Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3078464 A1 2019/04/11

(21) 3 078 464

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2018/10/03
(87) Date publication PCT/PCT Publication Date: 2019/04/11
(85) Entrée phase nationale/National Entry: 2020/04/03
(86) N° demande PCT/PCT Application No.: US 2018/054227
(87) N° publication PCT/PCT Publication No.: 2019/070894
(30) Priorités/Priorities: 2017/10/03 (US62/567,296); 2017/10/03 (US62/567,319); 2017/10/03 (US62/567,301); 2017/10/03 (US62/567,310); 2017/10/03 (US62/567,311)

(51) Cl.Int./Int.Cl. *C12N 15/85* (2006.01), *A61K 38/47* (2006.01), *A61P 25/16* (2006.01), *C07K 14/47* (2006.01), *C07K 14/53* (2006.01), *C07K 14/705* (2006.01), *C12N 15/113* (2010.01), *C12N 15/12* (2006.01), *C12N 15/24* (2006.01), *C12N 15/55* (2006.01), *C12N 15/56* (2006.01), *C12N 15/57* (2006.01), *C12N 15/86* (2006.01), *C12N 15/864* (2006.01), *C12N 15/866* (2006.01), *C12N 7/01* (2006.01), *C12N 9/16* (2006.01), *C12N 9/24* (2006.01), *C12N 9/42* (2006.01), *C12N 9/64* (2006.01), *C12N 9/78* (2006.01)

(71) Demandeur/Applicant:
PREVAIL THERAPEUTICS, INC., US

(72) Inventeurs/Inventors:

(54) Titre : THERAPIES GENIQUES POUR TROUBLES LYSOSOMIAUX
(54) Title: GENE THERAPIES FOR LYSOSOMAL DISORDERS

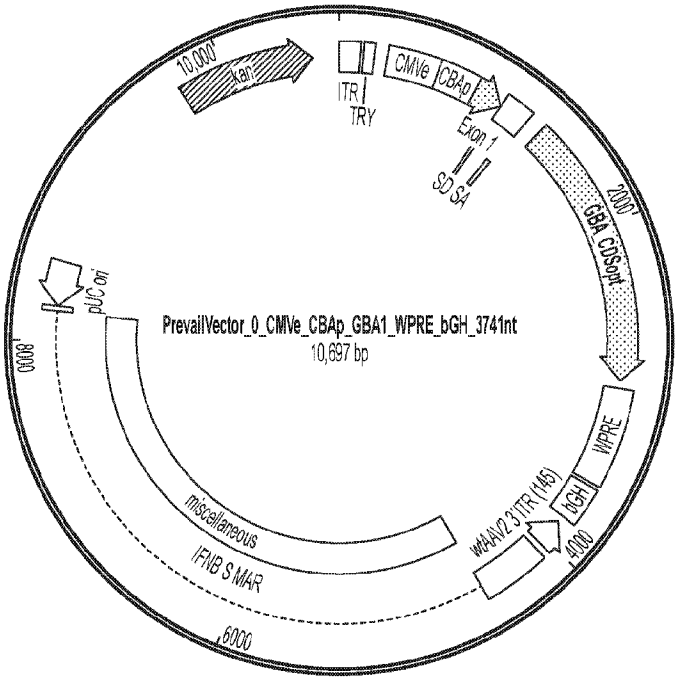


Figure 1

(57) Abrégé/Abstract:
The disclosure relates, in some aspects, to compositions and methods for treatment of diseases associated with aberrant lysosomal function, for example Parkinson's disease (PD) and Gaucher disease. In some embodiments, the disclosure provides expression constructs comprising a transgene encoding beta-Glucocerebrosidase (GBA) or a portion thereof alone or in combination with one or more PD-associated genes. In some embodiments, the disclosure provides methods of Parkinson's disease by administering such expression constructs to a subject in need thereof.

(21) 3 078 464

(13) A1

(72) Inventeurs(suite)/Inventors(continued): ABELIOVICH, ASA, US; HECKMAN, LAURA, US; RHINN, HERVE, US

(74) Agent: SMART & BIGGAR LLP

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 April 2019 (11.04.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/070894 A1

(51) International Patent Classification:
C12N 15/86 (2006.01) *A61K 48/00* (2006.01)
C12N 9/16 (2006.01) *C07K 14/00* (2006.01)

(21) International Application Number:
PCT/US2018/054227

(22) International Filing Date:
03 October 2018 (03.10.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/567,311	03 October 2017 (03.10.2017)	US
62/567,310	03 October 2017 (03.10.2017)	US
62/567,301	03 October 2017 (03.10.2017)	US
62/567,319	03 October 2017 (03.10.2017)	US
62/567,296	03 October 2017 (03.10.2017)	US

(71) Applicant: **PREVAIL THERAPEUTICS, INC.** [US/US]; 430 East 29th Street, Suite 940, New York, NY 10016 (US).

(72) Inventors: **ABELIOVICH, Asa**; 20 East 35th St., Apt. 15e, New York, NY 10016 (US). **HECKMAN, Laura**; 430 East 29th Street, Suite 940, New York, New York 10016 (US). **RHINN, Herve**; 430 East 29th Street, Suite 940, New York, New York 10016 (US).

(74) Agent: **MACDONALD, Kevin**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

(54) Title: GENE THERAPIES FOR LYSOSOMAL DISORDERS

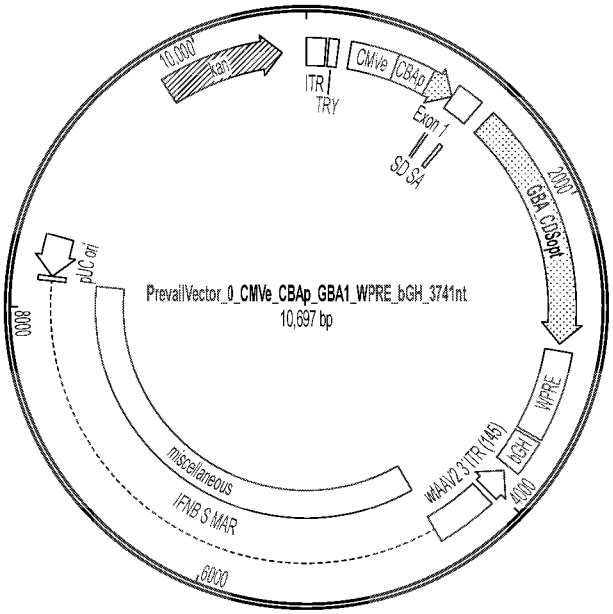


Figure 1

(57) Abstract: The disclosure relates, in some aspects, to compositions and methods for treatment of diseases associated with aberrant lysosomal function, for example Parkinson's disease (PD) and Gaucher disease. In some embodiments, the disclosure provides expression constructs comprising a transgene encoding beta-Glucocerebrosidase (GBA) or a portion thereof alone or in combination with one or more PD-associated genes. In some embodiments, the disclosure provides methods of Parkinson's disease by administering such expression constructs to a subject in need thereof.

[Continued on next page]

WO 2019/070894 A1

WO 2019/070894 A1

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

GENE THERAPIES FOR LYSOSOMAL DISORDERS

RELATED APPLICATIONS

This Application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S. Provisional Application Serial Numbers 62/567,296, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS", 62/567,311, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS", 62/567,319, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS", 62/567,301, filed October 3, 2018, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS", and 62/567,310, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS", the entire contents of each application which are incorporated herein by reference.

BACKGROUND

Gaucher disease is a rare inborn error of glycosphingolipid metabolism due to deficiency of lysosomal acid β-glucocerebrosidase (Gcase, "GBA"). Patients suffer from non-CNS symptoms and findings including hepatosplenomegly, bone marrow insufficiency leading to pancytopenia, lung disorders and fibrosis, and bone defects. In addition, a significant number of patients suffer from neurological manifestations, including defective saccadic eye movements and gaze, seizures, cognitive deficits, developmental delay, and movement disorders including Parkinson's disease.

Several therapeutics exist that address the peripheral disease and the principal clinical manifestations in hematopoietic bone marrow and viscera, including enzyme replacement therapies as described below, chaperone-like small molecule drugs that bind to defective Gcase and improve stability, and substrate reduction therapy that block the production of substrate that accumulate in Gaucher disease leading to symptoms and findings. However, other aspects of Gaucher disease (particularly those affecting the skeleton and brain) appear refractory to treatment.

SUMMARY

In addition to Gaucher disease patients (who possess mutations in both chromosomal alleles of *GBA1* gene), patients with mutations in only one allele of GBA1 are at highly increased risk of Parkinson's disease (PD). The severity of PD symptoms- which include gait difficulty, a tremor at rest, rigidity, and often depression, sleep difficulties, and cognitive decline - correlate with the degree of enzyme activity reduction. Thus, Gaucher disease patients have

the most severe course, whereas patient with a single mild mutation in *GBA1* typically have a more benign course. Mutation carriers are also at high risk of other PD-related disorders, including Lewy Body Dementia, characterized by executive dysfunction, psychosis, and a PD-like movement disorder, and multi-system atrophy, with characteristic motor and cognitive impairments. No therapies exist that alter the inexorable course of these disorders.

Deficits in enzymes such as Gcase (*e.g.*, the gene product of *GBA1* gene), as well as common variants in many genes implicated in lysosome function or trafficking of macromolecules to the lysosome (*e.g.*, Lysosomal Membrane Protein 1 (LIMP), also referred to as SCARB2), have been associated with increased PD risk. The disclosure is based, in part, on expression constructs (*e.g.*, vectors) encoding one or more PD-associated genes, for example Gcase, GBA2, prosaposin, progranulin, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, TMEM106B, or a combination of any of the foregoing (or portions thereof). In some embodiments, combinations of gene products described herein act together (*e.g.*, synergistically) to reduce one or more signs and symptoms of PD when expressed in a subject.

Accordingly, in some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a Gcase (*e.g.*, the gene product of *GBA1* gene). In some embodiments, the isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the Gcase encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 14 (*e.g.*, as set forth in NCBI Reference Sequence NP_000148.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 15. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the Gcase protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the prosaposin encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 16 (*e.g.*, as set forth in NCBI Reference Sequence NP_002769.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 17. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the prosaposin protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). In some embodiments, the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the LIMP2/SCARB2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 18 (*e.g.*, as set forth in NCBI Reference Sequence NP_005497.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 29. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the SCARB2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GBA2 protein (*e.g.*, the gene product of *GBA2* gene). In some embodiments, the isolated nucleic acid comprises a GBA2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GBA2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 30 (*e.g.*, as set forth in NCBI Reference Sequence NP_065995.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 31. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GBA2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GALC protein (*e.g.*, the gene product of *GALC* gene). In some embodiments, the isolated nucleic acid comprises a GALC-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GALC encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 33 (*e.g.*, as set forth in NCBI Reference Sequence NP_000144.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 34. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GALC protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding CTSB protein (*e.g.*, the gene product of *CTSB* gene). In some embodiments, the isolated nucleic acid comprises a CTSB-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human

cells). In some embodiments, the nucleic acid sequence encoding the CTSB encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 35 (*e.g.*, as set forth in NCBI Reference Sequence NP_001899.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 36. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the CTSB protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding SMPD1 protein (*e.g.*, the gene product of *SMPD1* gene). In some embodiments, the isolated nucleic acid comprises a SMPD1-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the SMPD1 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 37 (*e.g.*, as set forth in NCBI Reference Sequence NP_000534.3). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 38. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the SMPD1 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GCH1 protein (*e.g.*, the gene product of *GCH1* gene). In some embodiments, the isolated nucleic acid comprises a GCH1-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GCH1 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 45 (*e.g.*, as set forth in NCBI Reference Sequence NP_000534.3). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 46. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GCH1 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding RAB7L protein (*e.g.*, the gene product of *RAB7L* gene). In some embodiments, the isolated nucleic acid comprises a RAB7L-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the RAB7L encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 47 (*e.g.*, as set forth in NCBI Reference Sequence NP_003920.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 48. In some embodiments the expression construct

comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the RAB7L protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding VPS35 protein (*e.g.*, the gene product of *VPS35* gene). In some embodiments, the isolated nucleic acid comprises a VPS35-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the VPS35 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 49 (*e.g.*, as set forth in NCBI Reference Sequence NP_060676.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 50. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the VPS35 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding IL-34 protein (*e.g.*, the gene product of *IL34* gene). In some embodiments, the isolated nucleic acid comprises a IL-34-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the IL-34 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 55 (*e.g.*, as set forth in NCBI Reference Sequence NP_689669.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 56. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the IL_34 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding TREM2 protein (*e.g.*, the gene product of *TREM* gene). In some embodiments, the isolated nucleic acid comprises a TREM2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the TREM2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 57 (*e.g.*, as set forth in NCBI Reference Sequence NP_061838.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 58. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the TREM2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding TMEM106B protein (*e.g.*, the gene product of *TMEM106B* gene).

