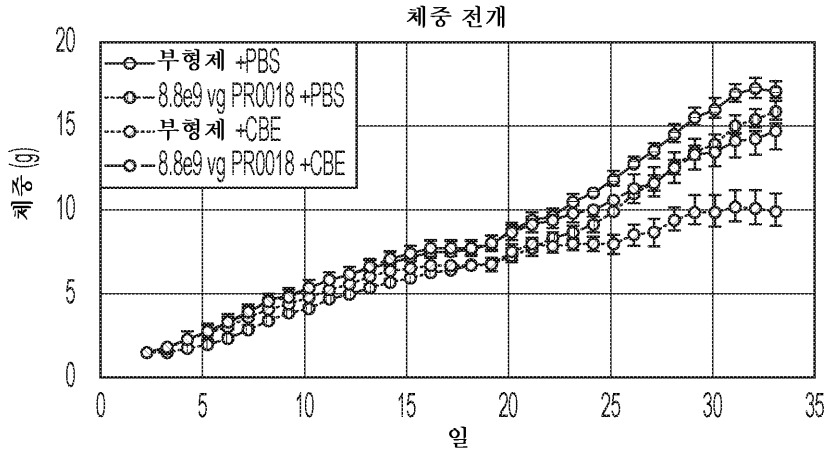


도면10

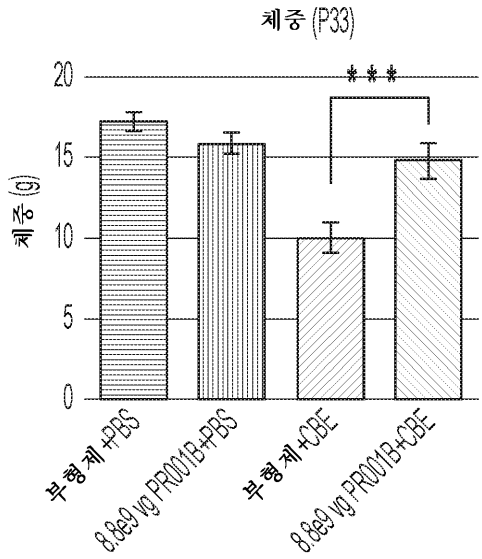


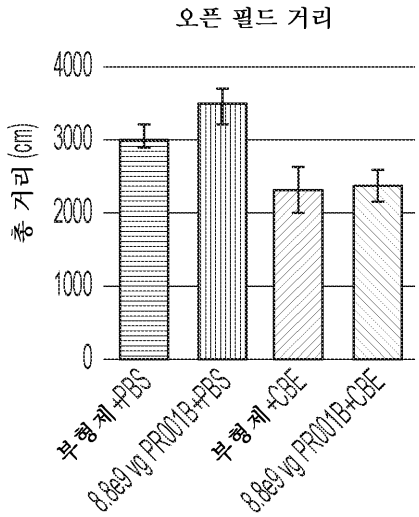
PR001B 용량	총 vg	vg/g 뇌	임상 대비 비율*
최대	8.8e9	5.9e10	0.8x

도면11i

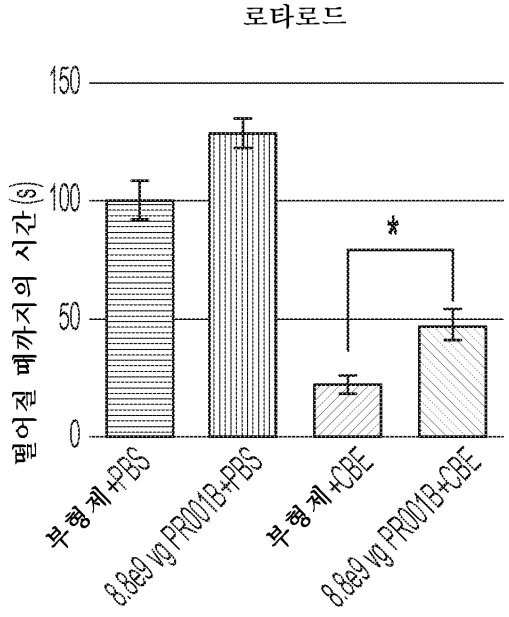


도면11ii

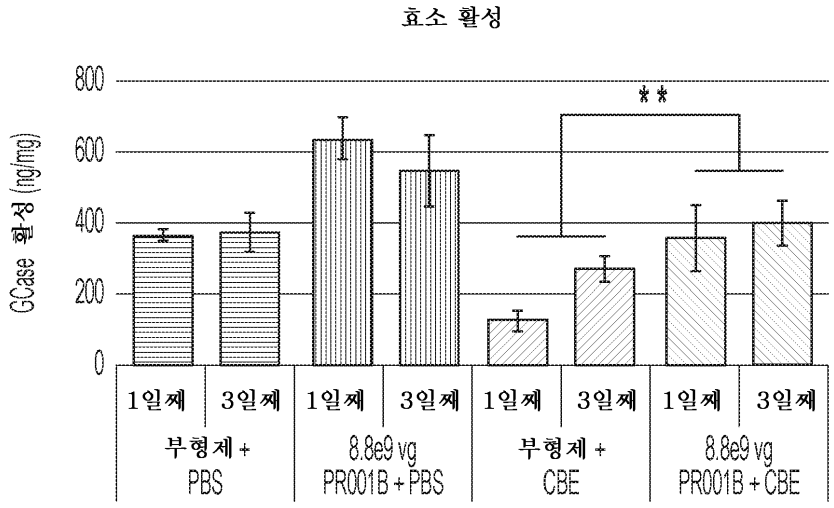




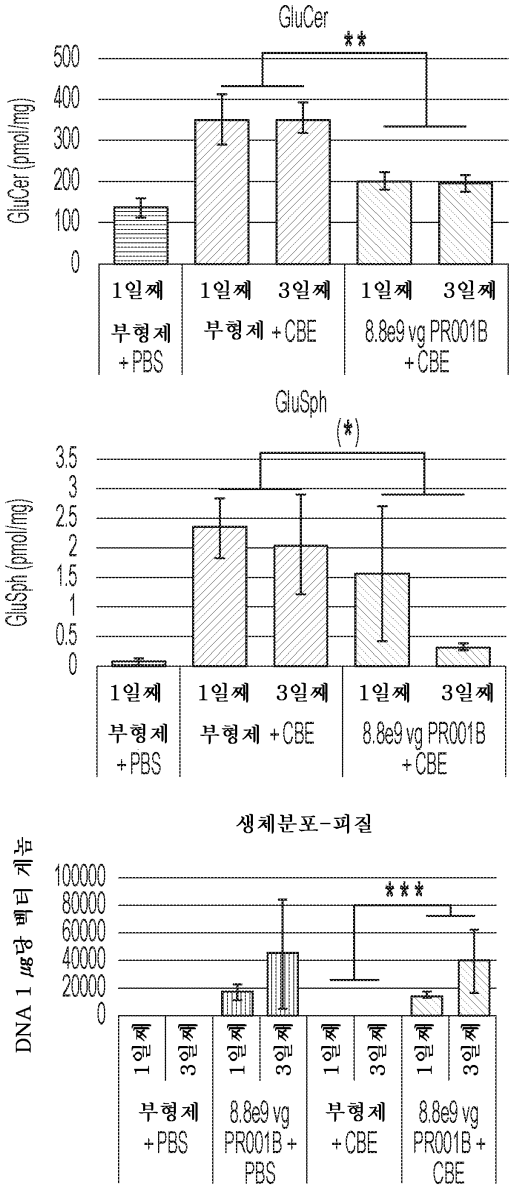
도면11iii



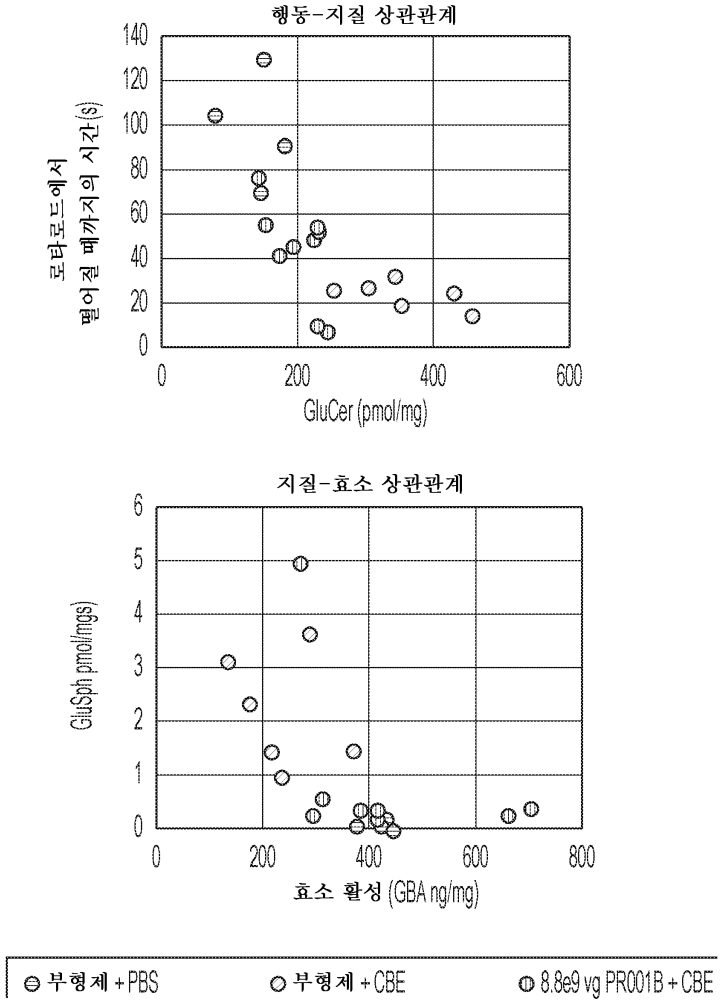
도면12i



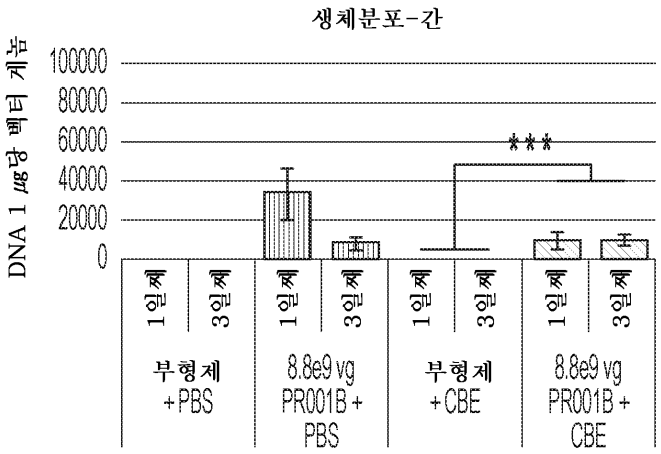
도면12ii

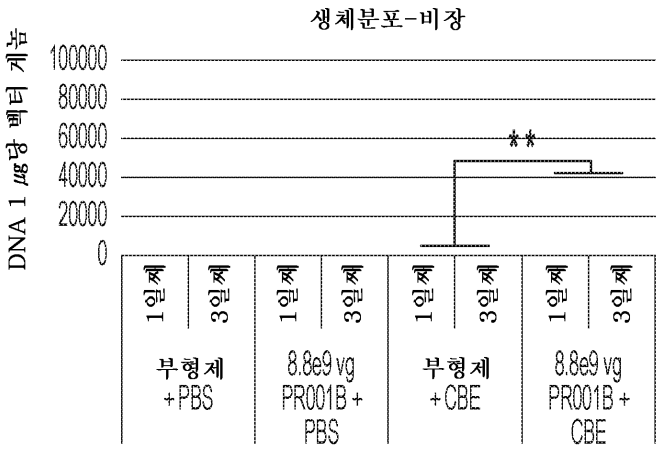

GluCer
**
GluCer (pmol/mg)
500
400
300
200
100
0
1일째
부형제 +PBS
1일째
3일째
부형제 +CBE
1일째
3일째
8.8e9 vg PR001B +CBE
GluSph
(*)
GluSph (pmol/mg)
3.5
3
2.5
2
1.5
1
0.5
0
1일째
부형제 +PBS
1일째
3일째
부형제 +CBE
1일째
3일째
8.8e9 vg PR001B +CBE
생체분포-피질
DNA 1 μg당 벡터 게놈
***
100000
80000
60000
40000
20000
0
1일째
3일째
1일째
3일째
1일째
3일째
1일째
3일째
부형제 +PBS
8.8e9 vg PR001B + PBS
부형제 +CBE
8.8e9 vg PR001B + CBE

도면13

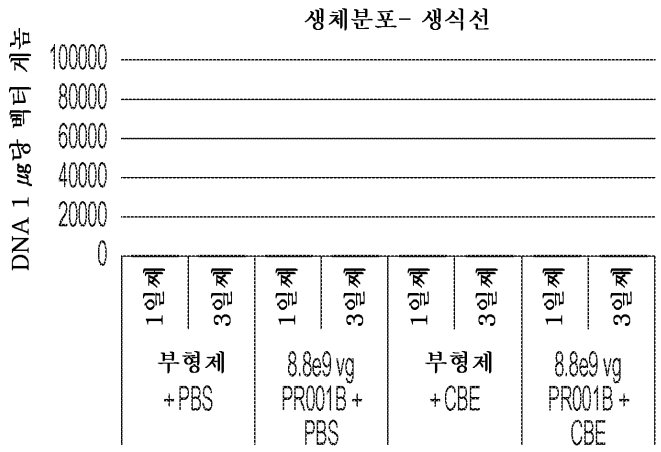

행동-지질 상관관계
로타로드에서
떨어질 때까지의 시간(s)
140
120
100
80
60
40
20
0
0
200
400
600
GluCer (pmol/mg)
지질-효소 상관관계
GluSph pmol/mgs)
6
5
4
3
2
1
0
0
200
400
600
800
효소 활성 (GBA ng/mg)
부형제 + PBS
부형제 + CBE
8.8e9 vg PR001B + CBE

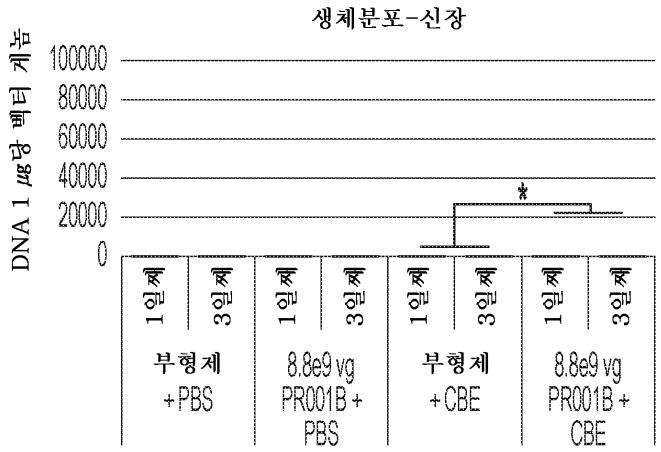
도면14i


생체분포-간
DNA 1 μg당 벡터 게놈
100000
80000
60000
40000
20000
0
***
1일째
3일째
1일째
3일째
1일째
3일째
1일째
3일째
부형제 +PBS
8.8e9 vg PR001B + PBS
부형제 +CBE
8.8e9 vg PR001B + CBE

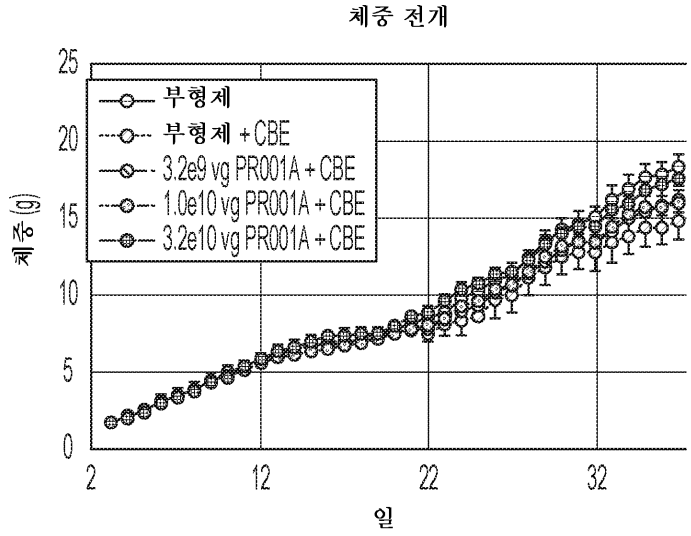

생체분포-비장
DNA 1 μg당 벡터 게놈
100000
80000
60000
40000
20000
0
**
1일째
3일째
1일째
3일째
1일째
3일째
1일째
3일째
부형제 +PBS
8.8e9 vg PR001B + PBS
부형제 +CBE
8.8e9 vg PR001B + CBE

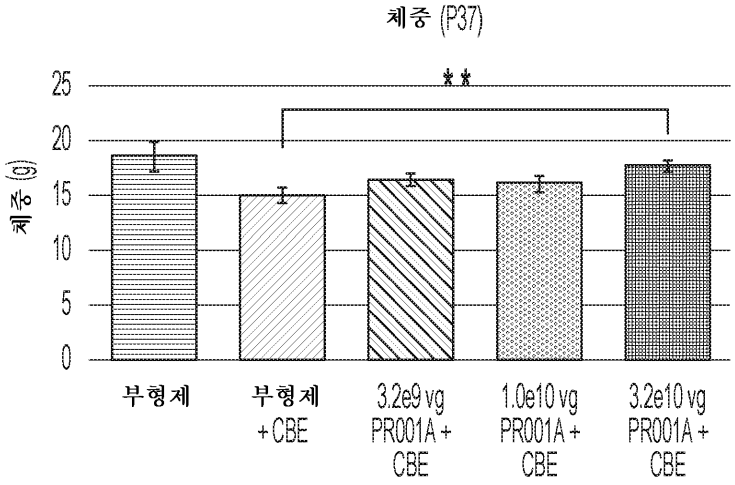
도면14ii


생체분포- 생식선
DNA 1 μg당 벡터 게놈
100000
80000
60000
40000
20000
0
1일째
3일째
부형제 +PBS
8.8e9 vg PR001B + PBS
부형제 +CBE
8.8e9 vg PR001B + CBE