In some embodiments, the isolated nucleic acid comprises a TMEM106B-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the TMEM106B encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 63 (*e.g.*, as set forth in NCBI Reference Sequence NP_060844.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 64. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the TMEM106B protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding progranulin (*e.g.*, the gene product of *PGRN* gene). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the progranulin (PRGN) encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 67 (*e.g.*, as set forth in NCBI Reference Sequence NP_002078.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 68. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the prosaposin protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

In some embodiments, a first gene product or a second gene product is a Gcase protein, or a portion thereof. In some embodiments, a first gene product is a Gcase protein and a second gene product is selected from GBA2, prosaposin, progranulin, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, and TMEM106B.

In some embodiments, an expression construct further encodes an interfering nucleic acid (*e.g.*, shRNA, miRNA, dsRNA, *etc.*). In some embodiments, an interfering nucleic acid inhibits expression of α-Synuclein (α-Synuclein). In some embodiments, an interfering nucleic acid that targets α-Synuclein comprises a sequence set forth in any one of SEQ ID NOs: 20-25. In some embodiments, an interfering nucleic acid that targets α-Synuclein binds to (*e.g.*, hybridizes with) a sequence set forth in any one of SEQ ID NO: 20-25.

In some embodiments, an interfering nucleic acid inhibits expression of TMEM106B. In some embodiments, an interfering nucleic acid that targets TMEM106B comprises a sequence set forth in SEQ ID NO: 64 or 65. In some embodiments, an interfering nucleic acid that targets TMEM106B binds to (*e.g.*, hybridizes with) a sequence set forth in SEQ ID NO: 64 or 65.

In some embodiments, an expression construct further comprises one or more promoters. In some embodiments, a promoter is a chicken-beta actin (CBA) promoter, a CAG promoter, a CD68 promoter, or a JeT promoter. In some embodiments, a promoter is a RNA pol II promoter (*e.g.*, or an RNA pol III promoter (*e.g.*, U6, *etc.*).

In some embodiments, an expression construct further comprises an internal ribosomal entry site (IRES). In some embodiments, an IRES is located between a first gene product and a second gene product.

In some embodiments, an expression construct further comprises a self-cleaving peptide coding sequence. In some embodiments, a self-cleaving peptide is a T2A peptide.

In some embodiments, an expression construct comprises two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences. In some embodiments, ITR sequences flank a first gene product and a second gene product (*e.g.*, are arranged as follows from 5'-end to 3'-end: ITR-first gene product-second gene product-ITR). In some embodiments, one of the ITR sequences of an isolated nucleic acid lacks a functional terminal resolution site (trs). For example, in some embodiments, one of the ITRs is a ΔITR.

The disclosure relates, in some aspects, to rAAV vectors comprising an ITR having a modified "D" region (*e.g.*, a D sequence that is modified relative to wild-type AAV2 ITR, SEQ ID NO: 29). In some embodiments, the ITR having the modified D region is the 5' ITR of the rAAV vector. In some embodiments, a modified "D" region comprises an "S" sequence, for example as set forth in SEQ ID NO: 26. In some embodiments, the ITR having the modified "D" region is the 3' ITR of the rAAV vector. In some embodiments, a modified "D" region comprises a 3'ITR in which the "D" region is positioned at the 3' end of the ITR (*e.g.*, on the outside or terminal end of the ITR relative to the transgene insert of the vector). In some embodiments, a modified "D" region comprises a sequence as set forth in SEQ ID NO: 26 or 27.

In some embodiments, an isolated nucleic acid (*e.g.*, an rAAV vector) comprises a TRY region. In some embodiments, a TRY region comprises the sequence set forth in SEQ ID NO: 28.

In some embodiments, an isolated nucleic acid described by the disclosure comprises or consists of, or encodes a peptide having, the sequence set forth in any one of SEQ ID NOs: 1-78.

In some aspects, the disclosure provides a vector comprising an isolated nucleic acid as described by the disclosure. In some embodiments, a vector is a plasmid, or a viral vector. In some embodiments, a viral vector is a recombinant AAV (rAAV) vector or a Baculovirus vector. In some embodiments, an rAAV vector is single-stranded (e.g., single-stranded DNA).

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid as described by the disclosure or a vector as described by the disclosure.

In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV) comprising a capsid protein and an isolated nucleic acid or a vector as described by the disclosure.

In some embodiments, a capsid protein is capable of crossing the blood-brain barrier, for example an AAV9 capsid protein or an AAVrh.10 capsid protein. In some embodiments, an rAAV transduces neuronal cells and non-neuronal cells of the central nervous system (CNS).

In some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

In some embodiments, administration comprises direct injection to the CNS of a subject. In some embodiments, direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna manga injection, or any combination thereof. In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED).

In some embodiments, administration comprises peripheral injection. In some embodiments, peripheral injection is intravenous injection.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof).

FIG. 2 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion thereof. The coding sequences of Gcase and LIMP2 are separated by an internal ribosomal entry site (IRES).

FIG. 3 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion

thereof. Expression of the coding sequences of Gcase and LIMP2 are each driven by a separate promoter.

FIG. 4 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), LIMP2 (SCARB2) or a portion thereof, and an interfering RNA for α-Syn.

FIG. 5 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Prosaposin (*e.g.*, *PSAP* or a portion thereof), and an interfering RNA for α-Syn.

FIG. 6 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Prosaposin (*e.g.*, *PSAP* or a portion thereof). The coding sequences of Gcase and Prosaposin are separated by an internal ribosomal entry site (IRES).

FIG. 7 is a schematic depicting one embodiment of a vector comprising an expression construct encoding a Gcase (*e.g.*, *GBA1* or a portion thereof). In this embodiment, the vector comprises a CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAp), Exon 1, and intron (int) to constitutively express the codon optimized coding sequence of human *GBA1*. The 3' region also contains a WPRE regulatory element followed by a bGH polyA tail. Three transcriptional regulatory activation sites are included at the 5' end of the promoter region: TATA, RBS, and YY1. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (inset box) were evaluated; these have several nucleotide differences within the 20-nucleotide "D" region of wild-type AAV2 ITR. In some embodiments, an rAAV vector contains the "D" domain nucleotide sequence shown on the top line. In some embodiments, a rAAV vector comprises a mutant "D" domain (*e.g.*, an "S" domain, with the nucleotide changes shown on the bottom line).

FIG. 8 is a schematic depicting one embodiment of the vector described in FIG. 6

FIG. 9 shows representative data for delivery of an rAAV comprising a transgene encoding a Gcase (*e.g.*, *GBA1* or a portion thereof) in a CBE mouse model of Parkinson's disease. Daily IP delivery of PBS vehicle, 25 mg/kg CBE, 37.5 mg/kg CBE, or 50 mg/kg CBE (left to right) initiated at P8. Survival (top left) was checked two times a day and weight (top right) was checked daily. All groups started with n = 8. Behavior was assessed by total distance traveled in Open Field (bottom left) at P23 and latency to fall on Rotarod (bottom middle) at P24. Levels of the GCase substrates were analyzed in the cortex of mice in the PBS and 25 mg/kg CBE treatment groups both with (Day 3) and without (Day 1) CBE withdrawal.

Aggregate GluSph and GalSph levels (bottom right) are shown as pmol per mg wet weight of the tissue. Means are presented. Error bars are SEM. *p<0.05; **p<0.01; ***p<0.001, nominal p-values for treatment groups by linear regression.

FIG. 10 is a schematic depicting one embodiment of a study design for maximal rAAV dose in a CBE mouse model. Briefly, rAAV was delivered by ICV injection at P3, and daily CBE treatment was initiated at P8. Behavior was assessed in the Open Field and Rotarod assays at P24-25 and substrate levels were measured at P36 and P38.

FIG. 11 shows representative data for in-life assessment of maximal rAAV dose in a CBE mouse model. At P3, mice were treated with either excipient or 8.8e9 vg rAAV-GBA1 via ICV delivery. Daily IP delivery of either PBS or 25 mg/kg CBE was initiated at P8. At the end of the study, half the mice were sacrificed one day after their last CBE dose at P36 (Day 1) while the remaining half went through 3 days of CBE withdrawal before sacrifice at P38 (Day3). All treatment groups (excipient + PBS n = 8, rAAV-GBA1+ PBS n = 7, excipient + CBE n = 8, and variant + CBE n = 9) were weighed daily (top left), and the weight at P36 was analyzed (top right). Behavior was assessed by total distance traveled in Open Field at P23 (bottom left) and latency to fall on Rotarod at P24 (bottom right), evaluated for each animal as the median across 3 trials. Due to lethality, n = 7 for the excipient + CBE group for the behavioral assays, while n=8 for all other groups. Means across animals are presented. Error bars are SEM. *p<0.05; ***p<0.001, nominal p-values for treatment groups by linear regression in the CBE-treated animals.

FIG. 12 shows representative data for biochemical assessment of maximal rAAV dose in a CBE mouse model. The cortex of all treatment groups (excipient + PBS n = 8, variant + PBS n = 7, excipient + CBE n = 7, and variant + CBE n = 9) was used to measure GCase activity (top left), GluSph levels (top right), GluCer levels (bottom left), and vector genomes (bottom right) in the groups before (Day 1) or after (Day 3) CBE withdrawal. Biodistribution is shown as vector genomes per 1 µg of genomic DNA. Means are presented. Error bars are SEM. (*)p<0.1; **p<0.01; ***p<0.001, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 13 shows representative data for behavioral and biochemical correlations in a CBE mouse model after administration of excipient + PBS, excipient + CBE, and variant + CBE treatment groups. Across treatment groups, performance on Rotarod was negatively correlated with GluCer accumulation (A, p=0.0012 by linear regression), and GluSph accumulation was negatively correlated with increased GCase activity (B, p=0.0086 by linear regression).

FIG. 14 shows representative data for biodistribution of variant in a CBE mouse model. Presence of vector genomes was assessed in the liver, spleen, kidney, and gonads for all treatment groups (excipient + PBS n = 8, variant+ PBS n = 7, excipient + CBE n = 7, and variant+ CBE n = 9). Biodistribution is shown as vector genomes per 1 μg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Means are presented. Error bars are SEM. *p<0.05; **p<0.01; ***p<0.001, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 15 shows representative data for in-life assessment of rAAV dose ranging in a CBE mouse model. Mice received excipient or one of three different doses of rAAV-GBA1 by ICV delivery at P3: 3.2e9 vg, 1.0e10vg, or 3.2e10 vg. At P8, daily IP treatment of 25 mg/kg CBE was initiated. Mice that received excipient and CBE or excipient and PBS served as controls. All treatment groups started with n = 10 (5M/5F) per group. All mice were sacrificed one day after their final CBE dose (P38-P40). All treatment groups were weighed daily, and their weight was analyzed at P36. Motor performance was assessed by latency to fall on Rotarod at P24 and latency to traverse the Tapered Beam at P30. Due to early lethality, the number of mice participating in the behavioral assays was: excipient + PBS n = 10, excipient + CBE n = 9, and 3.2e9 vg rAAV-GBA1+ CBE n = 6, 1.0e10 vg rAAV-GBA1+ CBE n = 10, 3.2e10 vg rAAV-GBA1+ CBE n = 7. Means are presented. Error bars are SEM; * p<0.05; **p<0.01 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 16 shows representative data for biochemical assessment of rAAV dose ranging in a CBE mouse model. The cortex of all treatment groups (excipient + PBS n = 10, excipient + CBE n = 9, and 3.2e9 vg rAAV-GBA1+ CBE n = 6, 1.0e10 vg rAAV-GBA1+ CBE n = 10, 3.2e10 vg rAAV-GBA1+ CBE n = 7) was used to measure GCase activity, GluSph levels, GluCer levels, and vector genomes. GCase activity is shown as ng of GCase per mg of total protein. GluSph and GluCer levels are shown as pmol per mg wet weight of the tissue. Biodistribution is shown as vector genomes per 1 μg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Vector genome presence was also measured in the liver (E). Means are presented. Error bars are SEM. **p<0.01; ***p<0.001 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 17 shows representative data for tapered beam analysis in maximal dose rAAV-GBA1 in a genetic mouse model. Motor performance of the treatment groups (WT + excipient, n = 5), 4L/PS-NA + excipient (n = 6), and 4L/PS-NA + rAAV-GBA1 (n = 5)) was assayed by Beam Walk 4 weeks post rAAV-GBA1 administration. The total slips and active time are shown as total over 5 trials on different beams. Speed and slips per speed are shown as the average over 5 trials on different beams. Means are presented. Error bars are SEM.

FIG. 18 shows representative data for *in vitro* expression of rAAV constructs encoding progranulin (PGRN) protein. The left panel shows a standard curve of progranulin (PGRN) ELISA assay. The bottom panel shows a dose-response of PGRN expression measured by ELISA assay in cell lysates of HEK293T cells transduced with rAAV. MOI = multiplicity of infection (vector genomes per cell).

FIG. 19 shows representative data for *in vitro* expression of rAAV constructs encoding *GBA1* in combination with Prosaposin (*PSAP*), *SCARB2*, and/or one or more inhibitory nucleic acids. Data indicate transfection of HEK293 cells with each construct resulted in overexpression of the transgenes of interest relative to mock transfected cells.

FIG. 20 is a schematic depicting an rAAV vectors comprising a "D" region located on the "outside" of the ITR (e.g., proximal to the terminus of the ITR relative to the transgene insert or expression construct) (top) and a wild-type rAAV vectors having ITRs on the "inside" of the vector (e.g., proximal to the transgene insert of the vector).

FIG. 21 a schematic depicting one embodiment of a vector comprising an expression construct encoding GBA2 or a portion thereof, and an interfering RNA for α-Syn.

FIG. 22 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Galactosylceramidase (*e.g.*, *GALC* or a portion thereof). Expression of the coding sequences of Gcase and Galactosylceramidase are separated by a T2A self-cleaving peptide sequence.

FIG. 23 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Galactosylceramidase (*e.g.*, *GALC* or a portion thereof). Expression of the coding sequences of Gcase and Galactosylceramidase are separated by a T2A self-cleaving peptide sequence.

FIG. 24 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Cathepsin B (*e.g.*, *CTSB* or a portion thereof), and an interfering RNA for α-Syn. Expression of the coding sequences of Gcase and Cathepsin B are separated by a T2A self-cleaving peptide sequence.

FIG. 25 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Sphingomyelin phosphodiesterase 1 (*e.g.*, *SMPD1* a portion thereof, and an interfering RNA for α-Syn.

FIG. 26 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Galactosylceramidase (*e.g.*, *GALC* or a portion thereof). The coding sequences of Gcase and Galactosylceramidase are separated by an internal ribosomal entry site (IRES).

FIG. 27 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Cathepsin B (*e.g.*, *CTSB* or a portion thereof). Expression of the coding sequences of Gcase and Cathepsin B are each driven by a separate promoter.

FIG. 28 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), GCH1 (*e.g.*, *GCH1* or a portion thereof), and an interfering RNA for α-Syn. The coding sequences of Gcase and GCH1 are separated by an T2A self-cleaving peptide sequence

FIG. 29 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), RAB7L1 (*e.g.*, *RAB7L1* or a portion thereof), and an interfering RNA for α-Syn . The coding sequences of Gcase and RAB7L1 are separated by an T2A self-cleaving peptide sequence.

FIG. 30 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), GCH1 (*e.g.*, *GCH1* or a portion thereof), and an interfering RNA for α-Syn. Expression of the coding sequences of Gcase and GCH1 are an internal ribosomal entry site (IRES).

FIG. 31 is a schematic depicting one embodiment of a vector comprising an expression construct encoding VPS35 (*e.g.*, *VPS35* or a portion thereof) and interfering RNAs for α-Syn and TMEM106B.

FIG. 32 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), IL-34 (*e.g.*, *IL34* or a portion thereof), and an interfering RNA for α-Syn. The coding sequences of Gcase and IL-34 are separated by T2A self-cleaving peptide sequence.

FIG. 33 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and IL-34 (*e.g.*, *IL34* or a portion thereof). The coding sequences of Gcase and IL-34 are separated by an internal ribosomal entry site (IRES).

FIG. 34 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and TREM2 (*e.g.*, *TREM2* or a portion thereof). Expression of the coding sequences of Gcase and TREM2 are each driven by a separate promoter.

FIG. 35 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and IL-34 (*e.g.*, *IL34* or a portion thereof). Expression of the coding sequences of Gcase and IL-34 are each driven by a separate promoter.

FIGs. 36A-36B show representative data for overexpression of TREM2 and GBA1 in HEK293 cells relative to control transduced cells, as measured by qPCR and ELISA. FIG. 36A shows data for overexpression of TREM2. FIG. 36B shows data for overexpression of GBA1 from the same construct.

FIG. 37 shows representative data indicating successful silencing of *SCNA in vitro* by GFP reporter assay (top) and α-Syn assay (bottom).

FIG. 38 shows representative data indicating successful silencing of *TMEM106B in vitro* by GFP reporter assay (top) and α-Syn assay (bottom).

FIG. 39 is a schematic depicting one embodiments of a vector comprising an expression construct encoding PGRN.

FIG. 40 shows data for transduction of HEK293 cells using rAAVs having ITRs with wild-type (circles) or alternative (e.g., "outside"; squares) placement of the "D" sequence. The rAAVs having ITRs placed on the "outside" were able to transduce cells as efficiently as rAAVs having wild-type ITRs.

DETAILED DESCRIPTION

The disclosure is based, in part, on compositions and methods for expression of combinations of PD-associated gene products in a subject. A gene product can be a protein, a fragment (*e.g.*, portion) of a protein, an interfering nucleic acid that inhibits a PD-associated gene, *etc.* In some embodiments, a gene product is a protein or a protein fragment encoded by a PD-associated gene. In some embodiments, a gene product is an interfering nucleic acid (*e.g.*, shRNA, siRNA, miRNA, amiRNA, *etc.*) that inhibits a PD-associated gene.

A PD-associated gene refers to a gene encoding a gene product that is genetically, biochemically or functionally associated with PD. For example, individuals having mutations in the *GBA1* gene (which encodes the protein Gcase), have been observed to be have an increased risk of developing PD compared to individuals that do not have a mutation in *GBA1*. In another

example, PD is associated with accumulation of protein aggregates comprising α-Synuclein (α-Syn) protein; accordingly, *SCNA* (which encodes α-Syn) is a PD-associated gene. In some embodiments, an expression cassette described herein encodes a wild-type or non-mutant form of a PD-associated gene (or coding sequence thereof). Examples of PD-associated genes are listed in Table 1.

Table 1: Examples of PD-associated genes

Name	**Gene**	**Function**	**NCBI Accession No.**
Lysosome membrane protein 2	*SCARB2/LIMP2*	lysosomal receptor for glucosylceramidase (GBA targeting)	NP_005497.1 (Isoform 1), NP_001191184.1 (Isoform 2)
Prosaposin	*PSAP*	precursor for saposins A, B, C, and D, which localize to the lysosomal compartment and facilitate the catabolism of glycosphingolipids with short oligosaccharide groups	AAH01503.1, AAH07612.1, AAH04275.1, AAA60303.1
beta-Glucocerebrosidase	*GBA1*	cleaves the beta-glucosidic linkage of glucocerebroside	NP_001005742.1 (Isoform 1), NP_001165282.1 (Isoform 2), NP_001165283.1 (Isoform 3)
Non-lysosomal Glucosylceramidase	*GBA2*	catalyzes the conversion of glucosylceramide to free glucose and ceramide	NP_065995.1 (Isoform 1), NP_001317589.1 (Isoform 2)
Galactosylceramidase	*GALC*	removes galactose from ceramide derivatives	EAW81359.1 (Isoform CRA_a), EAW81360.1 (Isoform CRA_b), EAW81362.1 (Isoform CRA_c)
Sphingomyelin phosphodiesterase 1	*SMPD1*	converts sphingomyelin to ceramide	EAW68726.1 (Isoform CRA_a), EAW68727.1 (Isoform CRA_b), EAW68728.1 (Isoform CRA_c), EAW68729.1 (Isoform CRA_d)

Cathepsin B	*CTSB*	thiol protease believed to participate in intracellular degradation and turnover of proteins; also implicated in tumor invasion and metastasis	AAC37547.1, AAH95408.1, AAH10240.1
RAB7, member RAS oncogene family-like 1	*RAB7L1*	regulates vesicular transport	AAH02585.1
Vacuolar protein sorting-associated protein 35	*VPS35*	component of retromer cargo-selective complex	NP_060676.2
GTP cyclohydrolase 1	*GCH1*	responsible for hydrolysis of guanosine triphosphate to form 7.8-dihydroneopterin triphosphate	AAH25415.1
Interleukin 34	*IL34*	increases growth or survival of monocytes; elicits activity by binding the Colony stimulating factor 1 receptor	AAH29804.1
Triggering receptor expressed on myeloid cells 2	*TREM2*	forms a receptor signaling complex with the TYRO protein tyrosine kinase binding protein; functions in immune response and may be involved in chronic inflammation	AAF69824.1
Progranulin	*PGRN*	plays a role in development, inflammation, cell proliferation and protein homeostasis	NP_002087.1

Isolated nucleic acids and vectors

An isolated nucleic acid may be DNA or RNA. The disclosure provides, in some aspects, an isolated nucleic acids (*e.g.*, rAAV vectors) comprising an expression construct encoding one or more PD-associated genes, for example a Gcase (*e.g.*, the gene product of *GBA1* gene) or a portion thereof. Gcase, also referred to as β-glucocerebrosidase or GBA, refers to a lysosomal protein that cleaves the beta-glucosidic linkage of the chemical glucocerebroside, an intermediate in glycolipid metabolism. In humans, Gcase is encoded by the *GBA1* gene, located on chromosome 1. In some embodiments, *GBA1* encodes a peptide that is represented by NCBI Reference Sequence NCBI Reference Sequence NP_000148.2 (SEQ ID NO: 14). In some embodiments, an isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 15.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). Prosaposin is a precursor glycoprotein for sphingolipid activator proteins (saposins) A, B, C, and D, which facilitate the catabolism of glycosphingolipids with short oligosaccharide groups. In humans, the *PSAP* gene is located on chromosome 10. In some embodiments, *PSAP* encodes a peptide that is represented by NCBI Reference Sequence NP_002769.1 (*e.g.*, SEQ ID NO: 16). In some embodiments, an isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 17.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). SCARB2 refers to a membrane protein that regulates lysosomal and endosomal transport within a cell. In humans, *SCARB2* gene is located on chromosome 4. In some embodiments, the *SCARB2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_005497.1 (SEQ ID NO: 18). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 19. In some embodiments the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GBA2 protein (*e.g.*, the gene product of *GBA2* gene). GBA2 protein refers to non-lysosomal glucosylceramidase. In humans, *GBA2* gene is located on chromosome 9. In some embodiments, the *GBA2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_065995.1 (SEQ ID NO: 30). In some embodiments, an isolated nucleic acid