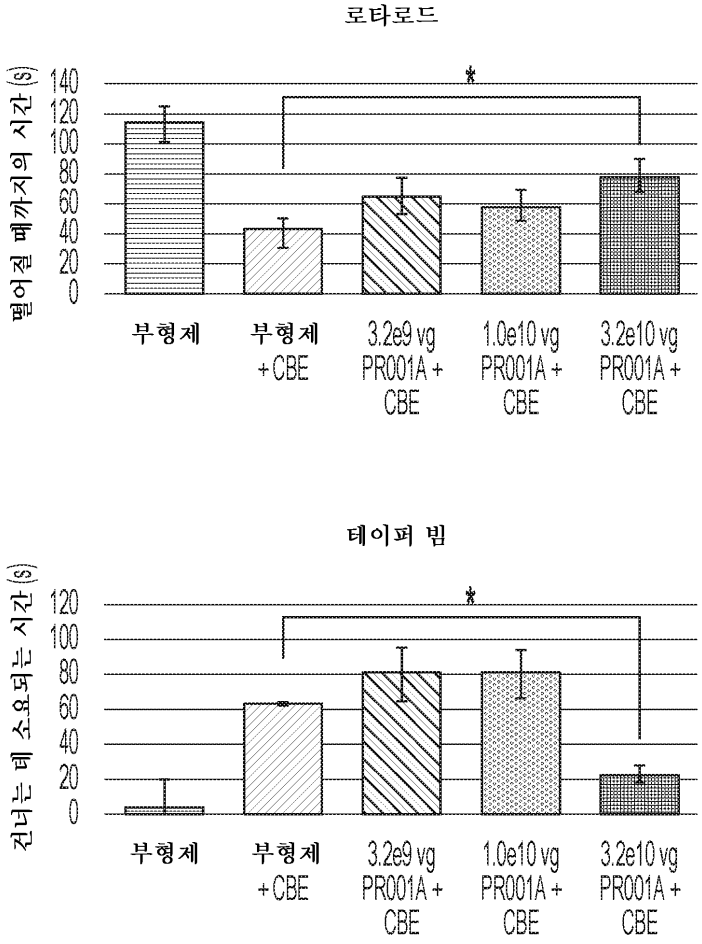

생체분포-신장
DNA 1 μg당 벡터 게놈
100000
80000
60000
40000
20000
0
*
1일째
3일째
부형제 +PBS
8.8e9 vg PR001B + PBS
부형제 +CBE
8.8e9 vg PR001B + CBE

도면15i

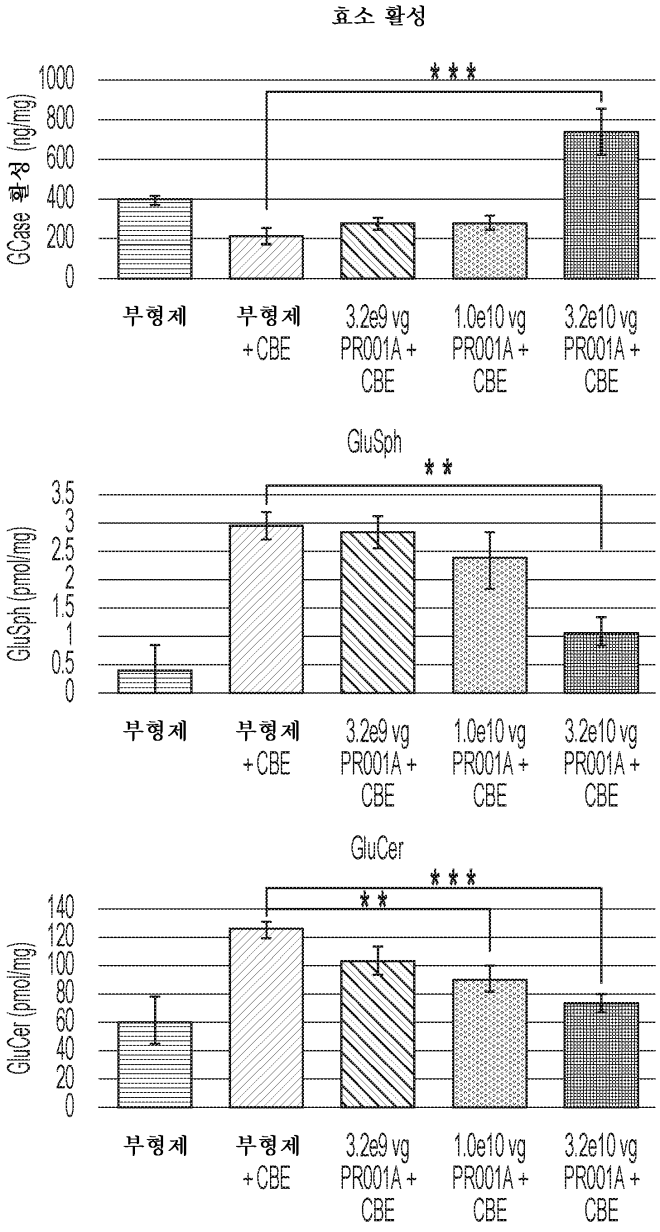

체중 전개
25
20
15
10
5
0
체중 (g)
부형제
부형제 + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
2
12
22
32
일


체중 (P37)
25
20
15
10
5
0
체중 (g)
**
부형제
부형제 + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

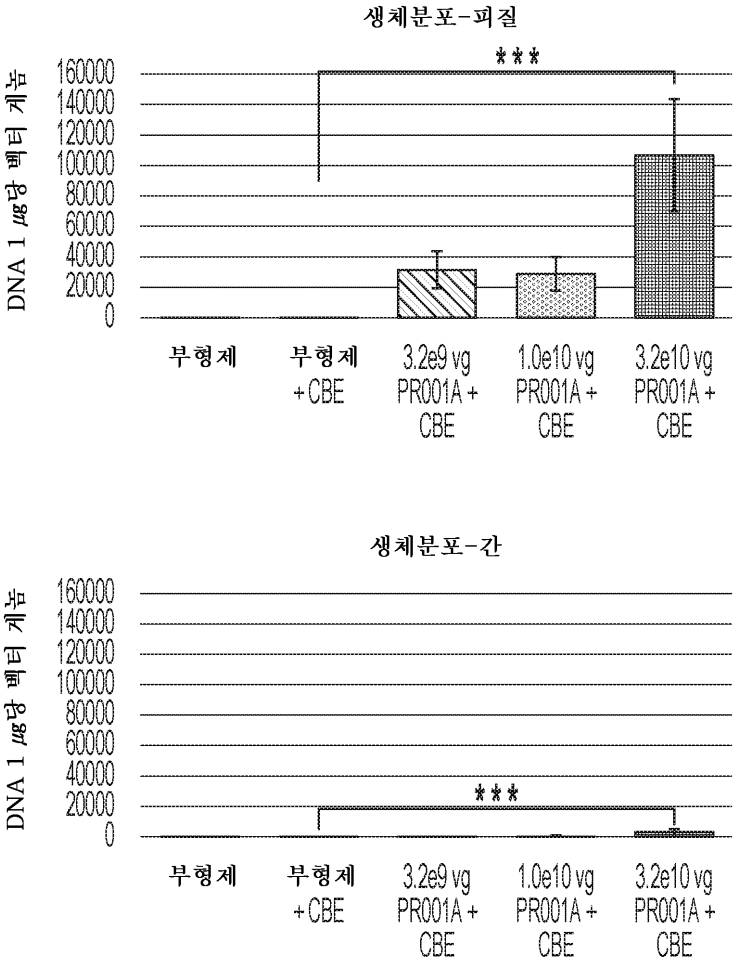
도면15ii


로타로드
떨어질 때까지의 시간 (s)
140
120
100
80
60
40
20
0
*
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
테이퍼 빔
건너는 데 소요되는 시간 (s)
120
100
80
60
40
20
0
*
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

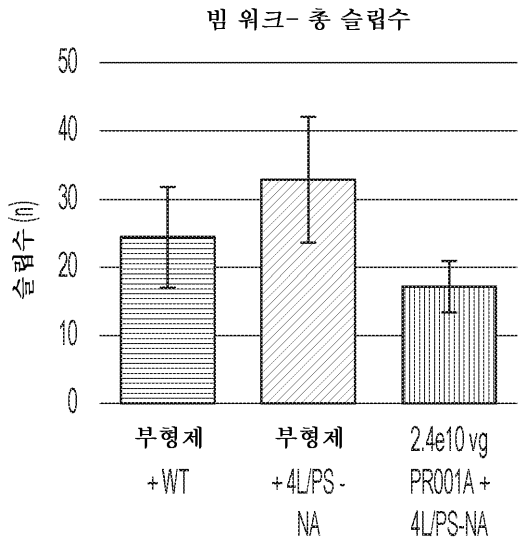
도면16i

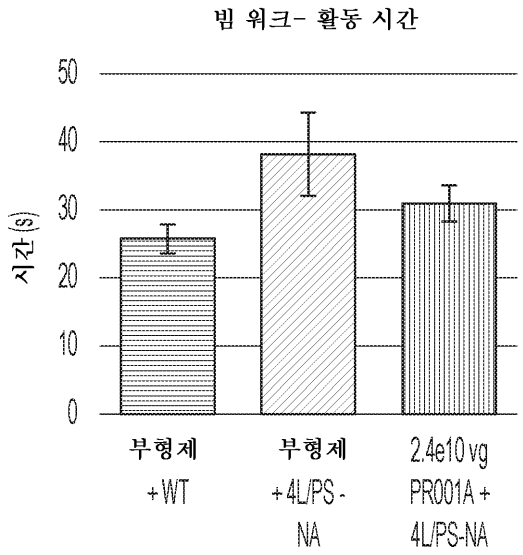

효소 활성
GCase 활성 (ng/mg)
1000
800
600
400
200
0
***
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
GluSph
GluSph (pmol/mg)
3.5
3
2.5
2
1.5
1
0.5
0
**
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
GluCer
GluCer (pmol/mg)
140
120
100
80
60
40
20
0
***
**
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

도면16ii


생체분포-피질
DNA 1 μg당 벡터 게놈
160000
140000
120000
100000
80000
60000
40000
20000
0
***
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
생체분포-간
DNA 1 μg당 벡터 게놈
160000
140000
120000
100000
80000
60000
40000
20000
0
***
부형제
부형제 +CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

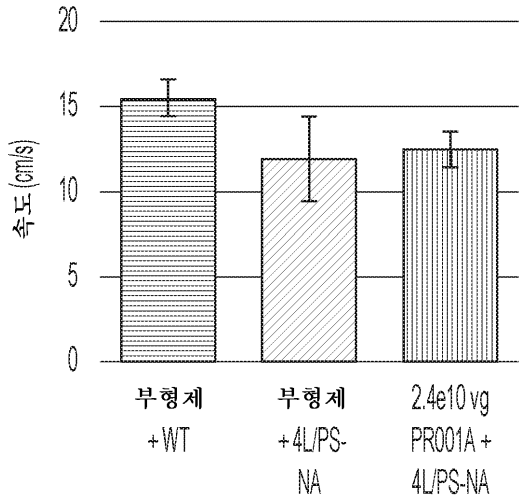
도면17i


빔 워크- 총 슬립수
슬립수 (n)
50
40
30
20
10
0
부형제 +WT
부형제 +4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA

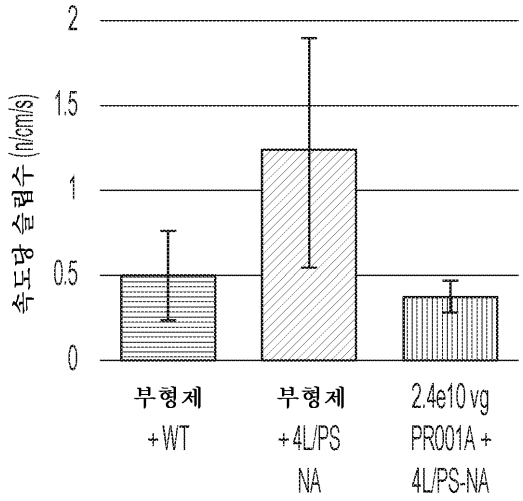

빔 워크- 활동 시간
시간 (s)
50
40
30
20
10
0
부형제 +WT
부형제 +4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA

도면17ii

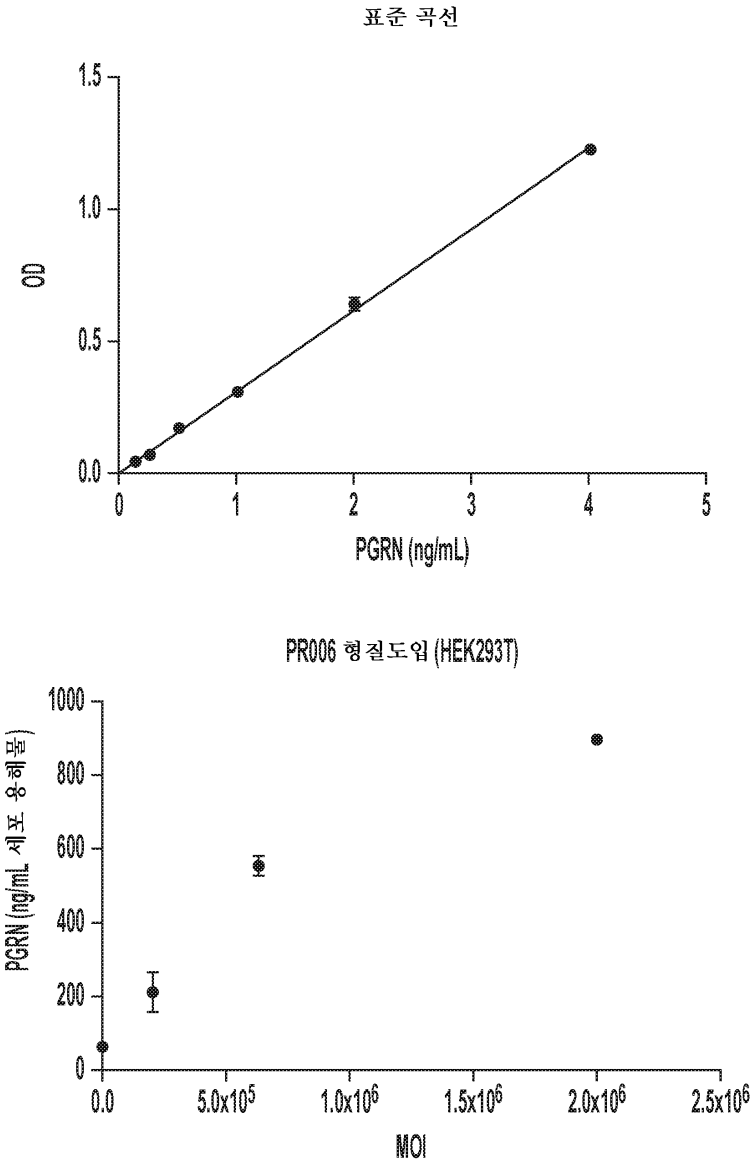
빔 워크- 속도



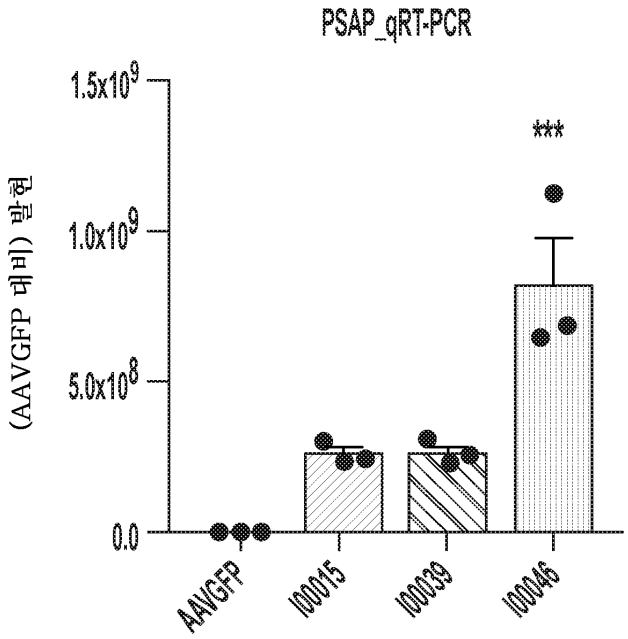
빔 워크- 속도당 슬립수



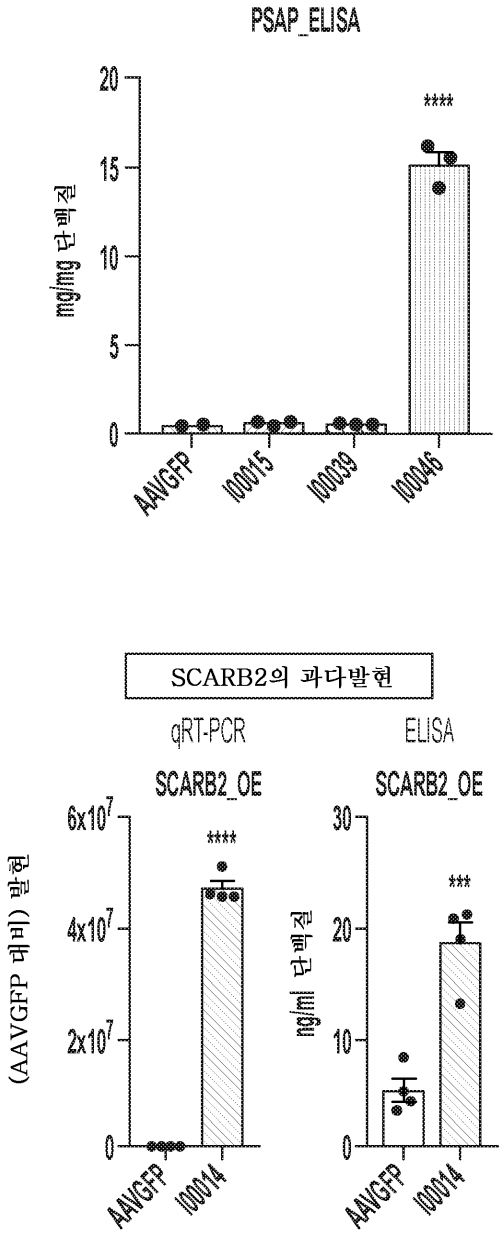
도면18



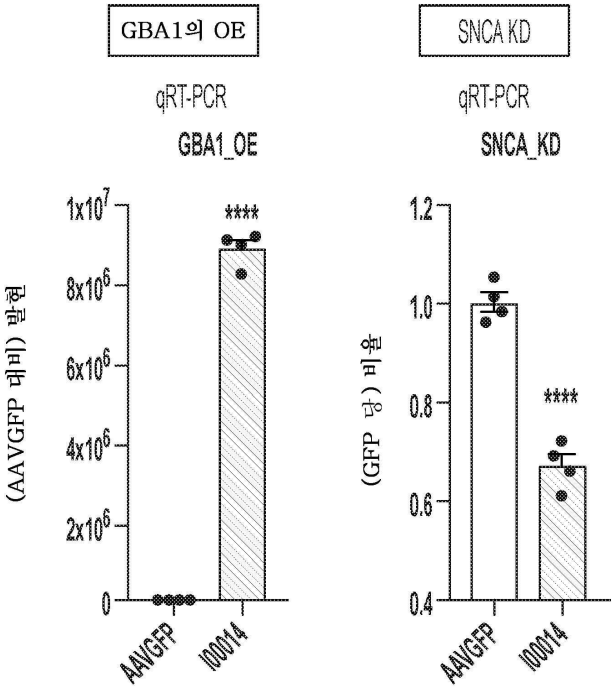
도면19i


PSAP_qRT-PCR
(AAVGFP 대비) 발현
1.5x10^9
1.0x10^9
5.0x10^8
0.0
***
AAVGFP
I00015
I00039
I00046