comprises the sequence set forth in SEQ ID NO: 31. In some embodiments the isolated nucleic acid comprises a GBA2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GALC protein (*e.g.*, the gene product of *GALC* gene). GALC protein refers to galactosylceramidase (or galactocerebrosidase), which is an enzyme that hydrolyzes galactose ester bonds of galactocerebroside, galactosylsphingosine, lactosylceramide, and monogalactosyldiglyceride. In humans, *GALC* gene is located on chromosome 14. In some embodiments, the *GALC* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000144.2 (SEQ ID NO: 33). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 34. In some embodiments the isolated nucleic acid comprises a GALC-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding CTSB protein (*e.g.*, the gene product of *CTSB* gene). CTSB protein refers to cathepsin B, which is a lysosomal cysteine protease that plays an important role in intracellular proteolysis. In humans, *CTSB* gene is located on chromosome 8. In some embodiments, the *CTSB* gene encodes a peptide that is represented by NCBI Reference Sequence NP_001899.1 (SEQ ID NO: 35). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 36. In some embodiments the isolated nucleic acid comprises a CTSB-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding SMPD1 protein (*e.g.*, the gene product of *SMPD1* gene). SMPD1 protein refers to sphingomyelin phosphodiesterase 1, which is a hydrolase enzyme that is involved in sphingolipid metabolism. In humans, *SMPD1* gene is located on chromosome 11. In some embodiments, the *SMPD1* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000534.3 (SEQ ID NO: 37). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 38. In some embodiments the isolated nucleic acid comprises a SMPD1-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GCH1 protein (*e.g.*, the gene product of *GCH1* gene). GCH1 protein refers to GTP cyclohydrolase I, which is a hydrolase enzyme that is part of the folate and biopterin biosynthesis pathways. In humans, *GCH1* gene is located on chromosome 14. In some embodiments, the *GCH1* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000152.1 (SEQ ID NO: 45). In some embodiments, an isolated nucleic acid

comprises the sequence set forth in SEQ ID NO: 46. In some embodiments the isolated nucleic acid comprises a GCH1-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding RAB7L protein (*e.g.*, the gene product of *RAB7L* gene). RAB7L protein refers to RAB7, member RAS oncogene family-like 1, which is a GTP binding protein. In humans, *RAB7L* gene is located on chromosome 1. In some embodiments, the *RAB7L* gene encodes a peptide that is represented by NCBI Reference Sequence NP_003920.1 (SEQ ID NO: 47). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 48. In some embodiments the isolated nucleic acid comprises a RAB7L-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding VPS35 protein (*e.g.*, the gene product of *VPS35* gene). VPS35 protein refers to vacuolar protein sorting-associated protein 35, which is part of a protein complex involved in retrograde transport of proteins from endosomes to the trans-Golgi network. In humans, *VPS35* gene is located on chromosome 16. In some embodiments, the *VPS35* gene encodes a peptide that is represented by NCBI Reference Sequence NP_060676.2 (SEQ ID NO: 49). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 50. In some embodiments the isolated nucleic acid comprises a VPS35-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding IL-34 protein (*e.g.*, the gene product of *IL34* gene). IL-34 protein refers to interleukin 34, which is a cytokine that increases growth and survival of monocytes. In humans, *IL34* gene is located on chromosome 16. In some embodiments, the *IL34* gene encodes a peptide that is represented by NCBI Reference Sequence NP_689669.2 (SEQ ID NO: 55). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 56. In some embodiments the isolated nucleic acid comprises a IL-34-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding TREM2 protein (*e.g.*, the gene product of *TREM2* gene). TREM2 protein refers to triggering receptor expressed on myeloid cells 2, which is an immunoglobulin superfamily receptor found on myeloid cells. In humans, *TREM2* gene is located on chromosome 6. In some embodiments, the *TREM2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_061838.1 (SEQ ID NO: 57). In some embodiments, the

isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 58. In some embodiments an isolated nucleic acid comprises a TREM2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding TMEM106B protein (*e.g.*, the gene product of *TMEM106B* gene). TMEM106B protein refers to transmembrane protein 106B, which is a protein involved in dendrite morphogenesis and regulation of lysosomal trafficking. In humans, *TMEM106B* gene is located on chromosome 7. In some embodiments, the *TMEM106B* gene encodes a peptide that is represented by NCBI Reference Sequence NP_060844.2 (SEQ ID NO: 62). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 63. In some embodiments the isolated nucleic acid comprises a TMEM106B-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding progranulin protein (*e.g.*, the gene product of *PRGN* gene). PGRN protein refers to progranulin, which is a protein involved in development, inflammation, cell proliferation and protein homeostasis. In humans, *PGRN* gene is located on chromosome 17. In some embodiments, the *PGRN* gene encodes a peptide that is represented by NCBI Reference Sequence NP_002078.1 (SEQ ID NO: 66). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 67. In some embodiments the isolated nucleic acid comprises a PGRN-encoding sequence that has been codon optimized.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

In some embodiments, a gene product is encoded by a coding portion (*e.g.*, a cDNA) of a naturally occurring gene. In some embodiments, a first gene product is a protein (or a fragment thereof) encoded by the *GBA1* gene. In some embodiments, a gene product is a protein (or a fragment thereof) encoded by another gene listed in Table 1, for example the *SCARB2/LIMP2* gene or the *PSAP* gene. However, the skilled artisan recognizes that the order of expression of a first gene product (*e.g.*, Gcase) and a second gene product (*e.g.*, LIMP2, *etc.*) can generally be reversed (*e.g.*, LIMP2 is the first gene product and Gcase is the second gene product). In some embodiments, a gene product is a fragment (*e.g.*, portion) of a gene listed in Table 1. A protein fragment may comprise about 50%, about 60%, about 70%, about 80% about 90% or about 99% of a protein encoded by the genes listed in Table 1. In some embodiments, a protein fragment

comprises between 50% and 99.9% (*e.g.*, any value between 50% and 99.9%) of a protein encoded by a gene listed in Table 1.

In some embodiments, an expression construct is monocistronic (*e.g.*, the expression construct encodes a single fusion protein comprising a first gene product and a second gene product). In some embodiments, an expression construct is polycistronic (*e.g.*, the expression construct encodes two distinct gene products, for example two different proteins or protein fragments).

A polycistronic expression vector may comprise a one or more (*e.g.*, 1, 2, 3, 4, 5, or more) promoters. Any suitable promoter can be used, for example, a constitutive promoter, an inducible promoter, an endogenous promoter, a tissue-specific promoter (*e.g.*, a CNS- specific promoter), *etc.* In some embodiments, a promoter is a chicken beta-actin promoter (CBA promoter), a CAG promoter (for example as described by Alexopoulou et al. (2008) *BMC Cell Biol.* 9:2; doi: 10.1186/1471-2121-9-2), a CD68 promoter, or a JeT promoter (for example as described by Tornøe et al. (2002) *Gene* 297(1-2):21-32). In some embodiments, a promoter is operably-linked to a nucleic acid sequence encoding a first gene product, a second gene product, or a first gene product and a second gene product. In some embodiments, an expression cassette comprises one or more additional regulatory sequences, including but not limited to transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences, repressor binding sites, or any combination of the foregoing.

In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding an internal ribosomal entry site (IRES). Examples of IRES sites are described, for example, by Mokrejs et al. (2006) *Nucleic Acids Res.* 34(Database issue):D125-30. In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding a self-cleaving peptide. Examples of self-cleaving peptides include but are not limited to T2A, P2A, E2A, F2A, BmCPV 2A, and BmIFV 2A, and those described by Liu et al. (2017) *Sci Rep.* 7: 2193. In some embodiments, the self-cleaving peptide is a T2A peptide.

Pathologically, disorders such as PD and Gaucher disease are associated with accumulation of protein aggregates composed largely of α-Synuclein (α-Syn) protein. Accordingly, in some embodiments, isolated nucleic acids described herein comprise an inhibitory nucleic acid that reduces or prevents expression of α-Syn protein. A sequence encoding an inhibitory nucleic acid may be placed in an untranslated region (*e.g.*, intron, 5'UTR, 3'UTR, *etc.*) of the expression vector.

In some embodiments, an inhibitory nucleic acid is positioned in an intron of an expression construct, for example in an intron upstream of the sequence encoding a first gene product. An inhibitory nucleic acid can be a double stranded RNA (dsRNA), siRNA, micro RNA (miRNA), artificial miRNA (amiRNA), or an RNA aptamer. Generally, an inhibitory nucleic acid binds to (*e.g.*, hybridizes with) between about 6 and about 30 (*e.g.*, any integer between 6 and 30, inclusive) contiguous nucleotides of a target RNA (*e.g.*, mRNA). In some embodiments, the inhibitory nucleic acid molecule is an miRNA or an amiRNA, for example an miRNA that targets *SNCA* (the gene encoding α-Syn protein) or *TMEM106B* (*e.g.*. the gene encoding TMEM106B protein). In some embodiments, the miRNA does not comprise any mismatches with the region of *SNCA* mRNA to which it hybridizes (*e.g.*, the miRNA is "perfected"). In some embodiments, the inhibitory nucleic acid is an shRNA (*e.g.*, an shRNA targeting *SNCA* or *TMEM106B*). In some embodiments, an inhibitory nucleic acid is an artificial miRNA (amiRNA) that includes a miR-155 scaffold and a *SCNA* or *TMEM106B* targeting sequence.

An isolated nucleic acid as described herein may exist on its own, or as part of a vector. Generally, a vector can be a plasmid, cosmid, phagemid, bacterial artificial chromosome (BAC), or a viral vector (*e.g.*, adenoviral vector, adeno-associated virus (AAV) vector, retroviral vector, baculoviral vector, *etc.*). In some embodiments, the vector is a plasmid (*e.g.*, a plasmid comprising an isolated nucleic acid as described herein). In some embodiments, an rAAV vector is single-stranded (*e.g.*, single-stranded DNA). In some embodiments, the vector is a recombinant AAV (rAAV) vector. In some embodiments, a vector is a Baculovirus vector (*e.g.*, an *Autographa californica* nuclear polyhedrosis (AcNPV) vector).

Typically an rAAV vector (*e.g.*, rAAV genome) comprises a transgene (*e.g.*, an expression construct comprising one or more of each of the following: promoter, intron, enhancer sequence, protein coding sequence, inhibitory RNA coding sequence, polyA tail sequence, etc.) flanked by two AAV inverted terminal repeat (ITR) sequences. In some embodiments the transgene of an rAAV vector comprises an isolated nucleic acid as described by the disclosure. In some embodiments, each of the two ITR sequences of an rAAV vector is a full-length ITR (*e.g.*, approximately 145 bp in length, and containing functional *Rep* binding site (RBS) and terminal resolution site (trs)). In some embodiments, one of the ITRs of an rAAV vector is truncated (*e.g.*, shortened or not full-length). In some embodiments, a truncated ITR lacks a functional terminal resolution site (trs) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a truncated ITR is a ΔITR, for example as described by McCarty et al. (2003) *Gene Ther.* 10(26):2112-8.

Aspects of the disclosure relate to isolated nucleic acids (*e.g.*, rAAV vectors) comprising an ITR having one or more modifications (*e.g.*, nucleic acid additions, deletions, substitutions, *etc.*) relative to a wild-type AAV ITR, for example relative to wild-type AAV2 ITR (*e.g.*, SEQ ID NO: 29). The structure of wild-type AAV2 ITR is shown in FIG. 20. Generally, a wild-type ITR comprises a 125 nucleotide region that self-anneals to form a palindromic double-stranded T-shaped, hairpin structure consisting of two cross arms (formed by sequences referred to as B/B′ and C/C′, respectively), a longer stem region (formed by sequences A/A′), and a single-stranded terminal region referred to as the "D" region (FIG. 20). Generally, the "D" region of an ITR is positioned between the stem region formed by the A/A′ sequences and the insert containing the transgene of the rAAV vector (*e.g.*, positioned on the "inside" of the ITR relative to the terminus of the ITR or proximal to the transgene insert or expression construct of the rAAV vector). In some embodiments, a "D" region comprises the sequence set forth in SEQ ID NO: 27. The "D" region has been observed to play an important role in encapsidation of rAAV vectors by capsid proteins, for example as disclosed by Ling et al. (2015) *J Mol Genet Med* 9(3).

The disclosure is based, in part, on the surprising discovery that rAAV vectors comprising a "D" region located on the "outside" of the ITR (*e.g.*, proximal to the terminus of the ITR relative to the transgene insert or expression construct) are efficiently encapsidated by AAV capsid proteins than rAAV vectors having ITRs with unmodified (*e.g.*, wild-type) ITRs In some embodiments, rAAV vectors having a modified "D" sequence (e.g., a "D" sequence in the "outside" position) have reduced toxicity relative to rAAV vectors having wild-type ITR sequences.

In some embodiments, a modified "D" sequence comprises at least one nucleotide substitution relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). A modified "D" sequence may have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 nucleotide substitutions relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence comprises at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 nucleic acid substitutions relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence is between about 10% and about 99% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%) identical to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence comprises the sequence set forth in SEQ ID NO: 26, also referred to as an "S" sequence as described in Wang et al. (1995) *J Mol Biol* 250(5):573-80.