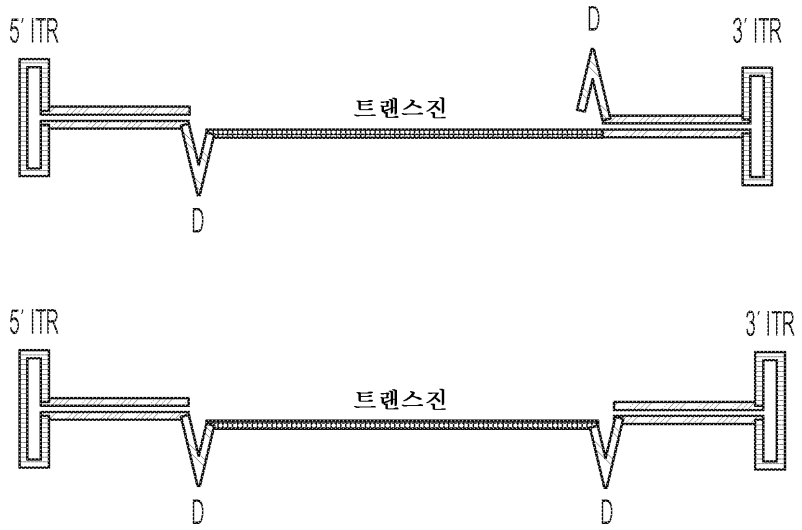
도면19ii


PSAP_ELISA
mg/mg 단백질
20
15
10
5
0
****
AAVGFP
I00015
I00039
I00046
SCARB2의 과다발현
qRT-PCR
SCARB2_OE
(AAVGFP 대비) 발현
6x10^7
4x10^7
2x10^7
0
****
AAVGFP
I00014
ELISA
SCARB2_OE
ng/ml 단백질
30
20
10
0
***
AAVGFP
I00014

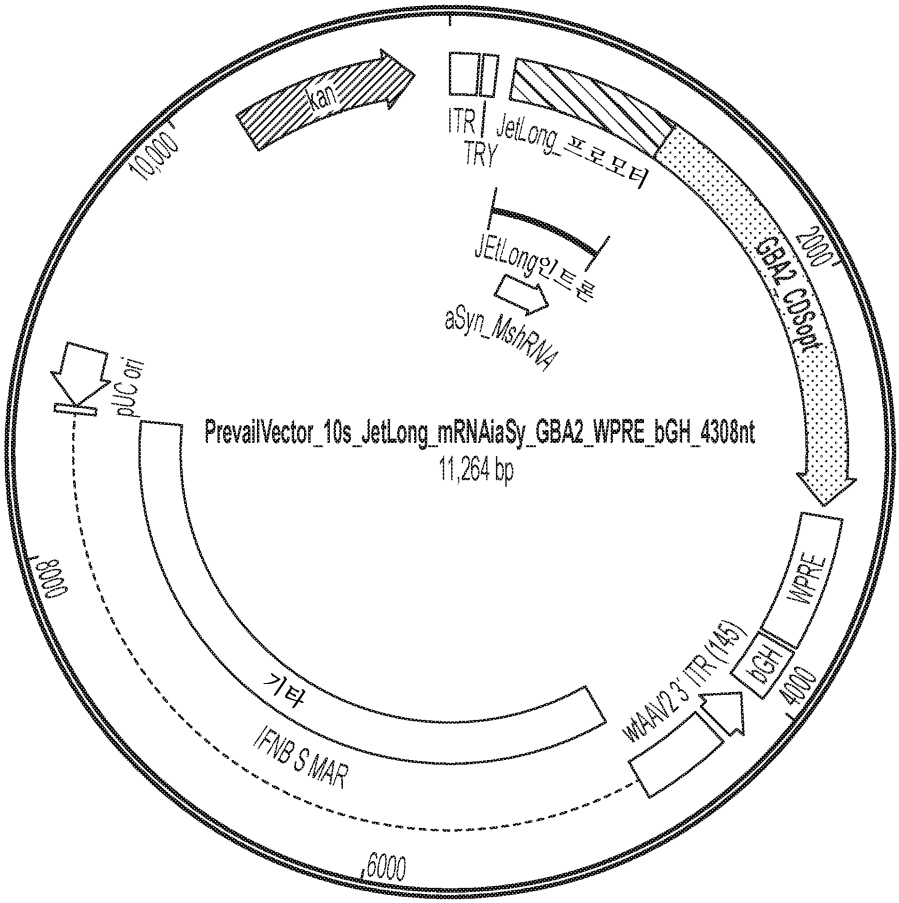
도면19iii



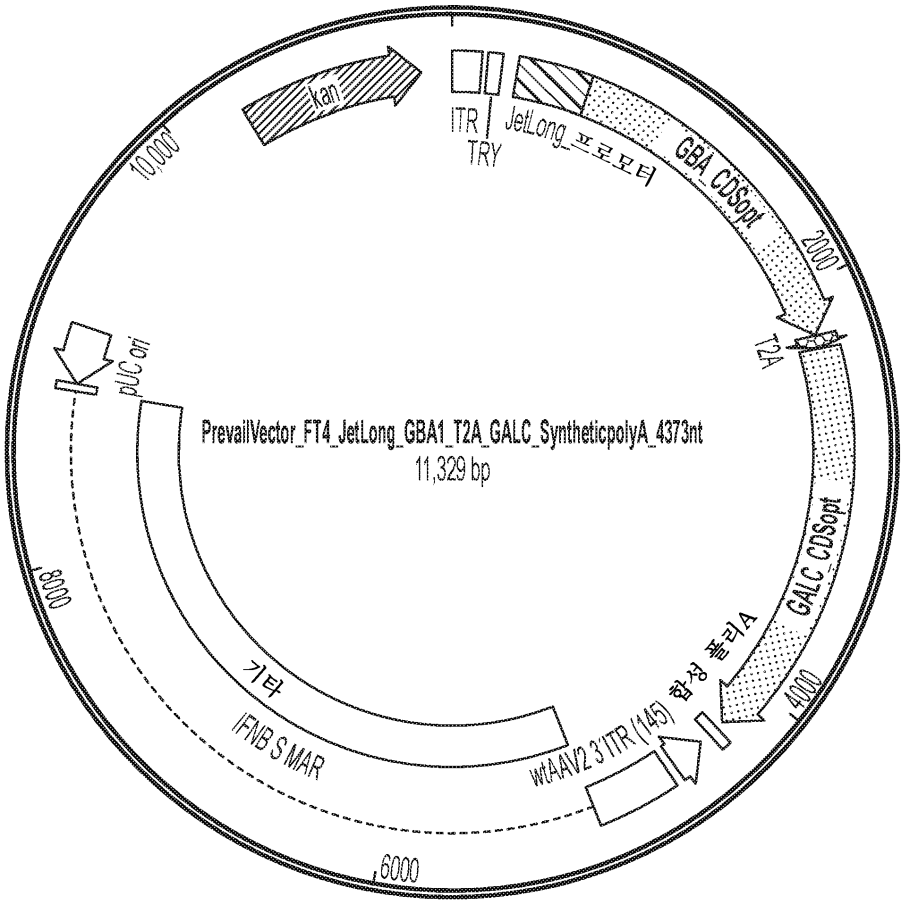
도면20



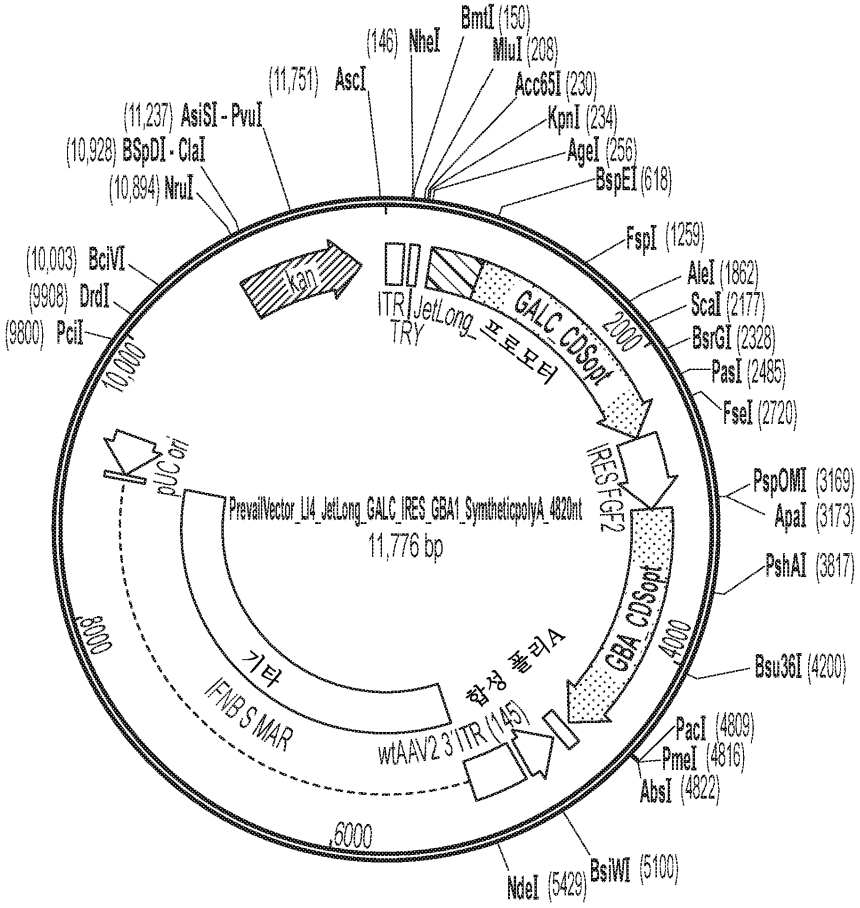
도면21


10,000
kan
ITR
TRY
JetLong_프로모터
2000
GBA2_COSopt
JEtLong인트론
aSyn_MshRN4
pUC ori
PrevailVector_10s_JetLong_mRNAiaSy_GBA2_WPRE_bGH_4308nt
11,264 bp
8000
WPRE
bGH
wtAAV2 3' ITR(145)
4000
기타
IFNB S MAR
6000

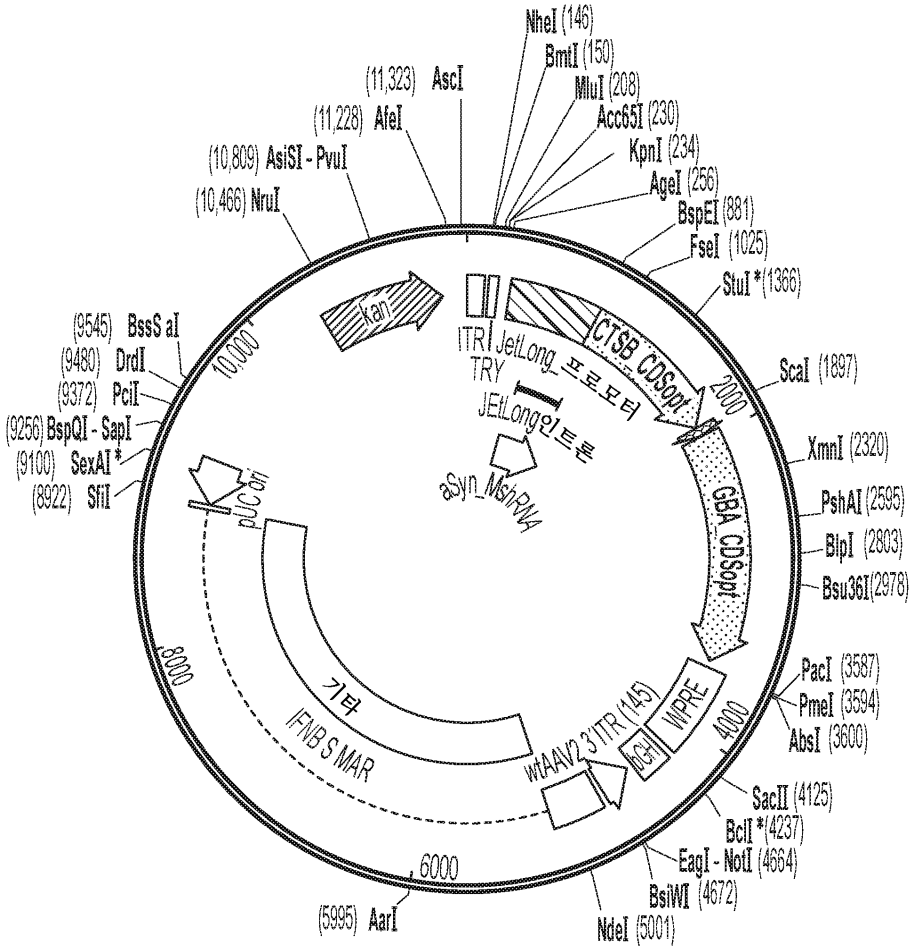
도면22


10,000
Kan
ITR
TRY
JetLong_프로모터
GBA_CDSopt
2000
T2A
GALC_CDSopt
4000
합성 폴리A
wtAAV2 3'ITR (145)
6000
IFNB S MAR
기타
8000
pUC ori
PrevailVector_FT4_JetLong_GBA1_T2A_GALC_SyntheticpolyA_4373nt
11,329 bp

도면23


BmtI (150)
(146) NheI
MluI (208)
(11,751) AscI
Acc65I (230)
(11,237) AsiSI - PvuI
KpnI (234)
(10,928) BSpDI - ClaI
AgeI (256)
(10,894) NruI
BspEI (618)
FspI (1259)
(10,003) BciVI
AleI (1862)
(9908) DrdI
ScaI (2177)
(9800) PciI
BsrGI (2328)
PasI (2485)
FseI (2720)
PspOMI (3169)
ApaI (3173)
PshAI (3817)
Bsu36I (4200)
PacI (4809)
PmeI (4816)
AbsI (4822)
NdeI (5429)
BsiWI (5100)
Kan
ITR
JetLong_프로모터
TRY
GALC CDSopt
IRES
FGF2
GBA CDSopt
pUC ori
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SyntheticpolyA_4820nt
11,776 bp
기타
IFNB S MAR
wtAAV2 3'ITR (145)
합성 폴리A
2000
4000
6000
8000
10,000