An isolated nucleic acid or rAAV vector as described by the disclosure may further comprise a "TRY" sequence, for example as set forth in SEQ ID NO: 28 or as described by

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. In some embodiments, a TRY sequence is positioned between an ITR (*e.g.* a 5' ITR) and an expression construct (*e.g.* a transgene-encoding insert) of an isolated nucleic acid or rAAV vector.

In some aspects, the disclosure relates to Baculovirus vectors comprising an isolated nucleic acid or rAAV vector as described by the disclosure. In some embodiments, the Baculovirus vector is an *Autographa californica* nuclear polyhedrosis (AcNPV) vector, for example as described by Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43 and Smith et al. (2009) *Mol Ther* 17(11):1888-1896.

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or vector as described herein. A host cell can be a prokaryotic cell or a eukaryotic cell. For example, a host cell can be a mammalian cell, bacterial cell, yeast cell, insect cell, *etc.* In some embodiments, a host cell is a mammalian cell, for example a HEK293T cell. In some embodiments, a host cell is a bacterial cell, for example an *E. coli* cell.

rAAVs

In some aspects, the disclosure relates to recombinant AAVs (rAAVs) comprising a transgene that encodes a nucleic acid as described herein (*e.g.*, an rAAV vector as described herein). The term "rAAVs" generally refers to viral particles comprising an rAAV vector encapsidated by one or more AAV capsid proteins. An rAAV described by the disclosure may comprise a capsid protein having a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAV10. In some embodiments, an rAAV comprises a capsid protein from a non-human host, for example a rhesus AAV capsid protein such as AAVrh.10, AAVrh.39, etc. In some embodiments, an rAAV described by the disclosure comprises a capsid protein that is a variant of a wild-type capsid protein, such as a capsid protein variant that includes at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 (e.g., 15, 20 25, 50, 100, etc.) amino acid substitutions (e.g., mutations) relative to the wild-type AAV capsid protein from which it is derived.

In some embodiments, rAAVs described by the disclosure readily spread through the CNS, particularly when introduced into the CSF space or directly into the brain parenchyma. Accordingly, in some embodiments, rAAVs described by the disclosure comprise a capsid protein that is capable of crossing the blood-brain barrier (BBB). For example, in some embodiments, an rAAV comprises a capsid protein having an AAV9 or AAVrh.10 serotype.

Production of rAAVs is described, for example, by Samulski et al. (1989) *J Virol.* 63(9):3822-8 and Wright (2009) *Hum Gene Ther.* 20(7): 698–706.

In some embodiments, an rAAV as described by the disclosure (*e.g.*, comprising a recombinant rAAV genome encapsidated by AAV capsid proteins to form an rAAV capsid particle) is produced in a Baculovirus vector expression system (BEVS). Production of rAAVs using BEVS are described, for example by Urabe et al. (2002) Hum Gene Ther 13(16):1935-43, Smith et al. (2009) Mol Ther 17(11):1888-1896, U.S. Patent No. 8,945,918, U.S. Patent No. 9,879,282, and International PCT Publication WO 2017/184879. However, an rAAV can be produced using any suitable method (*e.g.*, using recombinant rep and cap genes).

Pharmaceutical Compositions

In some aspects, the disclosure provides pharmaceutical compositions comprising an isolated nucleic acid or rAAV as described herein and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *e.g.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function. Additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

Compositions (*e.g.*, pharmaceutical compositions) provided herein can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration via blood and/or lymph supply, and/or direct administration to an affected site.

In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration). In certain embodiments, the compound or pharmaceutical composition described herein is suitable for topical administration to the eye of a subject.

Methods

The disclosure is based, in part, on compositions for expression of combinations of PD-associated gene products in a subject that act together (*e.g.*, synergistically) to treat Parkinson's disease. As used herein "treat" or "treating" refers to (a) preventing or delaying onset of Parkinson's disease; (b) reducing severity of Parkinson's disease; (c) reducing or preventing development of symptoms characteristic of Parkinson's disease; (d) and/or preventing worsening of symptoms characteristic of Parkinson's disease. Symptoms of Parkinson's disease include, for example, motor dysfunction (*e.g.*, shaking, rigidity, slowness of movement, difficulty with walking), cognitive dysfunction (*e.g.*, dementia, depression, anxiety), emotional and behavioral dysfunction.

Accordingly, in some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

In some embodiments, a composition is administered directly to the CNS of the subject, for example by direct injection into the brain and/or spinal cord of the subject. Examples of CNS-direct administration modalities include but are not limited to intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing. In some embodiments, direct injection into the CNS of a subject results in transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the midbrain, striatum and/or cerebral cortex of the subject. In some embodiments, direct injection into the CNS results in transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the spinal cord and/or CSF of the subject.

In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). Convection enhanced delivery is a therapeutic strategy that involves surgical exposure of the brain and placement of a small-diameter catheter directly into a target area of the brain, followed by infusion of a therapeutic agent (*e.g.*, a composition or rAAV as

described herein) directly to the brain of the subject. CED is described, for example by Debinski et al. (2009) *Expert Rev Neurother.* 9(10):1519-27.

In some embodiments, a composition is administered peripherally to a subject, for example by peripheral injection. Examples of peripheral injection include subcutaneous injection, intravenous injection, intra-arterial injection, intraperitoneal injection, or any combination of the foregoing. In some embodiments, the peripheral injection is intra-arterial injection, for example injection into the carotid artery of a subject.

In some embodiments, a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure is administered both peripherally and directly to the CNS of a subject. For example, in some embodiments, a subject is administered a composition by intra-arterial injection (*e.g.*, injection into the carotid artery) and by intraparenchymal injection (*e.g.*, intraparenchymal injection by CED). In some embodiments, the direct injection to the CNS and the peripheral injection are simultaneous (*e.g.*, happen at the same time). In some embodiments, the direct injection occurs prior (*e.g.*, between 1 minute and 1 week, or more before) to the peripheral injection. In some embodiments, the direct injection occurs after (*e.g.*, between 1 minute and 1 week, or more after) the peripheral injection.

The amount of composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure administered to a subject will vary depending on the administration method. For example, in some embodiments, a rAAV as described herein is administered to a subject at a titer between about 10^9 Genome copies (GC)/kg and about 10^{14} GC/kg (*e.g.*, about 10^9 GC/kg, about 10^{10} GC/kg, about 10^{11} GC/kg, about 10^{12} GC/kg, about 10^{12} GC/kg, or about 10^{14} GC/kg). In some embodiments, a subject is administered a high titer (*e.g.*, $>10^{12}$ Genome Copies GC/kg of an rAAV) by injection to the CSF space, or by intraparenchymal injection.

A composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure can be administered to a subject once or multiple times (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, or more) times. In some embodiments, a composition is administered to a subject continuously (*e.g.*, chronically), for example via an infusion pump.

EXAMPLES

Example 1: rAAV vectors

AAV vectors are generated using cells, such as HEK293 cells for triple-plasmid transfection. The ITR sequences flank an expression construct comprising a promoter/enhancer element for each transgene of interest, a 3' polyA signal, and posttranslational signals such as the WPRE element. Multiple gene products can be expressed simultaneously such as *GBA1* and *LIMP2* and/or Prosaposin, by fusion of the protein sequences; or using a 2A peptide linker, such as T2A or P2A, which leads 2 peptide fragments with added amino acids due to prevention of the creation of a peptide bond; or using an IRES element; or by expression with 2 separate expression cassettes. The presence of a short intronic sequence that is efficiently spliced, upstream of the expressed gene, can improve expression levels. shRNAs and other regulatory RNAs can potentially be included within these sequences. Examples of expression constructs described by the disclosure are shown in FIGs. 1-8 and 21-35, and in Table 2 below.

Table 2

Name	Promoter 1	shRNA	CDS1	PolyA 1	Bicistronic element	Promoter 2	CDS2	PolyA2	Length between ITRs
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	Synthetic 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	Synthetic pA	T2A	-	GALC	-	4373
PrevailVector_LT4_JetLong_GALC_T2A_GBA1_SyntheticpolyA_4373nt	JetLong	-	GALC	Synthetic pA	T2A	-	GBA1	-	4373
PrevailVector_LT5s_JetLong_mRNAiaSyn_CTSB-T2A-GBA1_WPRE_bGH_4392nt	JetLong	aSyn	CTSB	WPRE_bGH	T2A	-	GBA1	-	4392
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_4477nt	JetLong	aSyn	GBA1	Synthetic pA	T2A	-	SMPD1	-	4477
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SymtheticpolyA_4820nt	JetLong	-	GALC	Synthetic 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_G	JetLong	aSyn	GBA1	bGH	IRES	-	GCH1	-	3978

BA1-IRES-GCH1_bGH_3978nt									
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
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

Example 2: Cell based assays of viral transduction into GBA-deficient cells

Cells deficient in *GBA1* are obtained, for example as fibroblasts from GD patients, monocytes, or hES cells, or patient-derived induced pluripotent stem cells (iPSCs). These cells accumulate substrates such as glucosylceramide and glucosylsphingosine (GlcCer and GlcSph). Treatment of wild-type or mutant cultured cell lines with Gcase inhibitors, such as CBE, is also be used to obtain GBA deficient cells.

Using such cell models, lysosomal defects are quantified in terms of accumulation of protein aggregates, such as of α-Synuclein with an antibody for this protein or phospho-αSyn, followed by imaging using fluorescent microscopy. Imaging for lysosomal abnormalities by ICC for protein markers such as LAMP1, LAMP2, LIMP1, LIMP2, or using dyes such as Lysotracker, or by uptake through the endocytic compartment of fluorescent dextran or other markers is also performed. Imaging for autophagy marker accumulation due to defective fusion

with the lysosome, such as for LC3, can also be performed. Western blotting and/or ELISA is used to quantify abnormal accumulation of these markers. Also, the accumulation of glycolipid substrates and products of GBA1 is measured using standard approaches.

Therapeutic endpoints (*e.g.*, reduction of PD-associated pathology) are measured in the context of expression of transduction of the AAV vectors, to confirm and quantify activity and function. Gcase can is also quantified using protein ELISA measures, or by standard Gcase activity assays.

Example 3: In vivo assays using mutant mice

This example describes *in vivo* assays of AAV vectors using mutant mice. *In vivo* studies of AAV vectors as above in mutant mice are performed using assays described, for example, by Liou *et al.* (2006) *J. Biol. Chem.* 281(7): 4242–4253, Sun *et al.* (2005) *J. Lipid Res.* 46:2102–2113, and Farfel-Becker *et al.* (2011) *Dis. Model Mech.* 4(6):746–752.

The intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example at an injection volume between 5–10 μL. Intraparenchymal delivery by convection enhanced delivery is performed.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α-Synuclein monomers, protofibrils or fibrils.

Example 4: Chemical models of disease

This example describes *in vivo* assays of AAV vectors using a chemically-induced mouse model of Gaucher disease (*e.g.*, the CBE mouse model). *In vivo* studies of these AAV vectors are performed in a chemically-induced mouse model of Gaucher disease, for example as described by Vardi *et al.* (2016) *J Pathol.* 239(4):496-509.

Intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example with injection volume between 5–10 μL. Intraparenchymal delivery by convection enhanced delivery is performed. Peripheral delivery is achieved by tail vein injection.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of

Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α-Synuclein monomers, protofibrils or fibrils.

Example 5: Clinical trials in PD, LBD, Gaucher disease patients

In some embodiments, patients having certain forms of Gaucher disease (*e.g.*, GD1) have an increased risk of developing Parkinson's disease (PD) or Lewy body dementia (LBD). This Example describes clinical trials to assess the safety and efficacy of rAAVs as described by the disclosure, in patients having Gaucher disease, PD and/or LBD.

Clinical trials of such vectors for treatment of Gaucher disease, PD and/or LBD are performed using a study design similar to that described in Grabowski et al. (1995) *Ann. Intern. Med.* 122(1):33-39.

Example 6: Treatment of peripheral disease

In some embodiments, patients having certain forms of Gaucher disease exhibit symptoms of peripheral neuropathy, for example as described in Biegstraaten *et al.* (2010) *Brain* 133(10):2909–2919.

This example describes *in vivo* assays of AAV vectors as described herein for treatment of peripheral neuropathy associated with Gaucher disease (*e.g.*, Type 1 Gaucher disease). Briefly, Type 1 Gaucher disease patients identified as having signs or symptoms of peripheral neuropathy are administered a rAAV as described by the disclosure. In some embodiments, the peripheral neuropathic signs and symptoms of the subject are monitored, for example using methods described in Biegstraaten *et al.*, after administration of the rAAV.

Levels of transduced gene products as described by the disclosure present in patients (*e.g.*, in serum of a patient, in peripheral tissue (*e.g.*, liver tissue, spleen tissue, *etc.*)) of a patient are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 7: Treatment of CNS forms

This example describes *in vivo* assays of rAAVs as described herein for treatment of CNS forms of Gaucher disease. Briefly, Gaucher disease patients identified as having a CNS form of Gaucher disease (*e.g.*, Type 2 or Type 3 Gaucher disease) are administered a rAAV as described by the disclosure. Levels of transduced gene products as described by the disclosure present in the CNS of patients (*e.g.*, in serum of the CNS of a patient, in cerebrospinal fluid

(CSF) of a patient, or in CNS tissue of a patient) are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 8: Gene therapy of Parkinson's Disease in subjects having mutations in GBA1

This example describes administration of a recombinant adeno-associated virus (rAAV) encoding *GBA1* to a subject having Parkinson's disease characterized by a mutation in *GBA1*gene.

The rAAV-GBA1 vector insert contains the CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAp), Exon 1, and intron (int) to constitutively express the codon optimized coding sequence (CDS) of human GBA1 (maroon). The 3' region also contains a Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) posttranscriptional regulatory element followed by a bovine Growth Hormone polyA signal (bGH polyA) tail. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (FIG. 7, inset box, bottom sequence) were evaluated; these variants have several nucleotide differences within the 20-nucleotide "D" region of the ITR, which is believed to impact the efficiency of packaging and expression. The rAAV-GBA1 vector product contains the "D" domain nucleotide sequence shown in FIG. 7 (inset box, top sequence). A variant vector harbors a mutant "D" domain (termed an "S" domain herein, with the nucleotide changes shown by shading), performed similarly in preclinical studies. The backbone contains the gene to confer resistance to kanamycin as well as a stuffer sequence to prevent reverse packaging. A schematic depicting a rAAV-GBA1 vector is shown in FIG. 8. The rAAV-GBA1 vector is packaged into an rAAV using AAV9 serotype capsid proteins.

rAAV-GBA1 is administered to a subject as a single dose via a fluoroscopy guided sub-occipital injection into the cisterna magna (intracisternal magna; ICM). One embodiment of a rAAV-GBA1 dosing regimen study is as follows:

A single dose of rAAV-GBA1is administered to patients (N=12) at one of two dose levels (3e13 vg (low dose); 1e14 vg (high dose), *etc.*) which are determined based on the results of nonclinical pharmacology and toxicology studies.

Initial studies were conducted in a chemical mouse model involving daily delivery of conduritol-b-epoxide (CBE), an inhibitor of GCase to assess the efficacy and safety of the rAAV-GBA1 vector and a rAAV-GBA1 S-variant construct (as described further below). Additionally, initial studies were performed in a genetic mouse model, which carries a homozygous *GBA1* mutation and is partially deficient in saposins (4L/PS-NA). Additional

dose-ranging studies in mice and nonhuman primates (NHPs) are conducted to further evaluate vector safety and efficacy.

Two slightly different versions of the 5' inverted terminal repeat (ITR) in the AAV backbone were tested to assess manufacturability and transgene expression (FIG. 7). The 20 bp "D" domain within the 145 bp 5' ITR is thought to be necessary for optimal viral vector production, but mutations within the "D" domain have also been reported to increase transgene expression in some cases. Thus, in addition to the viral vector rAAV-GBA1, which harbors an intact "D" domain, a second vector form with a mutant D domain (termed an "S" domain herein) was also evaluated. Both rAAV-GBA1 and the variant express the same transgene. While both vectors produced virus that was efficacious in vivo as detailed below, rAAV-GBA1, which contains a wild-type "D" domain, was selected for further development.

To establish the CBE model of GCase deficiency, juvenile mice were dosed with CBE, a specific inhibitor of GCase. Mice were given CBE by IP injection daily, starting at postnatal day 8 (P8). Three different CBE doses (25 mg/kg, 37.5 mg/kg, 50 mg/kg) and PBS were tested to establish a model that exhibits a behavioral phenotype (FIG. 9). Higher doses of CBE led to lethality in a dose-dependent manner. All mice treated with 50 mg/kg CBE died by P23, and 5 of the 8 mice treated with 37.5 mg/kg CBE died by P27. There was no lethality in mice treated with 25 mg/kg CBE. Whereas CBE-injected mice showed no general motor deficits in the open field assay (traveling the same distance and at the same velocity as mice given PBS), CBE-treated mice exhibited a motor coordination and balance deficit as measured by the rotarod assay.

Mice surviving to the end of the study were sacrificed on the day after their last CBE dose (P27, "Day 1") or after three days of CBE withdrawal (P29, "Day 3"). Lipid analysis was performed on the cortex of mice given 25 mg/kg CBE to evaluate the accumulation of GCase substrates in both the Day 1 and Day 3 cohorts. GluSph and GalSph levels (measured in aggregate in this example) were significantly accumulated in the CBE-treated mice compared to PBS-treated controls, consistent with GCase insufficiency.

Based on the study described above, the 25 mg/kg CBE dose was selected since it produced behavioral deficits without impacting survival. To achieve widespread GBA1 distribution throughout the brain and transgene expression during CBE treatment, rAAV-GBA1 or excipient was delivered by intracerebroventricular (ICV) injection at postnatal day 3 (P3) followed by daily IP CBE or PBS treatment initiated at P8 (FIG. 10).

CBE-treated mice that received rAAV-GBA1 performed statistically significantly better on the rotarod than those that received excipient (FIG. 11). Mice in the variant treatment group

did not differ from excipient treated mice in terms of other behavioral measures, such as the total distance traveled during testing (FIG. 11).

At the completion of the in-life study, half of the mice were sacrificed the day after the last CBE dose (P36, "Day 1") or after three days of CBE withdrawal (P38, "Day 3") for biochemical analysis (FIG. 12). Using a fluorometric enzyme assay performed in biological triplicate, GCase activity was assessed in the cortex. GCase activity was increased in mice that were treated with rAAV-GBA1, while CBE treatment reduced GCase activity. Additionally, mice that received both CBE and rAAV-GBA1 had GCase activity levels that were similar to the PBS-treated group, indicating that delivery of rAAV-GBA1 is able to overcome the inhibition of GCase activity induced by CBE treatment. Lipid analysis was performed on the motor cortex of the mice to examine levels of the substrates GluCer and GluSph. Both lipids accumulated in the brains of mice given CBE, and rAAV-GBA1 treatment significantly reduced substrate accumulation.

Lipid levels were negatively correlated with both GCase activity and performance on the Rotarod across treatment groups. The increased GCase activity after rAAV-GBA1 administration was associated with substrate reduction and enhanced motor function (FIG. 13). As shown in FIG. 14, preliminary biodistribution was assessed by vector genome presence, as measured by qPCR (with >100 vector genomes per 1 μg genomic DNA defined as positive). Mice that received rAAV-GBA1, both with and without CBE, were positive for rAAV-GBA1 vector genomes in the cortex, indicating that ICV delivery results in rAAV-GBA1 delivery to the cortex. Additionally, vector genomes were detected in the liver, few in spleen, and none in the heart, kidney or gonads. For all measures, there was no statistically significant difference between the Day 1 and Day 3 groups.

A larger study in the CBE model further explored efficacious doses of rAAV-GBA1 in the CBE model. Using the 25 mg/kg CBE dose model, excipient or rAAV-GBA1 was delivered via ICV at P3, and daily IP PBS or CBE treatment initiated at P8. Given the similarity between the groups with and without CBE withdrawal observed in the previous studies, all mice were sacrificed one day after the final CBE dose (P38-40). The effect of three different rAAV-GBA1 doses was assessed, resulting in the following five groups, with 10 mice (5M/5F) per group:

Excipient ICV + PBS IP

Excipient ICV + 25 mg/kg CBE IP

3.2e9 vg (2.13e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

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

The highest dose of rAAV-GBA1 rescued the CBE treatment-related failure to gain weight at P37. Additionally, this dose resulted in a statistically significant increase in performance on the rotarod and tapered beam compared to the Excipient + CBE treated group (FIG. 15). Lethality was observed in several groups, including both excipient-treated and rAAV-GBA1-treated groups (Excipient + PBS: 0; Excipient + 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).

At the completion of the in-life study, mice were sacrificed for biochemical analysis (FIG. 16). GCase activity in the cortex was assessed in biological triplicates by a fluorometric assay. CBE-treated mice showed reduced GCase activity whereas mice that received a high rAAV-GBA1 dose showed a statistically significant increase in GCase activity compared to CBE treatment. CBE-treated mice also had accumulation of GluCer and GluSph, both of which were rescued by administering a high dose of rAAV-GBA1.

In addition to the established chemical CBE model, rAAV-GBA1is also evaluated in the 4L/PS-NA genetic model, which is homozygous for the V394L GD mutation in Gba1 and is also partially deficient in saposins, which affect GCase localization and activity. These mice exhibit motor strength, coordination, and balance deficits, as evidenced by their performance in the beam walk, rotarod, and wire hang assays. Typically the lifespan of these mice is less than 22 weeks. In an initial study, 3 µl of maximal titer virus was delivered by ICV at P23, with a final dose of 2.4e10 vg (6.0e10 vg/g brain). With 6 mice per group, the treatment groups were:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 2.4e10 vg (6.0e10 vg/g brain) rAAV-GBA1 ICV

Motor performance by the beam walk test was assessed 4 weeks post- rAAV-GBA1 delivery. The group of mutant mice that received rAAV-GBA1 showed a trend towards fewer total slips and fewer slips per speed when compared to mutant mice treated with excipient, restoring motor function to near WT levels (FIG. 17). Since the motor phenotypes become more severe as these mice age, their performance on this and other behavioral tests is assessed at later time points. At the completion of the in-life study, lipid levels, GCase activity, and biodistribution are assessed in these mice.

Additional lower doses of rAAV-GBA1 are currently being tested using the CBE model, corresponding to 0.03x, 0.1x, and 1x the proposed phase 1 high clinical dose. Each group includes 10 mice (5M/5F) per group:

Excipient ICV

Excipient ICV + 25 mg/kg CBE IP

3.2e8 vg (2.13e9 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e9 vg (6.67e9 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP.

In addition to motor phenotypes, lipid levels and GCase activity are assessed in the cortex. Time course of treatments and analyses are also performed.

A larger dose ranging study was initiated to evaluate efficacy and safety data. 10 4L/PS-NA mice (5M/5F per group) were injected with 10 µl of rAAV-GBA1. Using an allometric brain weight calculation, the doses correlate to 0.15x, 1.5x, 4.4x, and 14.5x the proposed phase 1 high clinical dose. The injection groups consist of:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 4.3e9 vg (1.1e10 vg/g brain) rAAV-GBA1 ICV

4L/PS-NA + 4.3e10 vg (1.1e11 vg/g/ brain) rAAV-GBA1 ICV

4L/PS-NA + 1.3e11 vg (3.2e11 vg/g brain) rAAV-GBA1 ICV

4L/PS-NA + 4.3e11 vg (1.1e12 vg/g brain) rAAV-GBA1 ICV.

A summary of nonclinical studies in the CBE model are shown in Table 3 below.

Table 3: Summary of Results in CBE Mouse Model

Test Material	Study Number	Dose Cohort	Behavioral Changes			Lipids	Enzyme	BD	
			Rotarod	Tapered Beam	Open Field			Brain	Liver
rAAV-GBA1	PRV-2018-005 Dose-ranging	3.2e9 vg (2.13e10 vg/g brain)	NS	NS	NS	NS	NS	+	-

	rAAV-GBA1 in CBE Model	1.10e10 vg (6.67e10 vg/g brain)	T	NS	NS	T/S	NS	+	+
		2.3e10vg (2.13e11 vg/g brain)	S	S	NS	S	S	+	+
Variant	PRV-2018-005 Dose-ranging Variant in CBE Model	8.8e9 vg (5.9e10 vg/g brain)	S	N/A	NS	S	S	+	+

Note that positive biodistribution is defined as >100 vg/1 µg genomic DNA.
Abbreviations: BD = biodistribution; NS = nonsignificant; T = trend; S = significant; N/A = not applicable; + = positive; - = negative.