도면24



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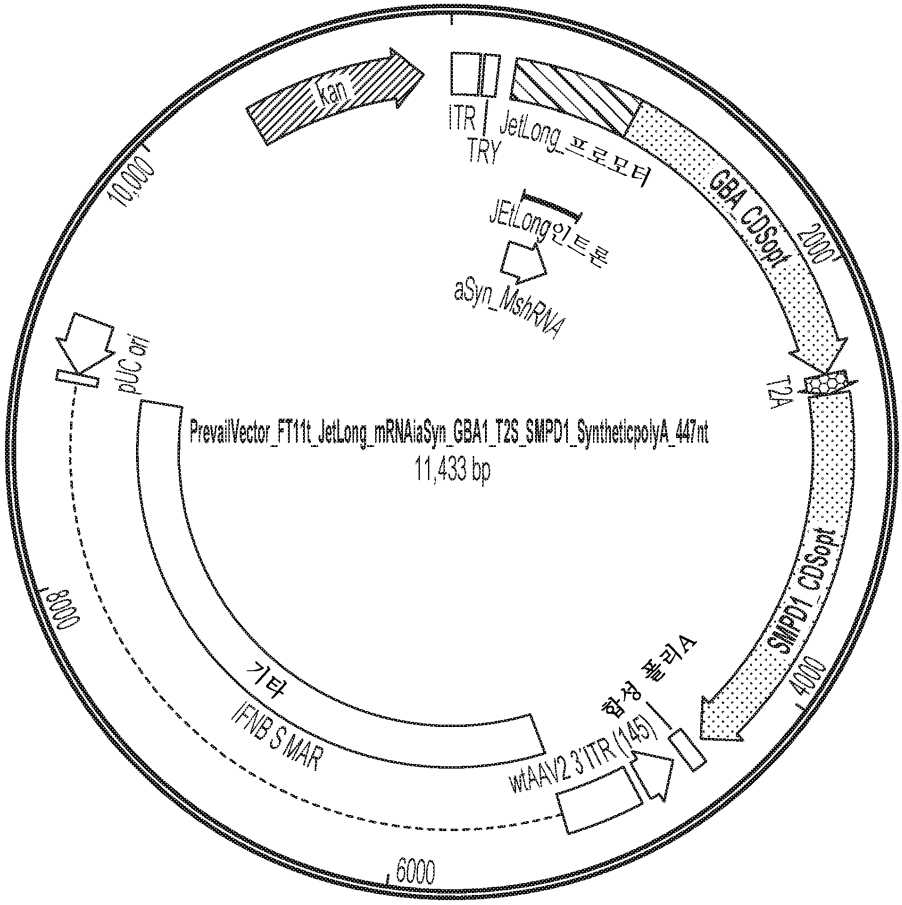
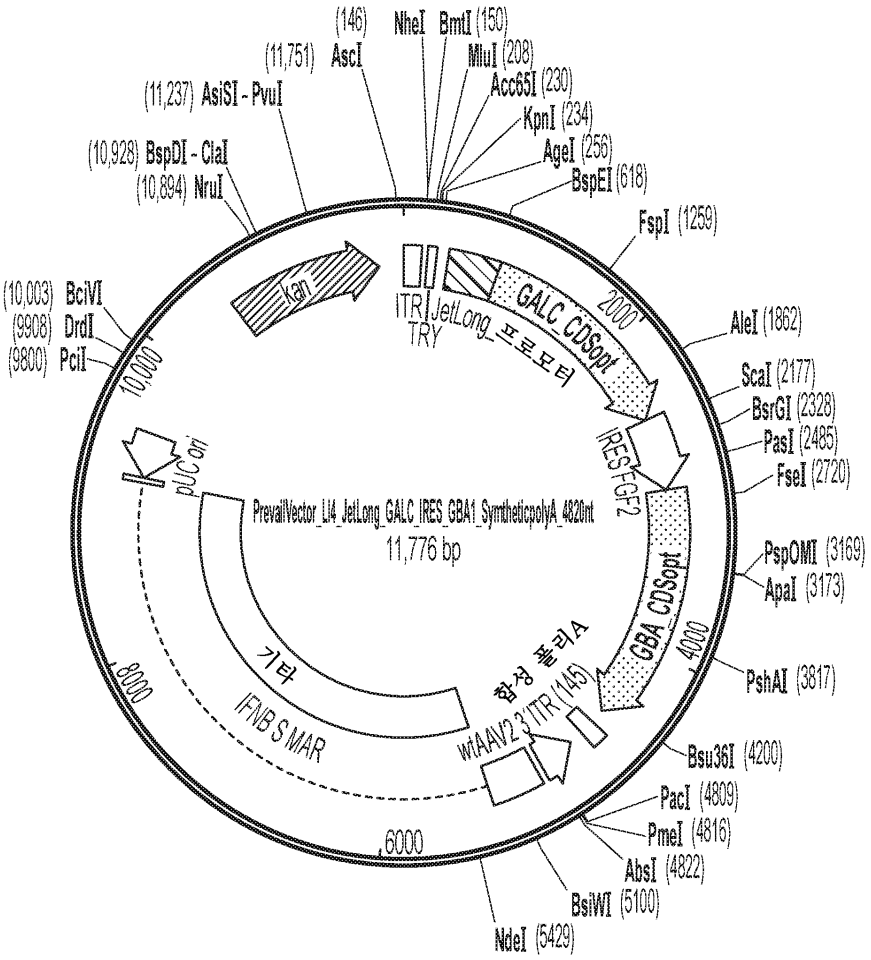
11,348 bp

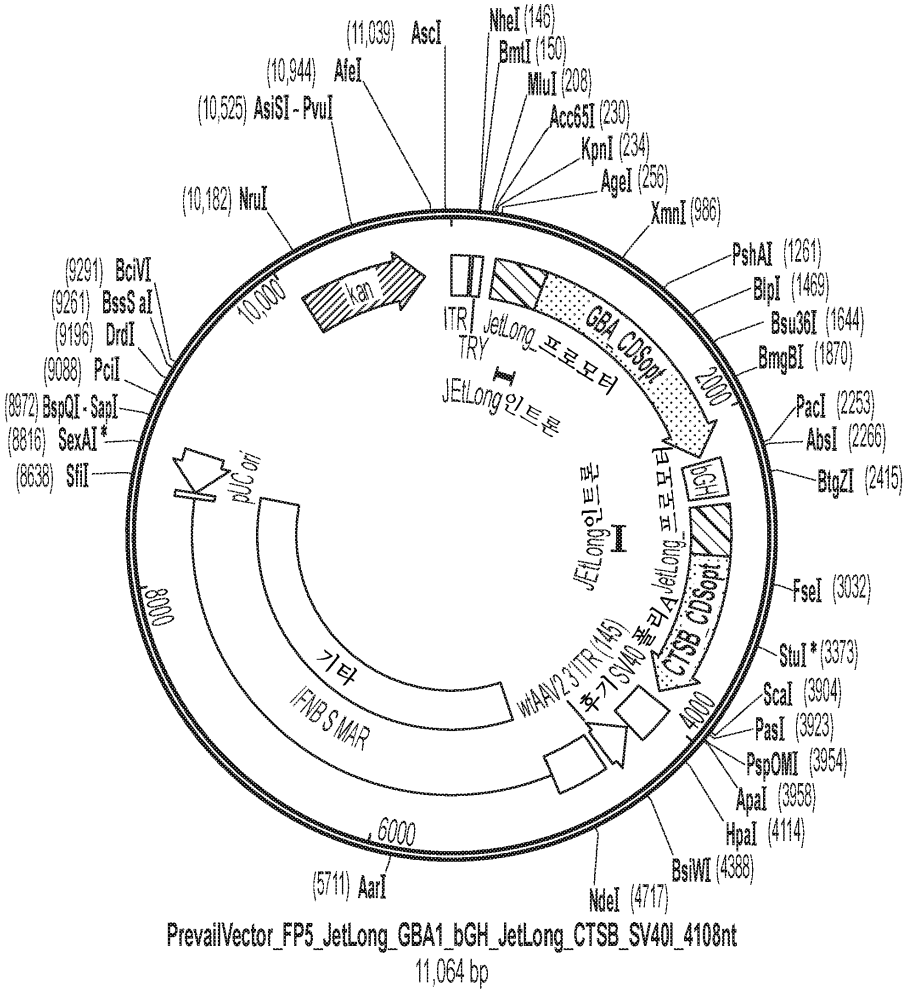
도면25


Kan
ITR
TRY
JetLong_프로모터
GBA_CDSopt
JEtLong인트론
aSyn_MshRNA
pUC ori
T2A
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_447nt
11,433 bp
SMPD1_CDSopt
기타
IFNB S MAR
합성 폴리A
wtAAV2 3'ITR (145)
10,000
2000
4000
6000
8000

도면26


(146) NheI BmtI (150)
(11,751) AscI MluI (208)
(11,237) AsiSI - PvuI Acc65I (230)
KpnI (234)
(10,928) BspDI - ClaI AgeI (256)
(10,894) NruI BspEI (618)
FspI (1259)
(10,003) BciVI
(9908) DrdI
(9800) PciI
Kan
ITR
TRY
JetLong_프로모터
GALC CDSopt
2000
AleI (1862)
ScaI (2177)
BsrGI (2328)
PasI (2485)
FseI (2720)
10,000
pUC ori
IRES FGF2
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SyntheticpolyA_4620nt
11,776 bp
PspOMI (3169)
ApaI (3173)
GBA CDSopt
4000
8000
기타
합성 폴리A
PshAI (3817)
IFNB S/MAR
wtAAV2 3'ITR (145)
Bsu36I (4200)
PacI (4809)
PmeI (4816)
6000
AbsI (4822)
BsiWI (5100)
NdeI (5429)

도면27



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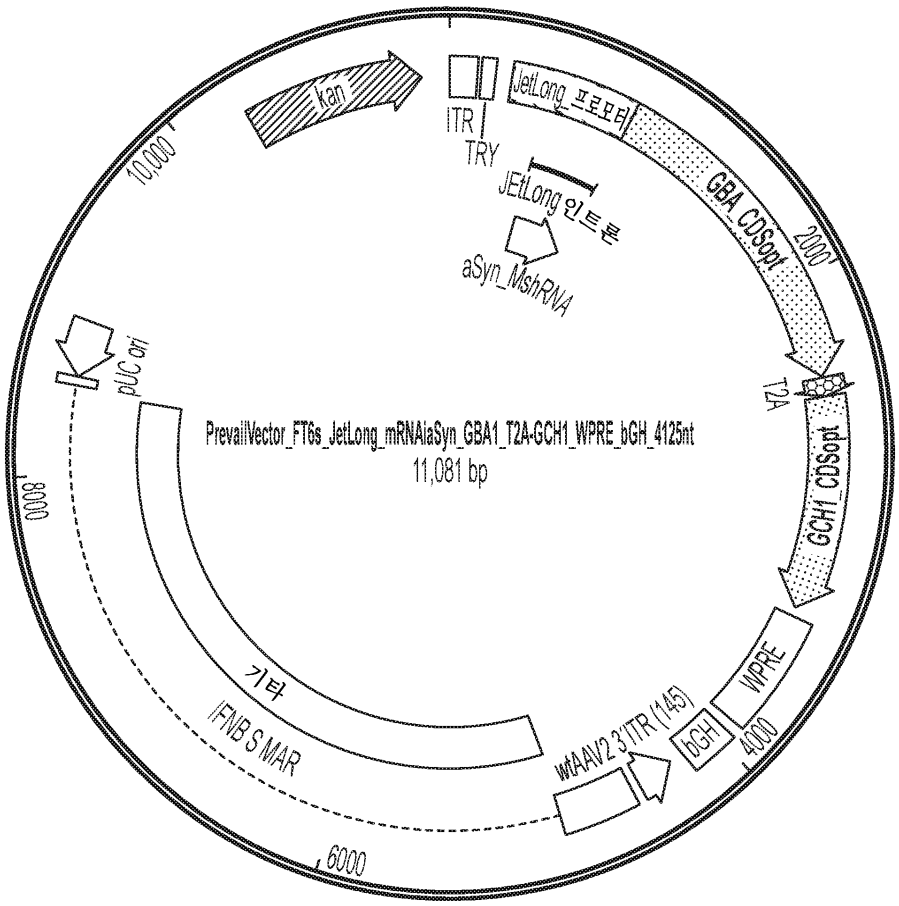
11,064 bp

도면28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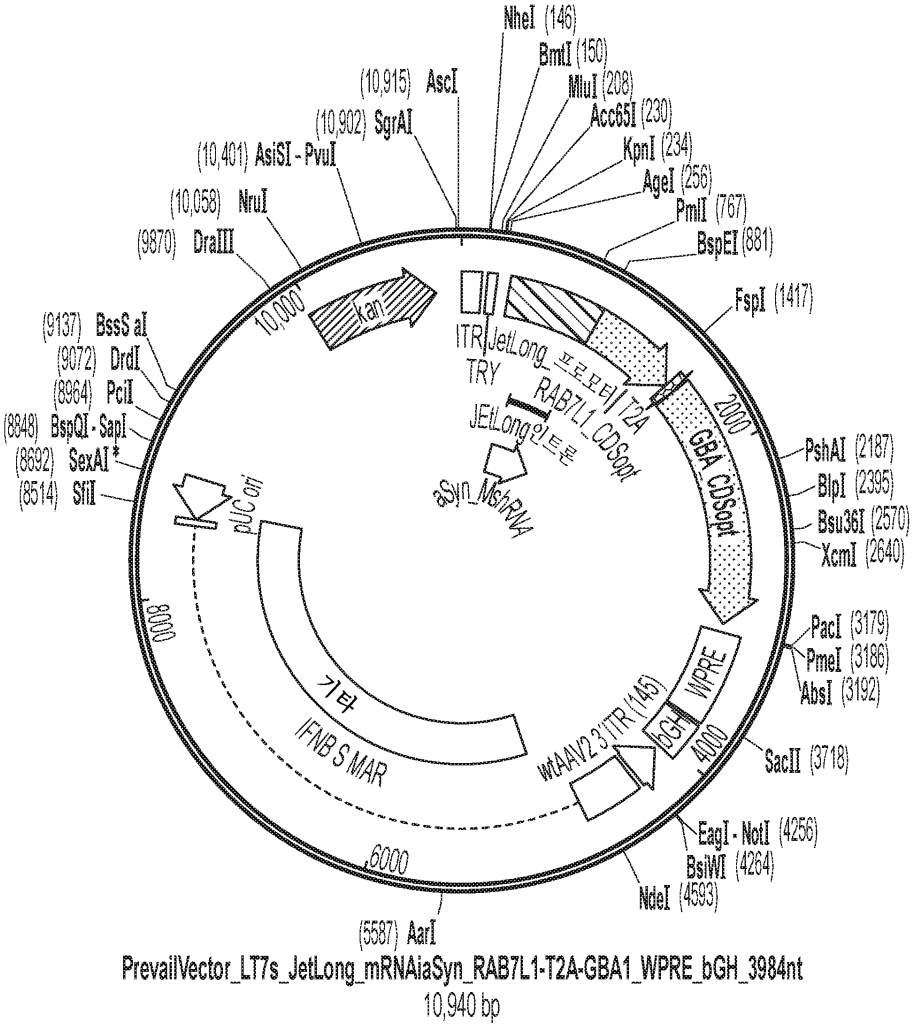
ITR
TRY
JetLong_프로모터
JEtLong 인트론
aSyn_MshRNA
GBA CDSopt
T2A
GCH1 CDSopt
WPRE
bGH
wtAAV2 3'ITR (145)
기타
IFNB S MAR
pUC ori
PrevailVector_FT6s_JetLong_mRNAiaSyn_GBA1_T2A-GCH1_WPRE_bGH_4125nt
11,081 bp
2000
4000
6000
8000
10000

도면29



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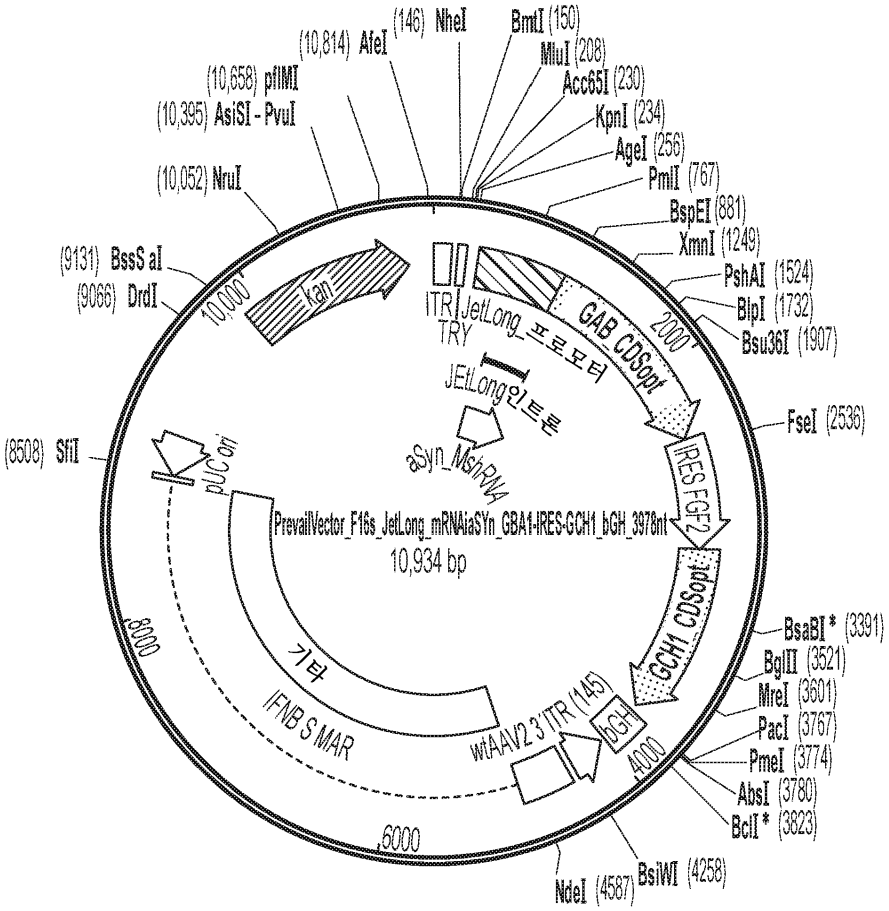
10,940 bp

도면30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BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
PmlI (767)
BspEI (881)
XmnI (1249)
PshAI (1524)
BlpI (1732)
Bsu36I (1907)
FseI (2536)
BsaBI* (3391)
BglII (3521)
MreI (3601)
PacI (3767)
PmeI (3774)
AbsI (3780)
BclI* (3823)
BsiWI (4258)
NdeI (4587)
(10,814) AfeI
(10,658) pflMI
(10,395) AsiSI - PvuI
(10,052) NruI
(9131) BssS aI
(9066) DrdI
(8508) SfiI
10,000
2000
4000
6000
8000
Kan
ITR
TRY
JetLong_프로모터
GAB CDSopt
JETLong인트론
aSyn_MshRNA
IRES FGF2
GCH1_CDSopt
pUC ori
PrevailVector_F16s_JetLong_mRNAiaSYn_GBA1-IRES-GCH1_bGH_3978nt
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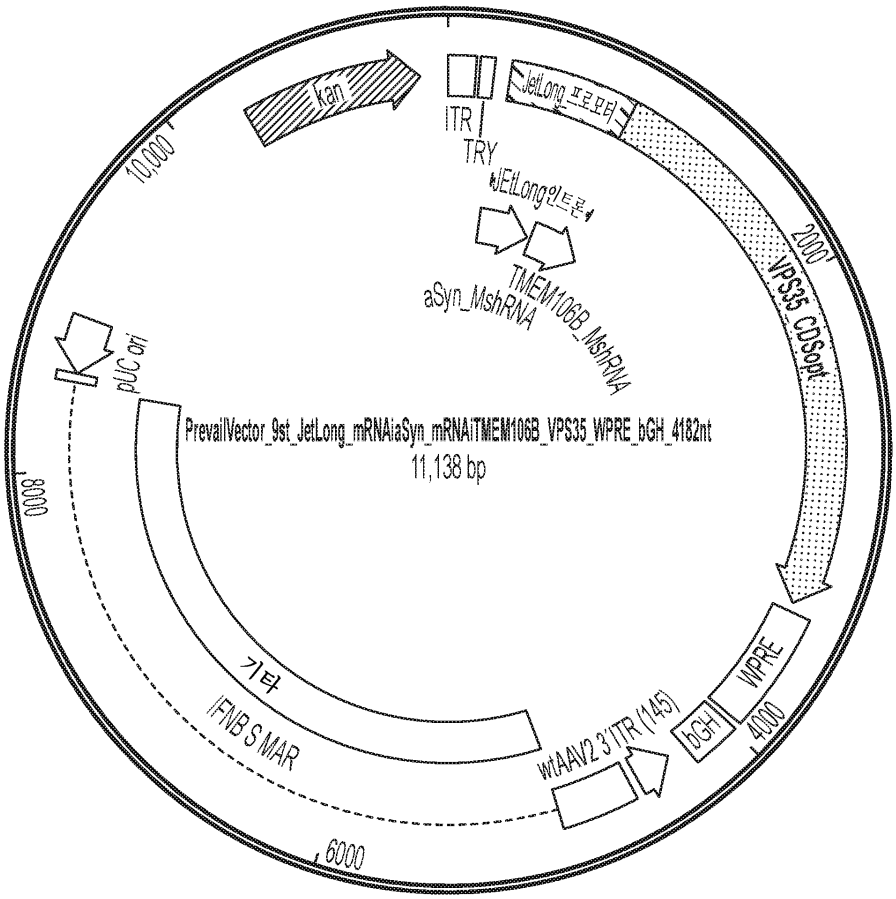
기타
IFNB S MAR
wtAAV2 3'TR (145)
bGH

도면31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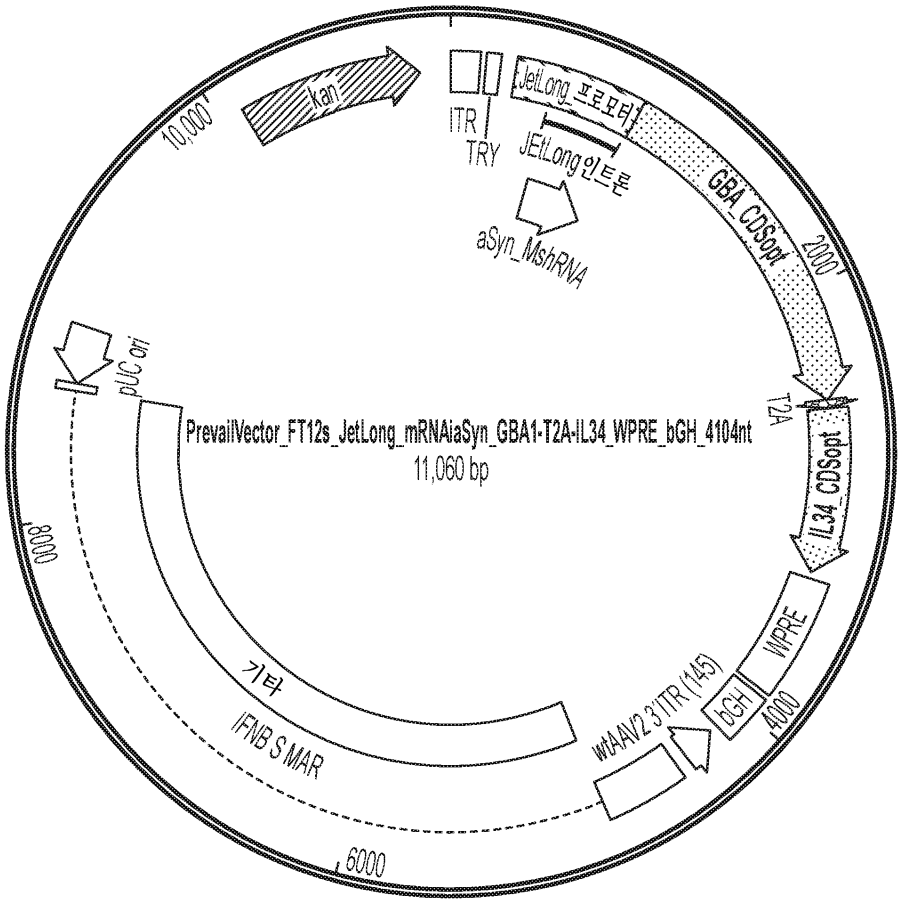
ITR
TRY
JetLong 프로모터
JEtLong인트론
TMEM106B_MshRNA
aSyn_MshRNA
VPS35_CDSopt
WPRE
bGH
wtAAV2 3'ITR (145)
기타
FVB S MAR
pUC ori
PrevailVector_9st_JetLong_mRNAiaSyn_mRNAiTMEM106B_VPS35_WPRE_bGH_4182nt
11,138 bp
10,000
2000
4000
6000
8000

도면32


10,000
kan
ITR
TRY
JetLong 프로모터
JETLong 인트론
aSyn_MshRNA
GBA_CDSopt
2000
T2A
IL34_CDSopt
WPRE
bGH
4000
wtAAV2 3'ITR (145)
6000
IFNB S MAR
기타
8000
pUC ori
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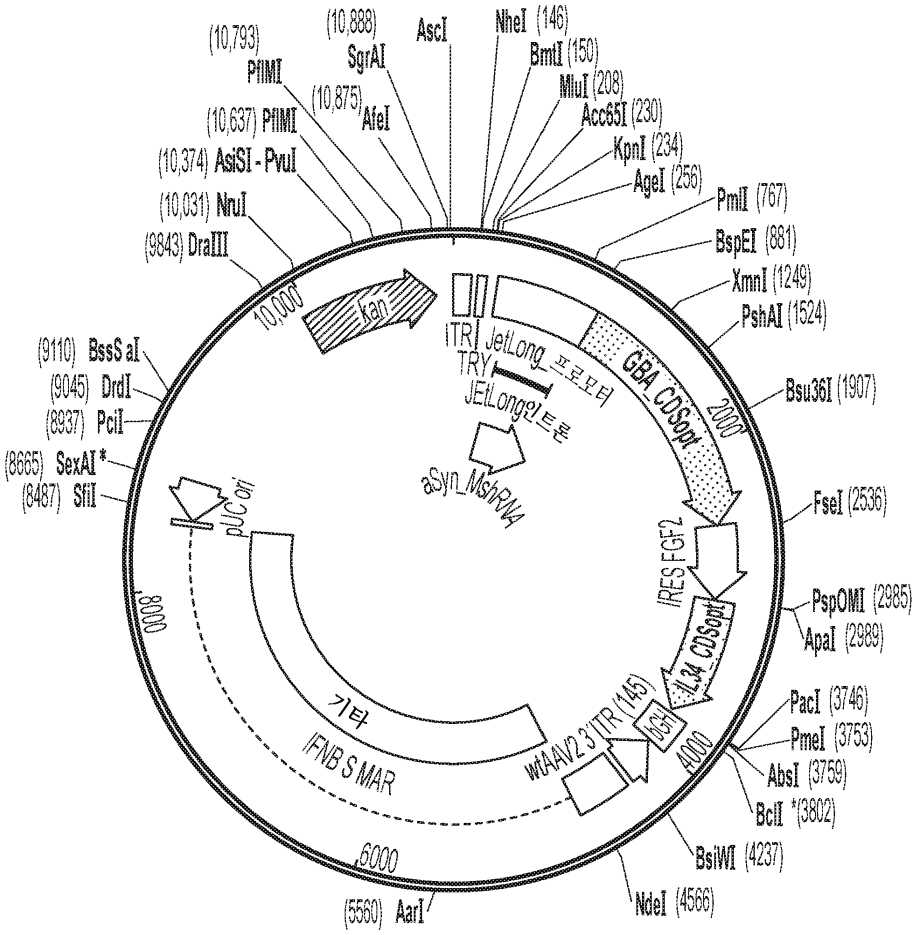
11,060 bp

도면33



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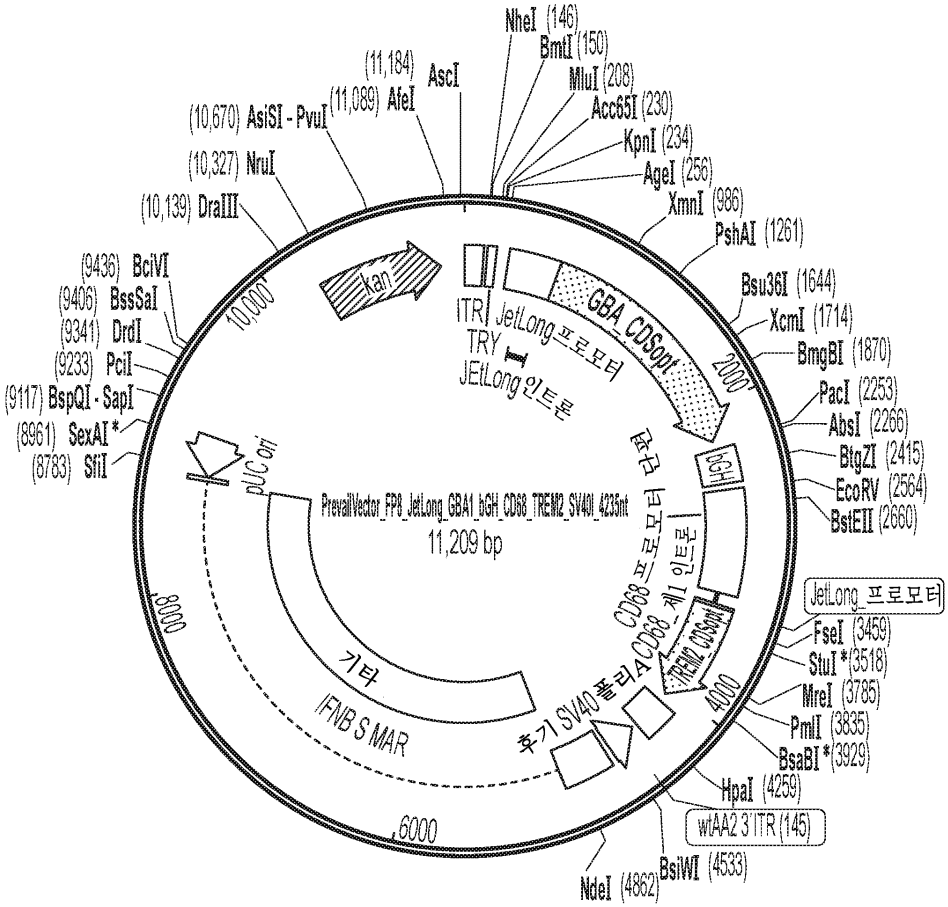
도면34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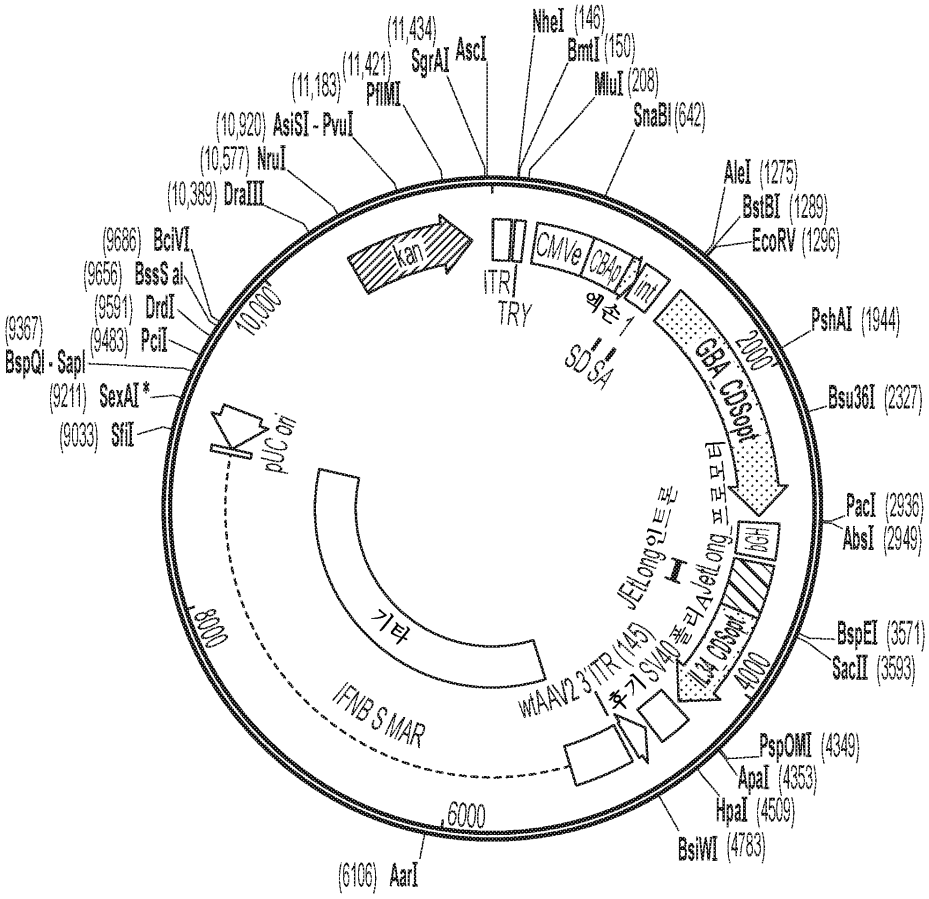
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
XmnI (986)
PshAI (1261)
Bsu36I (1644)
XcmI (1714)
BmgBI (1870)
PacI (2253)
AbsI (2266)
BtgZI (2415)
EcoRV (2564)
BstEII (2660)
JetLong_프로모터
FseI (3459)
StuI *(3518)
MreI (3785)
PmlI (3835)
BsaBI *(3929)
HpaI (4259)
wAA2 3'ITR (145)
BsiWI (4533)
NdeI (4862)
6000
8000
10,000
2000
4000
(11,184) AscI
(11,089) AfeI
(10,670) AsiSI - PvuI
(10,327) NruI
(10,139) DraIII
(9436) BciVI
(9406) BssSaI
(9341) DrdI
(9233) PciI
(9117) BspQI - SapI
(8961) SexAI *
(8783) SfiI
Kan
ITR
TRY
JEtLong 인트론
JetLong 프로모터
GBA CDSopt
pUC ori
PrevailVector_FP8_JetLong_GBA1_hGH_CD68_TREM2_SV40L_4235nt
11,209 bp
hGH
CD68 프로모터
CD68_제1 인트론
TREM2 CDSopt
폴리A
후기 SV40
기타
IFNB S MAR

도면35



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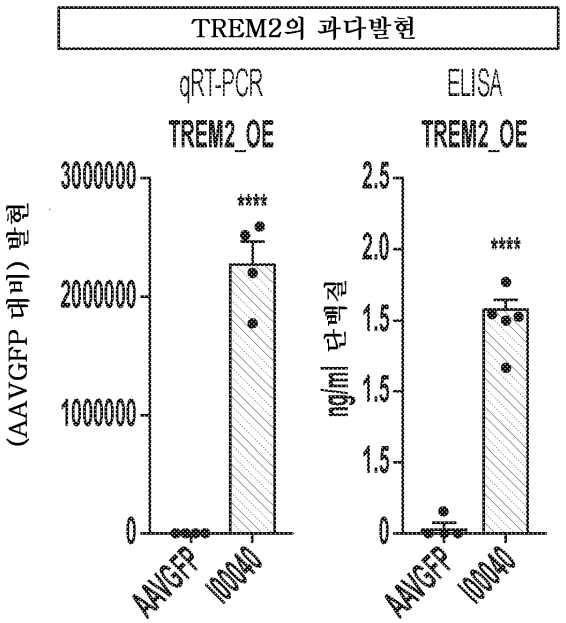
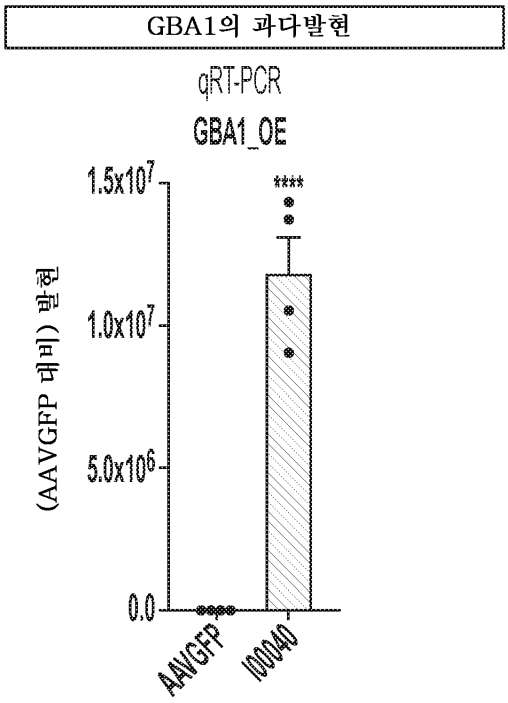
도면36a