Example 9: In vitro analysis of rAAV vectors

rAAV constructs were tested *in vitro* and *in vivo*. FIG. 18 shows representative data for in vitro expression of rAAV constructs encoding progranulin (PGRN) protein. The left panel shows a standard curve of progranulin (PGRN) ELISA assay. The bottom panel shows a dose-response of PGRN expression measured by ELISA assay in cell lysates of HEK293T cells transduced with rAAV. MOI = multiplicity of infection (vector genomes per cell).

A pilot study was performed to assess *in vitro* activity of rAAV vectors encoding Prosaposin (*PSAP*) and *SCARB2*, alone or in combination with *GBA1* and/or one or more inhibitory RNAs. One construct encoding PSAP and progranulin (PGRN) was also tested. Vectors tested include those shown in Table 4. "Opt" refers to a nucleic acid sequence codon optimized for expression in mammalian cells (*e.g.*, human cells). FIG. 19 shows representative data indicating that transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

A pilot study was performed to assess in vitro activity of rAAV vectors encoding TREM2, alone or in combination with one or more inhibitory RNAs. Vectors tested include those shown in Table 4. "Opt" refers to a nucleic acid sequence codon optimized for expression in mammalian cells (e.g., human cells). FIGs. 36A-36B show representative data indicating that

transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

Table 4

ID	Promoter	Inhibitory RNA	Promoter	Transgene
I00015	JL_intronic	*SCNA*	JetLong	Opt-PSAP_GBA1
I00039	-	-	JetLong	Opt-PSAP-GRN
I00046	-	-	CBA	Opt-PSAP
I00014	JetLong	*SCNA*	JetLong	Opt-SCARB2_GBA1
I00040			JL, CD68	opt-GBA1, TREM2

Example 10: Testing of SCNA and TMEM106B shRNA constructs

HEK293 cells

Human embryonic kidney 293 cell line (HEK293) were used in this study (#85120602, Sigma-Aldrich). HEK293 cells were maintained in culture media (D-MEM [#11995065, Thermo Fisher Scientific] supplemented with 10% fetal bovine serum [FBS] [#10082147, Thermo Fisher Scientific]) containing 100 units/ml penicillin and 100 µg/ml streptomycin (#15140122, Thermo Fisher Scientific).

Plasmid transfection

Plasmid transfection was performed using Lipofectamine 2000 transfection reagent (#11668019, Thermo Fisher Scientific) according to the manufacture's instruction. Briefly, HEK293 cells (#12022001, Sigma-Aldrich) were plated at the density of $3x10^5$ cells/ml in culture media without antibiotics. On the following day, the plasmid and Lipofectamine 2000 reagent were combined in Opti-MEM solution (#31985062, Thermo Fisher Scientific). After 5 minutes, the mixtures were added into the HEK293 culture. After 72 hours, the cells were harvested for RNA or protein extraction, or subjected to the imaging analyses. For imaging analyses, the plates were pre-coated with 0.01% poly-L-Lysine solution (P8920, Sigma-Aldrich) before the plating of cells.

Gene expression analysis by quantitative real-time PCR (qRT-PCR)

Relative gene expression levels were determined by quantitative real-time PCR (qRT-PCR) using Power SYBR Green Cells-to-CT Kit (#4402955, Thermo Fisher Scientific) according to the manufacturer's instruction. The candidate plasmids were transiently transfected into HEK293 cells plated on 48-well plates (7.5 x10^4 cells/well) using Lipofectamine 2000 transfection reagent (0.5 μg plasmid and 1.5 μl reagent in 50 μl Opti-MEM solution). After 72 hours, RNA was extracted from the cells and used for reverse transcription to synthesize cDNA according to the manufacturer's instruction. For quantitative PCR analysis, 2~5 μl of cDNA products were amplified in duplicates using gene specific primer pairs (250 nM final concentration) with Power SYBR Green PCR Master Mix (#4367659, Thermo Fisher Scientific). The primer sequences for *SNCA, TMEM106B*, and *GAPDH* genes were: 5'- AAG AGG GTG TTC TCT ATG TAG GC -3' (SEQ ID NO: 71), 5'- GCT CCT CCA ACA TTT GTC ACT T -3' (SEQ ID NO: 72) for *SNCA*, 5'-ACA CAG TAC CTA CCG TTA TAG CA-3' (SEQ ID NO: 73), 5'-TGT TGT CAC AGT AAC TTG CAT CA-3' (SEQ ID NO: 74) for *TMEM106B*, and 5'- CTG GGC TAC ACT GAG CAC C -3' (SEQ ID NO: 75), 5'- AAG TGG TCG TTG AGG GCA ATG -3' (SEQ ID NO: 76) for *GAPDH*. Quantitative PCR was performed in a QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific). Expression levels were normalized by the housekeeping gene *GAPDH* and calculated using the comparative CT method.

Fluorescence Imaging Analysis

EGFP reporter plasmids, which contain 3'-UTR of human *SNCA* gene at downstream of EGFP coding region, were used for the validation of *SNCA* and *TMEM106B* knockdown plasmids. EGFP reporter plasmids and candidate knockdown plasmids were simultaneously transfected into HEK293 cells plated on poly-L-Lysine coated 96-well plates (3.0 x10^4 cells/well) using Lipofectamine 2000 transfection reagent (0.04 μg reporter plasmid, 0.06 μg knockdown plasmid and 0.3 μl reagent in 10 μl Opti-MEM solution). After 72 hours, the fluorescent intensities of EGFP signal were measured at excitation 488 nm/emission 512 nm using Varioskan LUX multimode reader (Thermo Fisher Scientific). Cells were fixed with 4% PFA at RT for 10 minutes, and incubated with D-PBS containing 40 μg/ml 7-aminoactinomycin D (7-AAD) for 30 min at RT. After washing with D-PBS, the fluorescent intensities of 7-AAD signal were measured at excitation 546 nm/emission 647 nm using Varioskan reader to quantify cell number. Normalized EGFP signal per 7-AAD signal levels were compared with the control knockdown samples.

Enzyme-linked Immunosorbent Assay (ELISA)

α-Synuclein reporter plasmids, which contain 3'-UTR of human *SNCA* gene or *TMEM106B* gene downstream of *SNCA* coding region, were used for the validation of knockdown plasmids at the protein level. Levels of α-synuclein protein were determined by ELISA (#KHB0061, Thermo Fisher Scientific) using the lysates extracted from HEK293 cells. The candidate plasmids were transiently transfected into HEK293 cells plated on 48-well plates (7.5 $x10^4$ cells/well) using Lipofectamine 2000 transfection reagent (0.1 μg reporter plasmid, 0.15 μg knockdown plasmid and 0.75 μl reagent in 25 μl Opti-MEM solution). After 72 hours, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (#89900, Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (#P8340, Sigma-Aldrich), and sonicated for a few seconds. After incubation on ice for 30 min, the lysates were centrifuged at 20,000 ×g at 4°C for 15 min, and the supernatant was collected. Protein levels were quantified. Plates were read in a Varioskan plate reader at 450 nm, and concentrations were calculated using SoftMax Pro 5 software. Measured protein concentrations were normalized to total protein concentration determined with a bicinchoninic acid assay (#23225, Thermo Fisher Scientific).

FIG. 37 and Table 5 show representative data indicating successful silencing of *SCNA in vitro* by GFP reporter assay (top) and α-Syn assay (bottom). FIG. 38 and Table 6 show representative data indicating successful silencing of *TMEM106B in vitro* by GFP reporter assay (top) and α-Syn assay (bottom).

Table 5

ID	Promoter	Knockdown	Promoter	Overexpress
I00007	CMV_intronic	SNCA_mi	CMV	opt-GBA1
I00008	H1	SNCA_sh	CMV	opt-GBA1
I00009	H1	SNCA_Pubsh4	CMV	opt-GBA1
I00014	JL_intronic	SNCA_mi	JetLong	opt-SCARB2_GBA
I00015	JL_intronic	SNCA_mi	JetLong	opt-PSAP_GBA
I00016	JL_intronic	SNCA_mi	JetLong	opt-CTSB_GBA

I00019	JL_intronic	SNCA_TMEM_mi	JetLong	opt-VPS35
I00023	JL_intronic	SNCA_mi	JetLong	opt-GBA1_IL34
I00024	JL_intronic	SNCA_mi	JetLong	opt-GBA2
I00028	intronic	SNCA_Broadsh	CMV	opt-GBA1
I00029	intronic	SNCA_Pubsh4	CMV	opt-GBA1

Table 6

ID	Promoter	Knockdown	Promoter	Overexpress
I00010	H1	TMEM_Pubsh	CMV	opt-GRN
I00011	JL_intronic	TMEM_mi	JetLong	opt-GBA1_GRN
I00012	H1	TMEM_sh	CMV	opt-GRN
I00019	JL_intronic	SNCA_TMEM_mi	JetLong	opt-VPS35

Example 11: ITR "D" sequence placement and cell transduction

The effect of placement of ITR "D" sequence on cell transduction of rAAV vectors was investigated. HEK293 cells were transduced with Gcase-encoding rAAVs having 1) wild-type ITRs (e.g., "D" sequences proximal to the transgene insert and distal to the terminus of the ITR) or 2) ITRs with the "D" sequence located on the "outside" of the vector (e.g., "D" sequence located proximal to the terminus of the ITR and distal to the transgene insert), as shown in FIG. 20. Surprisingly, data indicate that rAAVs having the "D" sequence located in the "outside" position retain the ability to be packaged and transduce cells efficiently (FIG. 40).

Example 12: In vitro testing of Progranulin rAAVs

FIG. 39 is a schematic depicting one embodiments of a vector comprising an expression construct encoding PGRN. Progranulin is overexpressed in the CNS of rodents deficient in *GRN*, either heterozygous or homozygous for GRN deletion, by injection of an rAAV vector

encoding PGRN (*e.g.*, codon-optimized PGRN), either by intraparenchymal or intrathecal injection such as into the cisterna magna.

Mice are injected at 2 months or 6 months of age, and aged to 6 months or 12 months and analyzed for one or more of the following: expression level of GRN at the RNA and protein levels, behavioral assays (*e.g.*, improved movement), survival assays (*e.g.*, improved survival), microglia and inflammatory markers, gliosis, neuronal loss, Lipofuscinosis, and/or Lysosomal marker accumulation rescue, such as LAMP1. Assays on PGRN-deficient mice are described, for example by Arrant et al. (2017) *Brain* 140: 1477-1465; Arrant et al. (2018) *J. Neuroscience* 38(9):2341–2358; and Amado et al. (2018) doi:https://doi.org/10.1101/30869; the entire contents of which are incorporated herein by reference.

EQUIVALENTS

This Application incorporates by reference the contents of the following documents in their entirety: the International PCT Application referred to by Attorney Docket Number P1094.70002WO00, filed October 3 2018; International PCT Application referred to by Attorney Docket Number P1094.70004WO00, filed October 3, 2018; Provisional Application Serial Numbers 62/567,296, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,311, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,319, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,301, filed October 3, 2018, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,310, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; 62/567,303, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS"; and 62/567,305, filed October 3, 2017, entitled "GENE THERAPIES FOR LYSOSOMAL DISORDERS".

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be

within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "A and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); *etc.*

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of

exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); *etc.*

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Use of ordinal terms such as "first," "second," "third," *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

SEQUENCES

In some embodiments, an expression cassette encoding one or more gene products (*e.g.*, a first, second and/or third gene product) comprises or consists of (or encodes a peptide having) a sequence set forth in any one of SEQ ID NOs: 1-78. In some embodiments, a gene product is encoded by a portion (*e.g.*, fragment) of any one of SEQ ID NOs: 1-78.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising an expression construct encoding a Gcase protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the Gcase protein is encoded by a codon-optimized nucleic acid sequence.