도면36b


GBA1의 과다발현
qRT-PCR
GBA1_OE
(AAVGFP 대비) 발현
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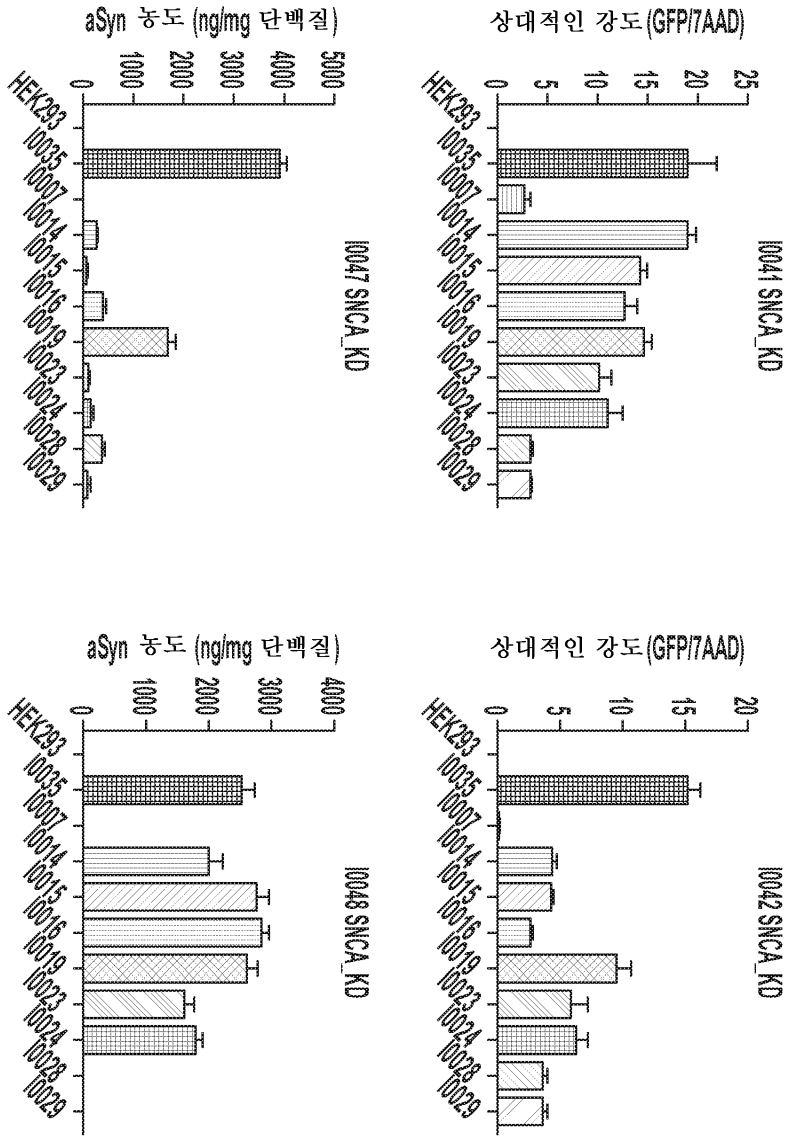
1.0x10^7
5.0x10^6
0.0
****
AAVGFP
100040

도면37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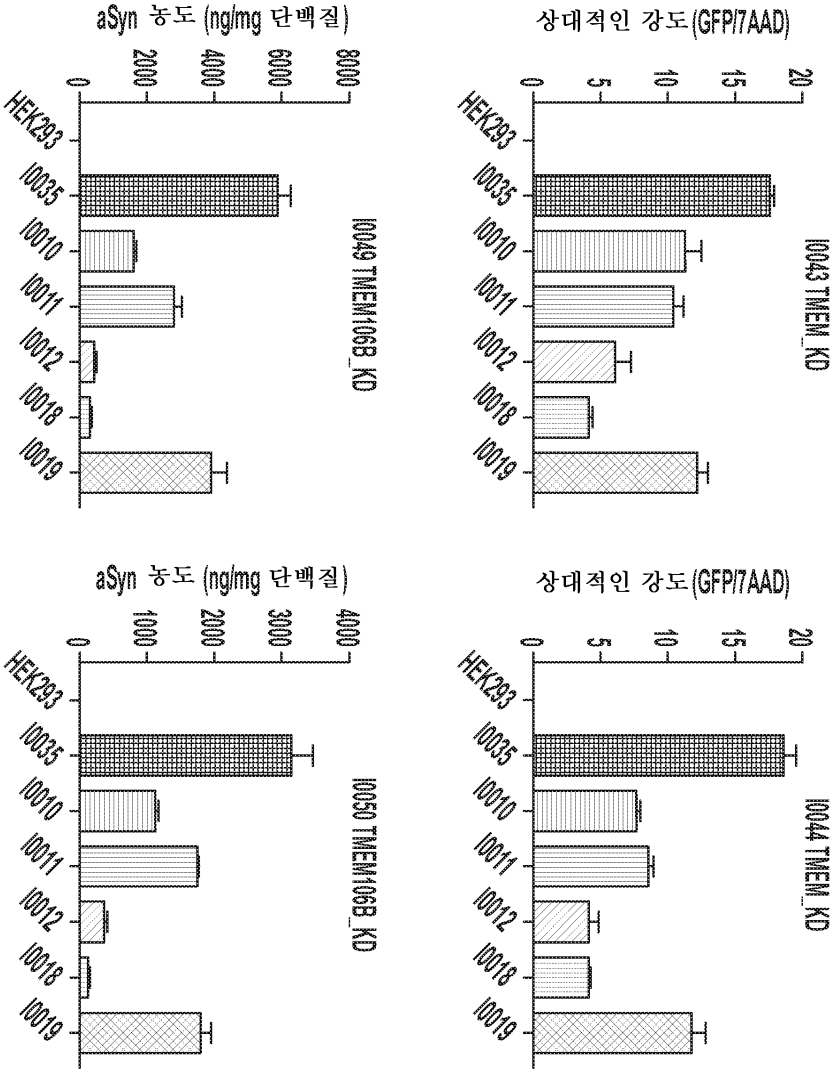
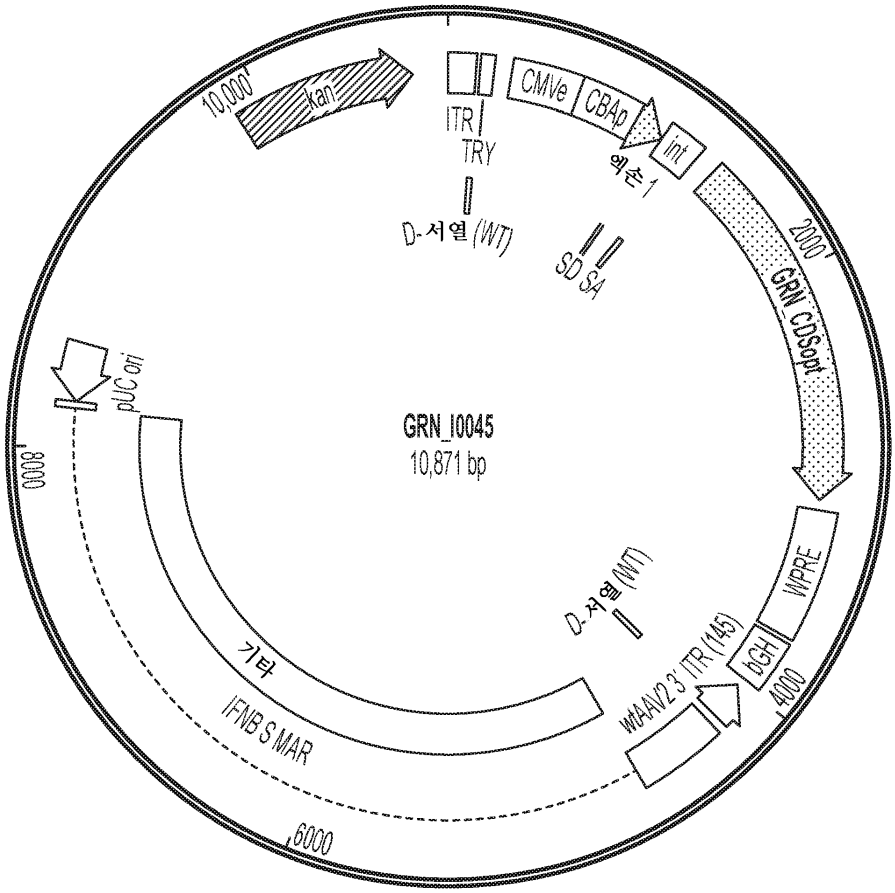
0
1000
2000
3000
4000
5000
HEK293
I0035
I0007
I0014
I0015
I0016
I0019
I0023
I0024
I0028
I0029
I0047 SNCA_KD
상대적인 강도(GFP/7AAD)
0
5
10
15
20
25
I0041 SNCA_KD
aSyn 농도 (ng/mg 단백질)
I0048 SNCA_KD
상대적인 강도(GFP/7AAD)
I0042 SNCA_KD

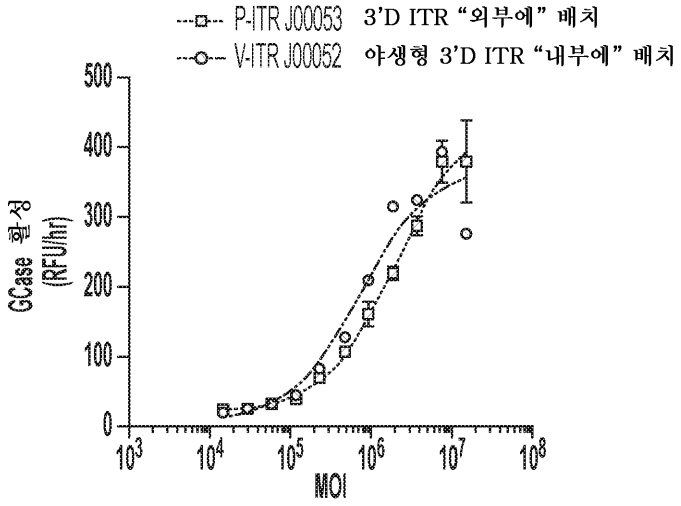
도면38


aSyn 농도 (ng/mg 단백질)
0
2000
4000
6000
8000
HEK293
I0035
I0010
I0011
I0012
I0018
I0019
I0049 TMEM106B_KD
상대적인 강도(GFP/7AAD)
0
5
10
15
20
I0043 TMEM_KD
aSyn 농도 (ng/mg 단백질)
0
1000
2000
3000
4000
I0050 TMEM106B_KD
상대적인 강도(GFP/7AAD)
I0044 TMEM_KD

도면39



도면40



서 열 목 록

SEQUENCE LISTING

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<120> GENE THERAPIES FOR LYSOSOMAL DISORDERS

<130> P1094.70003WO00

<140> Not Yet Assigned

<141> Concurrently herewith

<150> US 62/567,310

<151> 2017-10-03

<150> US 62/567,296

<151> 2017-10-03

<150> US 62/567,311

<151> 2017-10-03

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<151> 2017-10-03

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gaagcccaaa agacaataac aaaaatattc ttgtagaaca aaatgggaaa gaatgttcca 4140
ctaaatatca agatttagag caaagcatga gatgtgtggg gatagacagt gaggctgata 4200

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<211> 536

<212> PRT

<213> Homo sapiens

<400> 14

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Ala Val Ser Trp Ala Ser Gly Ala Arg Pro Cys Ile Pro Lys Ser Phe
35 40 45

Gly Tyr Ser Ser Val Val Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser
50 55 60

Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu
65 70 75 80

Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln
85 90 95

Ala Asn His Thr Gly Thr Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln
100 105 110

Lys Phe Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asp Ala Ala
115 120 125

Ala Leu Asn Ile Leu Ala Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu
130 135 140

Lys Ser Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val
145 150 155 160

Pro Met Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp
165 170 175

Thr Pro Asp Asp Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp
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Thr Lys Leu Lys Ile Pro Leu Ile His Arg Ala Leu Gln Leu Ala Gln
195 200 205

Arg Pro Val Ser Leu Leu Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu
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Lys Thr Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro

225 230 235 240

Gly Asp Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu

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Asp Ala Tyr Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu

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Gly Phe Thr Pro Glu His Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly

290 295 300

Pro Thr Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met Leu

305 310 315 320

Asp Asp Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr

325 330 335

Asp Pro Glu Ala Ala Lys Tyr Val His Gly Ile Ala Val His Trp Tyr

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Leu Asp Phe Leu Ala Pro Ala Lys Ala Thr Leu Gly Glu Thr His Arg

355 360 365

Leu Phe Pro Asn Thr Met Leu Phe Ala Ser Glu Ala Cys Val Gly Ser

370 375 380

Lys Phe Trp Glu Gln Ser Val Arg Leu Gly Ser Trp Asp Arg Gly Met

385 390 395 400

Gln Tyr Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly

405 410 415

Trp Thr Asp Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp

420 425 430

Val Arg Asn Phe Val Asp Ser Pro Ile Ile Val Asp Ile Thr Lys Asp

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Thr Phe Tyr Lys Gln Pro Met Phe Tyr His Leu Gly His Phe Ser Lys

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Phe Ile Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys

465 470 475 480

Asn Asp Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val

485 490 495

Val Val Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys

500 505 510

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<212> PRT

<213> Homo sapiens

<400> 16

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20 25 30

Cys Gln Asn Val Lys Thr Ala Ser Asp Cys Gly Ala Val Lys His Cys

35 40 45

Leu Gln Thr Val Trp Asn Lys Pro Thr Val Lys Ser Leu Pro Cys Asp

50 55 60

Ile Cys Lys Asp Val Val Thr Ala Ala Gly Asp Met Leu Lys Asp Asn

65 70 75 80

Ala Thr Glu Glu Glu Ile Leu Val Tyr Leu Glu Lys Thr Cys Asp Trp

85 90 95

Leu Pro Lys Pro Asn Met Ser Ala Ser Cys Lys Glu Ile Val Asp Ser

100 105 110

Tyr Leu Pro Val Ile Leu Asp Ile Ile Lys Gly Glu Met Ser Arg Pro

115 120 125

Gly Glu Val Cys Ser Ala Leu Asn Leu Cys Glu Ser Leu Gln Lys His

130 135 140

Leu Ala Glu Leu Asn His Gln Lys Gln Leu Glu Ser Asn Lys Ile Pro

145 150 155 160

Glu Leu Asp Met Thr Glu Val Val Ala Pro Phe Met Ala Asn Ile Pro

165 170 175

Leu Leu Leu Tyr Pro Gln Asp Gly Pro Arg Ser Lys Pro Gln Pro Lys

180 185 190

Asp Asn Gly Asp Val Cys Gln Asp Cys Ile Gln Met Val Thr Asp Ile

195 200 205

Gln Thr Ala Val Arg Thr Asn Ser Thr Phe Val Gln Ala Leu Val Glu

210 215 220

His Val Lys Glu Glu Cys Asp Arg Leu Gly Pro Gly Met Ala Asp Ile

225 230 235 240

Cys Lys Asn Tyr Ile Ser Gln Tyr Ser Glu Ile Ala Ile Gln Met Met

245 250 255

Met His Met Gln Pro Lys Glu Ile Cys Ala Leu Val Gly Phe Cys Asp

260 265 270

Glu Val Lys Glu Met Pro Met Gln Thr Leu Val Pro Ala Lys Val Ala

275 280 285

Ser Lys Asn Val Ile Pro Ala Leu Glu Leu Val Glu Pro Ile Lys Lys

290 295 300

His Glu Val Pro Ala Lys Ser Asp Val Tyr Cys Glu Val Cys Glu Phe

305 310 315 320

Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys

325 330 335

Glu Ile Leu Asp Ala Phe Asp Lys Met Cys Ser Lys Leu Pro Lys Ser

340 345 350

Leu Ser Glu Glu Cys Gln Glu Val Val Asp Thr Tyr Gly Ser Ser Ile

355 360 365

Leu Ser Ile Leu Leu Glu Glu Val Ser Pro Glu Leu Val Cys Ser Met

370 375 380

Leu His Leu Cys Ser Gly Thr Arg Leu Pro Ala Leu Thr Val His Val

385 390 395 400

Thr Gln Pro Lys Asp Gly Gly Phe Cys Glu Val Cys Lys Lys Leu Val

405 410 415

Gly Tyr Leu Asp Arg Asn Leu Glu Lys Asn Ser Thr Lys Gln Glu Ile

420 425 430

Leu Ala Ala Leu Glu Lys Gly Cys Ser Phe Leu Pro Asp Pro Tyr Gln

435 440 445

Lys Gln Cys Asp Gln Phe Val Ala Glu Tyr Glu Pro Val Leu Ile Glu

450 455 460

Ile Leu Val Glu Val Met Asp Pro Ser Phe Val Cys Leu Lys Ile Gly

465 470 475 480

Ala Cys Pro Ser Ala His Lys Pro Leu Leu Gly Thr Glu Lys Cys Ile

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Trp Gly Pro Ser Tyr Trp Cys Gln Asn Thr Glu Thr Ala Ala Gln Cys

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Asn Ala Val Glu His Cys Lys Arg His Val Trp Asn

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<211> 1572

<212> DNA

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<212> PRT

<213> Homo sapiens

<400> 18

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35 40 45

Ala Phe Asp Ser Trp Glu Lys Pro Pro Leu Pro Val Tyr Thr Gln Phe

50 55 60

Tyr Phe Phe Asn Val Thr Asn Pro Glu Glu Ile Leu Arg Gly Glu Thr
65 70 75 80

Pro Arg Val Glu Glu Val Gly Pro Tyr Thr Tyr Arg Glu Leu Arg Asn
85 90 95

Lys Ala Asn Ile Gln Phe Gly Asp Asn Gly Thr Thr Ile Ser Ala Val
100 105 110

Ser Asn Lys Ala Tyr Val Phe Glu Arg Asp Gln Ser Val Gly Asp Pro

115 120 125

Lys Ile Asp Leu Ile Arg Thr Leu Asn Ile Pro Val Leu Thr Val Ile
130 135 140

Glu Trp Ser Gln Val His Phe Leu Arg Glu Ile Ile Glu Ala Met Leu
145 150 155 160

Lys Ala Tyr Gln Gln Lys Leu Phe Val Thr His Thr Val Asp Glu Leu
165 170 175

Leu Trp Gly Tyr Lys Asp Glu Ile Leu Ser Leu Ile His Val Phe Arg

180 185 190

Pro Asp Ile Ser Pro Tyr Phe Gly Leu Phe Tyr Glu Lys Asn Gly Thr

195 200 205

Asn Asp Gly Asp Tyr Val Phe Leu Thr Gly Glu Asp Ser Tyr Leu Asn
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Phe Thr Lys Ile Val Glu Trp Asn Gly Lys Thr Ser Leu Asp Trp Trp
225 230 235 240

Ile Thr Asp Lys Cys Asn Met Ile Asn Gly Thr Asp Gly Asp Ser Phe

245 250 255

His Pro Leu Ile Thr Lys Asp Glu Val Leu Tyr Val Phe Pro Ser Asp

260 265 270

Phe Cys Arg Ser Val Tyr Ile Thr Phe Ser Asp Tyr Glu Ser Val Gln

275 280 285

Gly Leu Pro Ala Phe Arg Tyr Lys Val Pro Ala Glu Ile Leu Ala Asn

290 295 300

Thr Ser Asp Asn Ala Gly Phe Cys Ile Pro Glu Gly Asn Cys Leu Gly

305 310 315 320

Ser Gly Val Leu Asn Val Ser Ile Cys Lys Asn Gly Ala Pro Ile Ile

325 330 335

Met Ser Phe Pro His Phe Tyr Gln Ala Asp Glu Arg Phe Val Ser Ala

340 345 350

Ile Glu Gly Met His Pro Asn Gln Glu Asp His Glu Thr Phe Val Asp

355 360 365

Ile Asn Pro Leu Thr Gly Ile Ile Leu Lys Ala Ala Lys Arg Phe Gln

370 375 380

Ile Asn Ile Tyr Val Lys Lys Leu Asp Asp Phe Val Glu Thr Gly Asp

385 390 395 400

Ile Arg Thr Met Val Phe Pro Val Met Tyr Leu Asn Glu Ser Val His

405 410 415

Ile Asp Lys Glu Thr Ala Ser Arg Leu Lys Ser Met Ile Asn Thr Thr

420 425 430

Leu Ile Ile Thr Asn Ile Pro Tyr Ile Ile Met Ala Leu Gly Val Phe

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<212> DNA

<213> Homo sapiens

<400> 19

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<212> DNA

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<211> 685
<212> PRT
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<400> 33

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35 40 45
Asp Gly Leu Gly Arg Glu Phe Asp Gly Ile Gly Ala Val Ser Gly Gly
50 55 60
Gly Ala Thr Ser Arg Leu Leu Val Asn Tyr Pro Glu Pro Tyr Arg Ser
65 70 75 80
Gln Ile Leu Asp Tyr Leu Phe Lys Pro Asn Phe Gly Ala Ser Leu His

85 90 95
Ile Leu Lys Val Glu Ile Gly Gly Asp Gly Gln Thr Thr Asp Gly Thr
100 105 110
Glu Pro Ser His Met His Tyr Ala Leu Asp Glu Asn Tyr Phe Arg Gly
115 120 125
Tyr Glu Trp Trp Leu Met Lys Glu Ala Lys Lys Arg Asn Pro Asn Ile
130 135 140
Thr Leu Ile Gly Leu Pro Trp Ser Phe Pro Gly Trp Leu Gly Lys Gly

145 150 155 160
Phe Asp Trp Pro Tyr Val Asn Leu Gln Leu Thr Ala Tyr Tyr Val Val
165 170 175
Thr Trp Ile Val Gly Ala Lys Arg Tyr His Asp Leu Asp Ile Asp Tyr
180 185 190
Ile Gly Ile Trp Asn Glu Arg Ser Tyr Asn Ala Asn Tyr Ile Lys Ile
195 200 205
Leu Arg Lys Met Leu Asn Tyr Gln Gly Leu Gln Arg Val Lys Ile Ile

210 215 220
Ala Ser Asp Asn Leu Trp Glu Ser Ile Ser Ala Ser Met Leu Leu Asp
225 230 235 240
Ala Glu Leu Phe Lys Val Val Asp Val Ile Gly Ala His Tyr Pro Gly

245 250 255

Thr His Ser Ala Lys Asp Ala Lys Leu Thr Gly Lys Lys Leu Trp Ser

260 265 270

Ser Glu Asp Phe Ser Thr Leu Asn Ser Asp Met Gly Ala Gly Cys Trp

275 280 285

Gly Arg Ile Leu Asn Gln Asn Tyr Ile Asn Gly Tyr Met Thr Ser Thr

290 295 300

Ile Ala Trp Asn Leu Val Ala Ser Tyr Tyr Glu Gln Leu Pro Tyr Gly

305 310 315 320

Arg Cys Gly Leu Met Thr Ala Gln Glu Pro Trp Ser Gly His Tyr Val

325 330 335

Val Glu Ser Pro Val Trp Val Ser Ala His Thr Thr Gln Phe Thr Gln

340 345 350

Pro Gly Trp Tyr Tyr Leu Lys Thr Val Gly His Leu Glu Lys Gly Gly

355 360 365

Ser Tyr Val Ala Leu Thr Asp Gly Leu Gly Asn Leu Thr Ile Ile Ile

370 375 380

Glu Thr Met Ser His Lys His Ser Lys Cys Ile Arg Pro Phe Leu Pro

385 390 395 400

Tyr Phe Asn Val Ser Gln Gln Phe Ala Thr Phe Val Leu Lys Gly Ser

405 410 415

Phe Ser Glu Ile Pro Glu Leu Gln Val Trp Tyr Thr Lys Leu Gly Lys

420 425 430

Thr Ser Glu Arg Phe Leu Phe Lys Gln Leu Asp Ser Leu Trp Leu Leu

435 440 445

Asp Ser Asp Gly Ser Phe Thr Leu Ser Leu His Glu Asp Glu Leu Phe

450 455 460

Thr Leu Thr Thr Leu Thr Thr Gly Arg Lys Gly Ser Tyr Pro Leu Pro

465 470 475 480

Pro Lys Ser Gln Pro Phe Pro Ser Thr Tyr Lys Asp Asp Phe Asn Val

485 490 495

Asp Tyr Pro Phe Phe Ser Glu Ala Pro Asn Phe Ala Asp Gln Thr Gly
500 505 510

Val Phe Glu Tyr Phe Thr Asn Ile Glu Asp Pro Gly Glu His His Phe
515 520 525

Thr Leu Arg Gln Val Leu Asn Gln Arg Pro Ile Thr Trp Ala Ala Asp

530 535 540

Ala Ser Asn Thr Ile Ser Ile Ile Gly Asp Tyr Asn Trp Thr Asn Leu
545 550 555 560

Thr Ile Lys Cys Asp Val Tyr Ile Glu Thr Pro Asp Thr Gly Gly Val
565 570 575

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

Arg Gly Ile Phe Phe Trp Ile Phe Ala Asn Gly Ser Tyr Arg Val Thr

595 600 605

Gly Asp Leu Ala Gly Trp Ile Ile Tyr Ala Leu Gly Arg Val Glu Val
610 615 620

Thr Ala Lys Lys Trp Tyr Thr Leu Thr Leu Thr Ile Lys Gly His Phe
625 630 635 640

Thr Ser Gly Met Leu Asn Asp Lys Ser Leu Trp Thr Asp Ile Pro Val
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Asn Phe Pro Lys Asn Gly Trp Ala Ala Ile Gly Thr His Ser Phe Glu

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Phe Ala Gln Phe Asp Asn Phe Leu Val Glu Ala Thr Arg
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<210> 34

<211> 2055

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

<400> 34

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<211> 339

<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 35

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Tyr Val Asn Lys Arg Asn Thr Thr Trp Gln Ala Gly His Asn Phe Tyr
35 40 45

Asn Val Asp Met Ser Tyr Leu Lys Arg Leu Cys Gly Thr Phe Leu Gly
50 55 60

Gly Pro Lys Pro Pro Gln Arg Val Met Phe Thr Glu Asp Leu Lys Leu
65 70 75 80

Pro Ala Ser Phe Asp Ala Arg Glu Gln Trp Pro Gln Cys Pro Thr Ile
85 90 95

Lys Glu Ile Arg Asp Gln Gly Ser Cys Gly Ser Cys Trp Ala Phe Gly
100 105 110

Ala Val Glu Ala Ile Ser Asp Arg Ile Cys Ile His Thr Asn Ala His
115 120 125

Val Ser Val Glu Val Ser Ala Glu Asp Leu Leu Thr Cys Cys Gly Ser
130 135 140

Met Cys Gly Asp Gly Cys Asn Gly Gly Tyr Pro Ala Glu Ala Trp Asn
145 150 155 160

Phe Trp Thr Arg Lys Gly Leu Val Ser Gly Gly Leu Tyr Glu Ser His
165 170 175

Val Gly Cys Arg Pro Tyr Ser Ile Pro Pro Cys Glu His His Val Asn
180 185 190
Gly Ser Arg Pro Pro Cys Thr Gly Glu Gly Asp Thr Pro Lys Cys Ser
195 200 205

Lys Ile Cys Glu Pro Gly Tyr Ser Pro Thr Tyr Lys Gln Asp Lys His
210 215 220
Tyr Gly Tyr Asn Ser Tyr Ser Val Ser Asn Ser Glu Lys Asp Ile Met
225 230 235 240
Ala Glu Ile Tyr Lys Asn Gly Pro Val Glu Gly Ala Phe Ser Val Tyr
245 250 255
Ser Asp Phe Leu Leu Tyr Lys Ser Gly Val Tyr Gln His Val Thr Gly
260 265 270

Glu Met Met Gly Gly His Ala Ile Arg Ile Leu Gly Trp Gly Val Glu
275 280 285
Asn Gly Thr Pro Tyr Trp Leu Val Ala Asn Ser Trp Asn Thr Asp Trp
290 295 300
Gly Asp Asn Gly Phe Phe Lys Ile Leu Arg Gly Gln Asp His Cys Gly
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Ile Glu Ser Glu Val Val Ala Gly Ile Pro Arg Thr Asp Gln Tyr Trp
325 330 335

Glu Lys Ile

<210> 36
<211> 1017
<212> DNA
<213> Artificial sequence
<220><223> Synthetic polynucleotide
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<210> 37
<211> 631
<212> PRT
<213> Artificial sequence
<220><223> Synthetic polypeptide
<400> 37

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20 25 30

Met Gly Leu Val Leu Ala Leu Ala Leu Ala Leu Ala Leu Ala Leu Ala
35 40 45

Leu Ser Asp Ser Arg Val Leu Trp Ala Pro Ala Glu Ala His Pro Leu
50 55 60

Ser Pro Gln Gly His Pro Ala Arg Leu His Arg Ile Val Pro Arg Leu

65 70 75 80

Arg Asp Val Phe Gly Trp Gly Asn Leu Thr Cys Pro Ile Cys Lys Gly
85 90 95

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Leu Phe Thr Ala Ile Asn Leu Gly Leu Lys Lys Glu Pro Asn Val Ala
            100                 105                 110
Arg Val Gly Ser Val Ala Ile Lys Leu Cys Asn Leu Leu Lys Ile Ala
        115                 120                 125
Pro Pro Ala Val Cys Gln Ser Ile Val His Leu Phe Glu Asp Asp Met

    130                 135                 140
Val Glu Val Trp Arg Arg Ser Val Leu Ser Pro Ser Glu Ala Cys Gly
145                 150                 155                 160
Leu Leu Leu Gly Ser Thr Cys Gly His Trp Asp Ile Phe Ser Ser Trp
                165                 170                 175
Asn Ile Ser Leu Pro Thr Val Pro Lys Pro Pro Pro Lys Pro Pro Ser
            180                 185                 190
Pro Pro Ala Pro Gly Ala Pro Val Ser Arg Ile Leu Phe Leu Thr Asp

        195                 200                 205
Leu His Trp Asp His Asp Tyr Leu Glu Gly Thr Asp Pro Asp Cys Ala
    210                 215                 220
Asp Pro Leu Cys Cys Arg Arg Gly Ser Gly Leu Pro Pro Ala Ser Arg
225                 230                 235                 240
Pro Gly Ala Gly Tyr Trp Gly Glu Tyr Ser Lys Cys Asp Leu Pro Leu
                245                 250                 255
Arg Thr Leu Glu Ser Leu Leu Ser Gly Leu Gly Pro Ala Gly Pro Phe

            260                 265                 270
Asp Met Val Tyr Trp Thr Gly Asp Ile Pro Ala His Asp Val Trp His
        275                 280                 285
Gln Thr Arg Gln Asp Gln Leu Arg Ala Leu Thr Thr Val Thr Ala Leu
    290                 295                 300
Val Arg Lys Phe Leu Gly Pro Val Pro Val Tyr Pro Ala Val Gly Asn
305                 310                 315                 320
His Glu Ser Thr Pro Val Asn Ser Phe Pro Pro Pro Phe Ile Glu Gly

                325                 330                 335
Asn His Ser Ser Arg Trp Leu Tyr Glu Ala Met Ala Lys Ala Trp Glu
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340 345 350

Pro Trp Leu Pro Ala Glu Ala Leu Arg Thr Leu Arg Ile Gly Gly Phe

355 360 365

Tyr Ala Leu Ser Pro Tyr Pro Gly Leu Arg Leu Ile Ser Leu Asn Met

370 375 380

Asn Phe Cys Ser Arg Glu Asn Phe Trp Leu Leu Ile Asn Ser Thr Asp

385 390 395 400

Pro Ala Gly Gln Leu Gln Trp Leu Val Gly Glu Leu Gln Ala Ala Glu

405 410 415

Asp Arg Gly Asp Lys Val His Ile Ile Gly His Ile Pro Pro Gly His

420 425 430

Cys Leu Lys Ser Trp Ser Trp Asn Tyr Tyr Arg Ile Val Ala Arg Tyr

435 440 445

Glu Asn Thr Leu Ala Ala Gln Phe Phe Gly His Thr His Val Asp Glu

450 455 460

Phe Glu Val Phe Tyr Asp Glu Glu Thr Leu Ser Arg Pro Leu Ala Val

465 470 475 480

Ala Phe Leu Ala Pro Ser Ala Thr Thr Tyr Ile Gly Leu Asn Pro Gly

485 490 495

Tyr Arg Val Tyr Gln Ile Asp Gly Asn Tyr Ser Gly Ser Ser His Val

500 505 510

Val Leu Asp His Glu Thr Tyr Ile Leu Asn Leu Thr Gln Ala Asn Ile

515 520 525

Pro Gly Ala Ile Pro His Trp Gln Leu Leu Tyr Arg Ala Arg Glu Thr

530 535 540

Tyr Gly Leu Pro Asn Thr Leu Pro Thr Ala Trp His Asn Leu Val Tyr

545 550 555 560

Arg Met Arg Gly Asp Met Gln Leu Phe Gln Thr Phe Trp Phe Leu Tyr

565 570 575

His Lys Gly His Pro Pro Ser Glu Pro Cys Gly Thr Pro Cys Arg Leu

580 585 590

Ala Thr Leu Cys Ala Gln Leu Ser Ala Arg Ala Asp Ser Pro Ala Leu
595 600 605

Cys Arg His Leu Met Pro Asp Gly Ser Leu Pro Glu Ala Gln Ser Leu
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Trp Pro Arg Pro Leu Phe Cys
625 630

<210> 38

<211> 1896

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

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<211> 250
<212> PRT
<213> Artificial sequence
<220><223> Synthetic polypeptide
<400> 45

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Arg Cys Ser Asn Gly Phe Pro Glu Arg Asp Pro Pro Arg Pro Gly Pro
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Ser Arg Pro Ala Glu Lys Pro Pro Arg Pro Glu Ala Lys Ser Ala Gln
35 40 45
Pro Ala Asp Gly Trp Lys Gly Glu Arg Pro Arg Ser Glu Glu Asp Asn
50 55 60
Glu Leu Asn Leu Pro Asn Leu Ala Ala Ala Tyr Ser Ser Ile Leu Ser
65 70 75 80

Ser Leu Gly Glu Asn Pro Gln Arg Gln Gly Leu Leu Lys Thr Pro Trp
85 90 95
Arg Ala Ala Ser Ala Met Gln Phe Phe Thr Lys Gly Tyr Gln Glu Thr

100 105 110

Ile Ser Asp Val Leu Asn Asp Ala Ile Phe Asp Glu Asp His Asp Glu

115 120 125

Met Val Ile Val Lys Asp Ile Asp Met Phe Ser Met Cys Glu His His

130 135 140

Leu Val Pro Phe Val Gly Lys Val His Ile Gly Tyr Leu Pro Asn Lys

145 150 155 160

Gln Val Leu Gly Leu Ser Lys Leu Ala Arg Ile Val Glu Ile Tyr Ser

165 170 175

Arg Arg Leu Gln Val Gln Glu Arg Leu Thr Lys Gln Ile Ala Val Ala

180 185 190

Ile Thr Glu Ala Leu Arg Pro Ala Gly Val Gly Val Val Val Glu Ala

195 200 205

Thr His Met Cys Met Val Met Arg Gly Val Gln Lys Met Asn Ser Lys

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Thr Val Thr Ser Thr Met Leu Gly Val Phe Arg Glu Asp Pro Lys Thr

225 230 235 240

Arg Glu Glu Phe Leu Thr Leu Ile Arg Ser

245 250

<210> 46

<211> 750

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

<400> 46

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cgcgaggagt tcctgaccct gatccgcagc 750

<210> 47

<211> 203

<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 47

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Ala Val Gly Lys Thr Ser Leu Val Gln Arg Tyr Ser Gln Asp Ser Phe

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Ser Lys His Tyr Lys Ser Thr Val Gly Val Asp Phe Ala Leu Lys Val

35 40 45

Leu Gln Trp Ser Asp Tyr Glu Ile Val Arg Leu Gln Leu Trp Asp Ile

50 55 60

Ala Gly Gln Glu Arg Phe Thr Ser Met Thr Arg Leu Tyr Tyr Arg Asp

65 70 75 80

Ala Ser Ala Cys Val Ile Met Phe Asp Val Thr Asn Ala Thr Thr Phe

85 90 95

Ser Asn Ser Gln Arg Trp Lys Gln Asp Leu Asp Ser Lys Leu Thr Leu

100 105 110

Pro Asn Gly Glu Pro Val Pro Cys Leu Leu Leu Ala Asn Lys Cys Asp

115 120 125

Leu Ser Pro Trp Ala Val Ser Arg Asp Gln Ile Asp Arg Phe Ser Lys

130 135 140

Glu Asn Gly Phe Thr Gly Trp Thr Glu Thr Ser Val Lys Glu Asn Lys

145 150 155 160

Asn Ile Asn Glu Ala Met Arg Val Leu Ile Glu Lys Met Met Arg Asn

165 170 175

Ser Thr Glu Asp Ile Met Ser Leu Ser Thr Gln Gly Asp Tyr Ile Asn

180 185 190

Leu Gln Thr Lys Ser Ser Ser Trp Ser Cys Cys

195 200

<210> 48

<211> 609

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

<400> 48

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cgcttcagca aggagaacgg cttcaccggc tggaccgaga ccagcgtgaa ggagaacaag 480

aacatcaacg aggccatgcg cgtgctgatc gagaagatga tgcgcaacag caccgaggac 540

atcatgagcc tgagcaccca gggcgactac atcaacctgc agaccaagag cagcagctgg 600

agctgctgc 609

<210> 49

<211> 796

<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 49

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Asp Glu Ala Ile Gln Ala Val Lys Val Gln Ser Phe Gln Met Lys Arg

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Cys Leu Asp Lys Asn Lys Leu Met Asp Ala Leu Lys His Ala Ser Asn

35 40 45

Met Leu Gly Glu Leu Arg Thr Ser Met Leu Ser Pro Lys Ser Tyr Tyr

50 55 60

Glu Leu Tyr Met Ala Ile Ser Asp Glu Leu His Tyr Leu Glu Val Tyr

65 70 75 80

Leu Thr Asp Glu Phe Ala Lys Gly Arg Lys Val Ala Asp Leu Tyr Glu

85 90 95

Leu Val Gln Tyr Ala Gly Asn Ile Ile Pro Arg Leu Tyr Leu Leu Ile

100 105 110

Thr Val Gly Val Val Tyr Val Lys Ser Phe Pro Gln Ser Arg Lys Asp

115 120 125

Ile Leu Lys Asp Leu Val Glu Met Cys Arg Gly Val Gln His Pro Leu

130 135 140

Arg Gly Leu Phe Leu Arg Asn Tyr Leu Leu Gln Cys Thr Arg Asn Ile

145 150 155 160

Leu Pro Asp Glu Gly Glu Pro Thr Asp Glu Glu Thr Thr Gly Asp Ile

165 170 175

Ser Asp Ser Met Asp Phe Val Leu Leu Asn Phe Ala Glu Met Asn Lys

180 185 190

Leu Trp Val Arg Met Gln His Gln Gly His Ser Arg Asp Arg Glu Lys

195 200 205

Arg Glu Arg Glu Arg Gln Glu Leu Arg Ile Leu Val Gly Thr Asn Leu

210 215 220

Val Arg Leu Ser Gln Leu Glu Gly Val Asn Val Glu Arg Tyr Lys Gln

225 230 235 240

Ile Val Leu Thr Gly Ile Leu Glu Gln Val Val Asn Cys Arg Asp Ala

245 250 255

Leu Ala Gln Glu Tyr Leu Met Glu Cys Ile Ile Gln Val Phe Pro Asp

260 265 270

Glu Phe His Leu Gln Thr Leu Asn Pro Phe Leu Arg Ala Cys Ala Glu

275 280 285

Leu His Gln Asn Val Asn Val Lys Asn Ile Ile Ile Ala Leu Ile Asp

290 295 300

Arg Leu Ala Leu Phe Ala His Arg Glu Asp Gly Pro Gly Ile Pro Ala

305 310 315 320

Asp Ile Lys Leu Phe Asp Ile Phe Ser Gln Gln Val Ala Thr Val Ile

325 330 335

Gln Ser Arg Gln Asp Met Pro Ser Glu Asp Val Val Ser Leu Gln Val

340 345 350

Ser Leu Ile Asn Leu Ala Met Lys Cys Tyr Pro Asp Arg Val Asp Tyr

355 360 365

Val Asp Lys Val Leu Glu Thr Thr Val Glu Ile Phe Asn Lys Leu Asn

370 375 380

Leu Glu His Ile Ala Thr Ser Ser Ala Val Ser Lys Glu Leu Thr Arg

385 390 395 400

Leu Leu Lys Ile Pro Val Asp Thr Tyr Asn Asn Ile Leu Thr Val Leu

405 410 415

Lys Leu Lys His Phe His Pro Leu Phe Glu Tyr Phe Asp Tyr Glu Ser

420 425 430

Arg Lys Ser Met Ser Cys Tyr Val Leu Ser Asn Val Leu Asp Tyr Asn

435 440 445

Thr Glu Ile Val Ser Gln Asp Gln Val Asp Ser Ile Met Asn Leu Val

450 455 460

Ser Thr Leu Ile Gln Asp Gln Pro Asp Gln Pro Val Glu Asp Pro Asp

465 470 475 480

Pro Glu Asp Phe Ala Asp Glu Gln Ser Leu Val Gly Arg Phe Ile His

485 490 495

Leu Leu Arg Ser Glu Asp Pro Asp Gln Gln Tyr Leu Ile Leu Asn Thr

500 505 510

Ala Arg Lys His Phe Gly Ala Gly Gly Asn Gln Arg Ile Arg Phe Thr

515 520 525

Leu Pro Pro Leu Val Phe Ala Ala Tyr Gln Leu Ala Phe Arg Tyr Lys

530 535 540

Glu Asn Ser Lys Val Asp Asp Lys Trp Glu Lys Lys Cys Gln Lys Ile

545 550 555 560

Phe Ser Phe Ala His Gln Thr Ile Ser Ala Leu Ile Lys Ala Glu Leu

565 570 575

Ala Glu Leu Pro Leu Arg Leu Phe Leu Gln Gly Ala Leu Ala Ala Gly

580 585 590

Glu Ile Gly Phe Glu Asn His Glu Thr Val Ala Tyr Glu Phe Met Ser

595 600 605

Gln Ala Phe Ser Leu Tyr Glu Asp Glu Ile Ser Asp Ser Lys Ala Gln

610 615 620

Leu Ala Ala Ile Thr Leu Ile Ile Gly Thr Phe Glu Arg Met Lys Cys

625 630 635 640

Phe Ser Glu Glu Asn His Glu Pro Leu Arg Thr Gln Cys Ala Leu Ala

645 650 655

Ala Ser Lys Leu Leu Lys Lys Pro Asp Gln Gly Arg Ala Val Ser Thr

660 665 670

Cys Ala His Leu Phe Trp Ser Gly Arg Asn Thr Asp Lys Asn Gly Glu

675 680 685

Glu Leu His Gly Gly Lys Arg Val Met Glu Cys Leu Lys Lys Ala Leu

690 695 700

Lys Ile Ala Asn Gln Cys Met Asp Pro Ser Leu Gln Val Gln Leu Phe

705 710 715 720

Ile Glu Ile Leu Asn Arg Tyr Ile Tyr Phe Tyr Glu Lys Glu Asn Asp

725 730 735

Ala Val Thr Ile Gln Val Leu Asn Gln Leu Ile Gln Lys Ile Arg Glu

740 745 750

Asp Leu Pro Asn Leu Glu Ser Ser Glu Glu Thr Glu Gln Ile Asn Lys

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His Phe His Asn Thr Leu Glu His Leu Arg Leu Arg Arg Glu Ser Pro

770 775 780

Glu Ser Glu Gly Pro Ile Tyr Glu Gly Leu Ile Leu

785 790 795

<210> 50

<211> 2388

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

<400> 50

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gacgccctga agcacgccag caacatgctg ggcgagctgc gcaccagcat gctgagcccc 180

aagagctact acgagctgta catggccatc agcgacgagc tgcactacct ggaggtgtac 240

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cagcaccccc tgcgcggcct gttcctgcgc aactacctgc tgcagtgcac ccgcaacatc 480

ctgcccgacg agggcgagcc caccgacgag gagaccaccg gcgacatcag cgacagcatg 540

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<211> 11081

<212> DNA

<213> Artificial sequence

<220><223> Synthetic polynucleotide

<400> 51

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<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 55

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Glu Glu Cys Thr Val Thr Gly Phe Leu Arg Asp Lys Leu Gln Tyr Arg
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Ser Arg Leu Gln Tyr Met Lys His Tyr Phe Pro Ile Asn Tyr Lys Ile
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Ser Val Pro Tyr Glu Gly Val Phe Arg Ile Ala Asn Val Thr Arg Leu
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Gln Arg Ala Gln Val Ser Glu Arg Glu Leu Arg Tyr Leu Trp Val Leu
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Val Ser Leu Ser Ala Thr Glu Ser Val Gln Asp Val Leu Leu Glu Gly
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His Pro Ser Trp Lys Tyr Leu Gln Glu Val Glu Thr Leu Leu Leu Asn
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145 150 155 160

Pro Lys Ala Leu Leu Asp Asn Cys Phe Arg Val Met Glu Leu Leu Tyr
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Cys Ser Cys Cys Lys Gln Ser Ser Val Leu Asn Trp Gln Asp Cys Glu
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Val Pro Ser Pro Gln Ser Cys Ser Pro Glu Pro Ser Leu Gln Tyr Ala
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Ala Thr Gln Leu Tyr Pro Pro Pro Pro Trp Ser Pro Ser Ser Pro Pro
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Gln Val Ser Cys Pro Tyr Asp Ser Met Lys His Trp Gly Arg Arg Lys
35 40 45

Ala Trp Cys Arg Gln Leu Gly Glu Lys Gly Pro Cys Gln Arg Val Val
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Ser Thr His Asn Leu Trp Leu Leu Ser Phe Leu Arg Arg Trp Asn Gly

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Ser Thr Ala Ile Thr Asp Asp Thr Leu Gly Gly Thr Leu Thr Ile Thr

85 90 95

Leu Arg Asn Leu Gln Pro His Asp Ala Gly Leu Tyr Gln Cys Gln Ser

100 105 110

Leu His Gly Ser Glu Ala Asp Thr Leu Arg Lys Val Leu Val Glu Val

115 120 125

Leu Ala Asp Pro Leu Asp His Arg Asp Ala Gly Asp Leu Trp Phe Pro

130 135 140

Gly Glu Ser Glu Ser Phe Glu Asp Ala His Val Glu His Ser Ile Ser

145 150 155 160

Arg Ser Leu Leu Glu Gly Glu Ile Pro Phe Pro Pro Thr Ser Ile Leu

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Leu Trp Ala Ala Ala Trp His Gly Gln Lys Pro Gly Thr His Pro Pro

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Pro Gly Leu Arg Asp Thr

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<211> 274

<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 63

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35 40 45

Pro Tyr Val Glu Phe Thr Gly Arg Asp Ser Val Thr Cys Pro Thr Cys

50 55 60

Gln Gly Thr Gly Arg Ile Pro Arg Gly Gln Glu Asn Gln Leu Val Ala
65 70 75 80
Leu Ile Pro Tyr Ser Asp Gln Arg Leu Arg Pro Arg Arg Thr Lys Leu
85 90 95
Tyr Val Met Ala Ser Val Phe Val Cys Leu Leu Leu Ser Gly Leu Ala
100 105 110

Val Phe Phe Leu Phe Pro Arg Ser Ile Asp Val Lys Tyr Ile Gly Val
115 120 125
Lys Ser Ala Tyr Val Ser Tyr Asp Val Gln Lys Arg Thr Ile Tyr Leu
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Asn Ile Thr Asn Thr Leu Asn Ile Thr Asn Asn Asn Tyr Tyr Ser Val
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Glu Val Glu Asn Ile Thr Ala Gln Val Gln Phe Ser Lys Thr Val Ile
165 170 175

Gly Lys Ala Arg Leu Asn Asn Ile Thr Ile Ile Gly Pro Leu Asp Met
180 185 190
Lys Gln Ile Asp Tyr Thr Val Pro Thr Val Ile Ala Glu Glu Met Ser
195 200 205
Tyr Met Tyr Asp Phe Cys Thr Leu Ile Ser Ile Lys Val His Asn Ile
210 215 220
Val Leu Met Met Gln Val Thr Val Thr Thr Thr Tyr Phe Gly His Ser
225 230 235 240

Glu Gln Ile Ser Gln Glu Arg Tyr Gln Tyr Val Asp Cys Gly Arg Asn
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<211> 267

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<211> 593

<212> PRT

<213> Artificial sequence

<220><223> Synthetic polypeptide

<400> 67

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35 40 45

Trp Pro Thr Thr Leu Ser Arg His Leu Gly Gly Pro Cys Gln Val Asp

50 55 60

Ala His Cys Ser Ala Gly His Ser Cys Ile Phe Thr Val Ser Gly Thr

65 70 75 80

Ser Ser Cys Cys Pro Phe Pro Glu Ala Val Ala Cys Gly Asp Gly His

85 90 95

His Cys Cys Pro Arg Gly Phe His Cys Ser Ala Asp Gly Arg Ser Cys

100 105 110

Phe Gln Arg Ser Gly Asn Asn Ser Val Gly Ala Ile Gln Cys Pro Asp

115 120 125

Ser Gln Phe Glu Cys Pro Asp Phe Ser Thr Cys Cys Val Met Val Asp

130 135 140

Gly Ser Trp Gly Cys Cys Pro Met Pro Gln Ala Ser Cys Cys Glu Asp

145 150 155 160

Arg Val His Cys Cys Pro His Gly Ala Phe Cys Asp Leu Val His Thr

165 170 175

Arg Cys Ile Thr Pro Thr Gly Thr His Pro Leu Ala Lys Lys Leu Pro

180 185 190

Ala Gln Arg Thr Asn Arg Ala Val Ala Leu Ser Ser Ser Val Met Cys

195 200 205

Pro Asp Ala Arg Ser Arg Cys Pro Asp Gly Ser Thr Cys Cys Glu Leu

210 215 220

Pro Ser Gly Lys Tyr Gly Cys Cys Pro Met Pro Asn Ala Thr Cys Cys

225 230 235 240

Ser Asp His Leu His Cys Cys Pro Gln Asp Thr Val Cys Asp Leu Ile

245 250 255

Gln Ser Lys Cys Leu Ser Lys Glu Asn Ala Thr Thr Asp Leu Leu Thr

260 265 270

Lys Leu Pro Ala His Thr Val Gly Asp Val Lys Cys Asp Met Glu Val

275 280 285

Ser Cys Pro Asp Gly Tyr Thr Cys Cys Arg Leu Gln Ser Gly Ala Trp

290 295 300

Gly Cys Cys Pro Phe Thr Gln Ala Val Cys Cys Glu Asp His Ile His

305 310 315 320

Cys Cys Pro Ala Gly Phe Thr Cys Asp Thr Gln Lys Gly Thr Cys Glu

325 330 335

Gln Gly Pro His Gln Val Pro Trp Met Glu Lys Ala Pro Ala His Leu

340 345 350

Ser Leu Pro Asp Pro Gln Ala Leu Lys Arg Asp Val Pro Cys Asp Asn

355 360 365

Val Ser Ser Cys Pro Ser Ser Asp Thr Cys Cys Gln Leu Thr Ser Gly

370 375 380

Glu Trp Gly Cys Cys Pro Ile Pro Glu Ala Val Cys Cys Ser Asp His

385 390 395 400

Gln His Cys Cys Pro Gln Gly Tyr Thr Cys Val Ala Glu Gly Gln Cys

405 410 415

Gln Arg Gly Ser Glu Ile Val Ala Gly Leu Glu Lys Met Pro Ala Arg

420 425 430

Arg Ala Ser Leu Ser His Pro Arg Asp Ile Gly Cys Asp Gln His Thr

435 440 445

Ser Cys Pro Val Gly Gln Thr Cys Cys Pro Ser Leu Gly Gly Ser Trp

450 455 460

Ala Cys Cys Gln Leu Pro His Ala Val Cys Cys Glu Asp Arg Gln His

465 470 475 480

Cys Cys Pro Ala Gly Tyr Thr Cys Asn Val Lys Ala Arg Ser Cys Glu

485 490 495

Lys Glu Val Val Ser Ala Gln Pro Ala Thr Phe Leu Ala Arg Ser Pro

500 505 510

His Val Gly Val Lys Asp Val Glu Cys Gly Glu Gly His Phe Cys His

515 520 525

Asp Asn Gln Thr Cys Cys Arg Asp Asn Arg Gln Gly Trp Ala Cys Cys

530 535 540

Pro Tyr Arg Gln Gly Val Cys Cys Ala Asp Arg Arg His Cys Cys Pro

545 550 555 560

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Leu

<210> 68

<211> 1779

<212> DNA

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