2. The isolated nucleic acid of claim 1, wherein the Gcase protein comprises the amino acid sequence set forth in SEQ ID NO: 14 or a portion thereof.

3. The isolated nucleic acid of claim 1 or 2, wherein the Gcase protein is encoded by a codon-optimized nucleic acid sequence, optionally the nucleic acid sequence set forth in SEQ ID NO: 15.

4. The isolated nucleic acid of any one of claims 1 to 3, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

5. The isolated nucleic acid of any one of claims 1 to 4, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

6. The isolated nucleic acid of any one of claims 1 to 5, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

7. An isolated nucleic acid comprising an expression construct encoding a prosaposin protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the prosaposin protein is encoded by a codon-optimized nucleic acid sequence.

8. The isolated nucleic acid of claim 7, wherein the prosaposin protein comprises the amino acid sequence set forth in SEQ ID NO: 16 or a portion thereof.

9. The isolated nucleic acid of claim 7 or 8, wherein the prosaposin protein is encoded by a codon-optimized nucleic acid sequence, optionally the nucleic acid sequence set forth in SEQ ID NO: 17.

10. The isolated nucleic acid of any one of claims 7 to 9, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

11. The isolated nucleic acid of any one of claims 7 to 10, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

12. The isolated nucleic acid of any one of claims 7 to 11, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

13. An isolated nucleic acid comprising an expression construct encoding a SCARB2 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the SCARB2 protein is encoded by a codon-optimized nucleic acid sequence.

14. The isolated nucleic acid of claim 13, wherein the SCARB2 protein comprises the amino acid sequence set forth in SEQ ID NO: 18 or a portion thereof.

15. The isolated nucleic acid of claim 13 or 14, wherein the SCARB2 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 19.

16. The isolated nucleic acid of any one of claims 13 to 15, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

17. The isolated nucleic acid of any one of claims 13 to 16, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

18. The isolated nucleic acid of any one of claims 13 to 17, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

19. An isolated nucleic acid comprising an expression construct encoding a GBA2 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the GBA2 protein is encoded by a codon-optimized nucleic acid sequence.

20. The isolated nucleic acid of claim 19, wherein the GBA2 protein comprises the amino acid sequence set forth in SEQ ID NO: 30 or a portion thereof.

21. The isolated nucleic acid of claim 19 or 20, wherein the GBA2 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 31.

22. The isolated nucleic acid of any one of claims 19 to 21, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

23. The isolated nucleic acid of any one of claims 19 to 22, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

24. The isolated nucleic acid of any one of claims 19 to 23, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

25. An isolated nucleic acid comprising an expression construct encoding a GALC protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the GALC protein is encoded by a codon-optimized nucleic acid sequence.

26. The isolated nucleic acid of claim 25, wherein the GALC protein comprises the amino acid sequence set forth in SEQ ID NO: 33 or a portion thereof.

27. The isolated nucleic acid of claim 25 or 26, wherein the GALC protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 34.

28. The isolated nucleic acid of any one of claims 25 to 27, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

29. The isolated nucleic acid of any one of claims 25 to 28, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

30. The isolated nucleic acid of any one of claims 25 to 29, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

31. An isolated nucleic acid comprising an expression construct encoding a CTSB protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the CTSB protein is encoded by a codon-optimized nucleic acid sequence.

32. The isolated nucleic acid of claim 31, wherein the CTSB protein comprises the amino acid sequence set forth in SEQ ID NO: 30 or a portion thereof.

33. The isolated nucleic acid of claim 31 or 32, wherein the CTSB protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 36.

34. The isolated nucleic acid of any one of claims 31 to 33, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

35. The isolated nucleic acid of any one of claims 31 to 34, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

36. The isolated nucleic acid of any one of claims 31 to 35, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

37. An isolated nucleic acid comprising an expression construct encoding a SMPD1 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the SMPD1 protein is encoded by a codon-optimized nucleic acid sequence.

38. The isolated nucleic acid of claim 37, wherein the SMPD1 protein comprises the amino acid sequence set forth in SEQ ID NO: 37 or a portion thereof.

39. The isolated nucleic acid of claim 37 or 38, wherein the SMPD1 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 38.

40. The isolated nucleic acid of any one of claims 37 to 39, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

41. The isolated nucleic acid of any one of claims 37 to 40, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

42. The isolated nucleic acid of any one of claims 37 to 41, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

43. An isolated nucleic acid comprising an expression construct encoding a GCH1 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the GCH1 protein is encoded by a codon-optimized nucleic acid sequence.

44. The isolated nucleic acid of claim 43, wherein the GCH1 protein comprises the amino acid sequence set forth in SEQ ID NO: 45 or a portion thereof.

45. The isolated nucleic acid of claim 43 or 44, wherein the GCH1 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 46.

46. The isolated nucleic acid of any one of claims 43 to 45, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

47. The isolated nucleic acid of any one of claims 43 to 46, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

48. The isolated nucleic acid of any one of claims 43 to 47, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

49. An isolated nucleic acid comprising an expression construct encoding a RAB7L protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the RAB7L protein is encoded by a codon-optimized nucleic acid sequence.

50. The isolated nucleic acid of claim 49, wherein the RAB7L protein comprises the amino acid sequence set forth in SEQ ID NO: 47 or a portion thereof.

51. The isolated nucleic acid of claim 49 or 50, wherein the RAB7L protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 48.

52. The isolated nucleic acid of any one of claims 49 to 51, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

53. The isolated nucleic acid of any one of claims 49 to 52, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

54. The isolated nucleic acid of any one of claims 49 to 53, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

55. An isolated nucleic acid comprising an expression construct encoding a VPS35 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the VPS35 protein is encoded by a codon-optimized nucleic acid sequence.

56. The isolated nucleic acid of claim 55, wherein the VPS35 protein comprises the amino acid sequence set forth in SEQ ID NO: 49 or a portion thereof.

57. The isolated nucleic acid of claim 55 or 56, wherein the VPS35 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 50.

58. The isolated nucleic acid of any one of claims 55 to 57, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

59. The isolated nucleic acid of any one of claims 55 to 58, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

60. The isolated nucleic acid of any one of claims 55 to 59, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

61. An isolated nucleic acid comprising an expression construct encoding a IL-34 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the IL-34 protein is encoded by a codon-optimized nucleic acid sequence.

62. The isolated nucleic acid of claim 61, wherein the IL-34 protein comprises the amino acid sequence set forth in SEQ ID NO: 55 or a portion thereof.

63. The isolated nucleic acid of claim 61 or 62, wherein the IL-34 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 56.

64. The isolated nucleic acid of any one of claims 61 to 63, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

65. The isolated nucleic acid of any one of claims 61 to 64, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

66. The isolated nucleic acid of any one of claims 61 to 65, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

67. An isolated nucleic acid comprising an expression construct encoding a TREM2 protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the TREM2 protein is encoded by a codon-optimized nucleic acid sequence.

68. The isolated nucleic acid of claim 67, wherein the TREM2 protein comprises the amino acid sequence set forth in SEQ ID NO: 57 or a portion thereof.

69. The isolated nucleic acid of claim 67 or 68, wherein the TREM2 protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 58.

70. The isolated nucleic acid of any one of claims 67 to 69, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

71. The isolated nucleic acid of any one of claims 67 to 70, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

72. The isolated nucleic acid of any one of claims 67 to 71, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

73. An isolated nucleic acid comprising an expression construct encoding a TMEM106B protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the TMEM106B protein is encoded by a codon-optimized nucleic acid sequence.

74. The isolated nucleic acid of claim 73, wherein the TMEM106B protein comprises the amino acid sequence set forth in SEQ ID NO: 63 or a portion thereof.

75. The isolated nucleic acid of claim 73 or 74, wherein the TMEM106B protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 64.

76. The isolated nucleic acid of any one of claims 73 to 75, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

77. The isolated nucleic acid of any one of claims 73 to 76, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

78. The isolated nucleic acid of any one of claims 73 to 77, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

79. An isolated nucleic acid comprising an expression construct encoding a Progranulin (PGRN) protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein

(i) at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29); and/or

(ii) the PGRN protein is encoded by a codon-optimized nucleic acid sequence.

80. The isolated nucleic acid of claim 79, wherein the PGRN protein comprises the amino acid sequence set forth in SEQ ID NO: 67 or a portion thereof.

81. The isolated nucleic acid of claim 79 or 80, wherein the PGRN protein is encoded by a codon-optimized nucleic acid sequence or the nucleic acid sequence set forth in SEQ ID NO: 68.

82. The isolated nucleic acid of any one of claims 79 to 81, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

83. The isolated nucleic acid of any one of claims 79 to 82, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

84. The isolated nucleic acid of any one of claims 79 to 83, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

85. An isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

86. The isolated nucleic acid of claim 85, wherein the first gene product is a Gcase protein, or a portion thereof.

87. The isolated nucleic acid of claim 85 or 86, wherein the second gene product is LIMP2 or a portion thereof, or Prosaposin or a portion thereof.

88. The isolated nucleic acid of any one of claims 85 to 87, further encoding an interfering nucleic acid (*e.g.*, shRNA, miRNA, dsRNA, *etc.*), optionally wherein the interfering nucleic acid inhibits expression of α-Syn or TMEM106B.

89. The isolated nucleic acid of any one of claims 85 to 88, further comprising one or more promoters, optionally wherein each of the one or more promoters is independently a chicken-beta actin (CBA) promoter, a CAG promoter, a CD68 promoter, or a JeT promoter.

90. The isolated nucleic acid of any one of claims 85 to 89, further comprising an internal ribosomal entry site (IRES), optionally wherein the IRES is located between the first gene product and the second gene product.

91. The isolated nucleic acid of any one of claims 85 to 90, further comprising a self-cleaving peptide coding sequence, optionally wherein the self-cleaving peptide is T2A.

92. The isolated nucleic acid of any one of claims 85 to 91, wherein the expression construct comprises two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences flanking the first gene product and the second gene product, optionally wherein one of the ITR sequences lacks a functional terminal resolution site.

93. The isolated nucleic acid of claim 92, wherein at least one of the ITRs comprises a modified "D" region relative to a wild-type AAV2 ITR (SEQ ID NO: 29).

94. The isolated nucleic acid of claim 93, wherein the modified "D" region is a "D" sequence located on the outside of the ITR relative to the expression construct.

95. The isolated nucleic acid of claim 93 or 94, wherein the ITR comprising the modified "D" sequence is a 3' ITR.

96. The isolated nucleic acid of any one of claims 85 to 95, further comprising a TRY sequence, optionally wherein the TRY sequence is set forth in SEQ ID NO: 28.

97. An isolated nucleic acid having the sequence set forth in any one of SEQ ID NOs: 1 to 78.

98. A vector comprising the isolated nucleic acid of any one of claims 1 to 97.

99. The vector of claim 98, wherein the vector is a plasmid.

100. The vector of claim 98, wherein the vector is a viral vector, optionally wherein the viral vector is a recombinant AAV (rAAV) vector or a Baculovirus vector.

101. A composition comprising the isolated nucleic acid of any one of claims 1 to 97 or the vector of any one of claims 98 to 100.

102. A host cell comprising the isolated nucleic acid of any one of claims 1 to 97 or the vector of any one of claims 98 to 100.

103. A recombinant adeno-associated virus (rAAV) comprising:

(i) a capsid protein; and

(ii) the isolated nucleic acid of any one of claims 1 to 97, or the vector of any one of claims 98 to 100.

104. The rAAV of claim 103, wherein the capsid protein is capable of crossing the blood-brain barrier, optionally wherein the capsid protein is an AAV9 capsid protein or an AAVrh.10 capsid protein.

105. The rAAV of claim 103 or claim 104, wherein the rAAV transduces neuronal cells and non-neuronal cells of the central nervous system (CNS).

106. A method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject an isolated nucleic acid of any one of claims 1 to 97, the vector of any one of claims 98 to 100, the composition of claim 101, or the rAAV of any one of claims 103 to 105.

107. The method of claim 106, wherein the administration comprises direct injection to the CNS of the subject, optionally wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection or any combination thereof.

108. The method of claim 107, wherein the direct injection to the CNS of the subject comprises convection enhanced delivery (CED).

109. The method of any one of claims 106 to 108, wherein the administration comprises peripheral injection, optionally wherein the peripheral injection is intravenous injection.

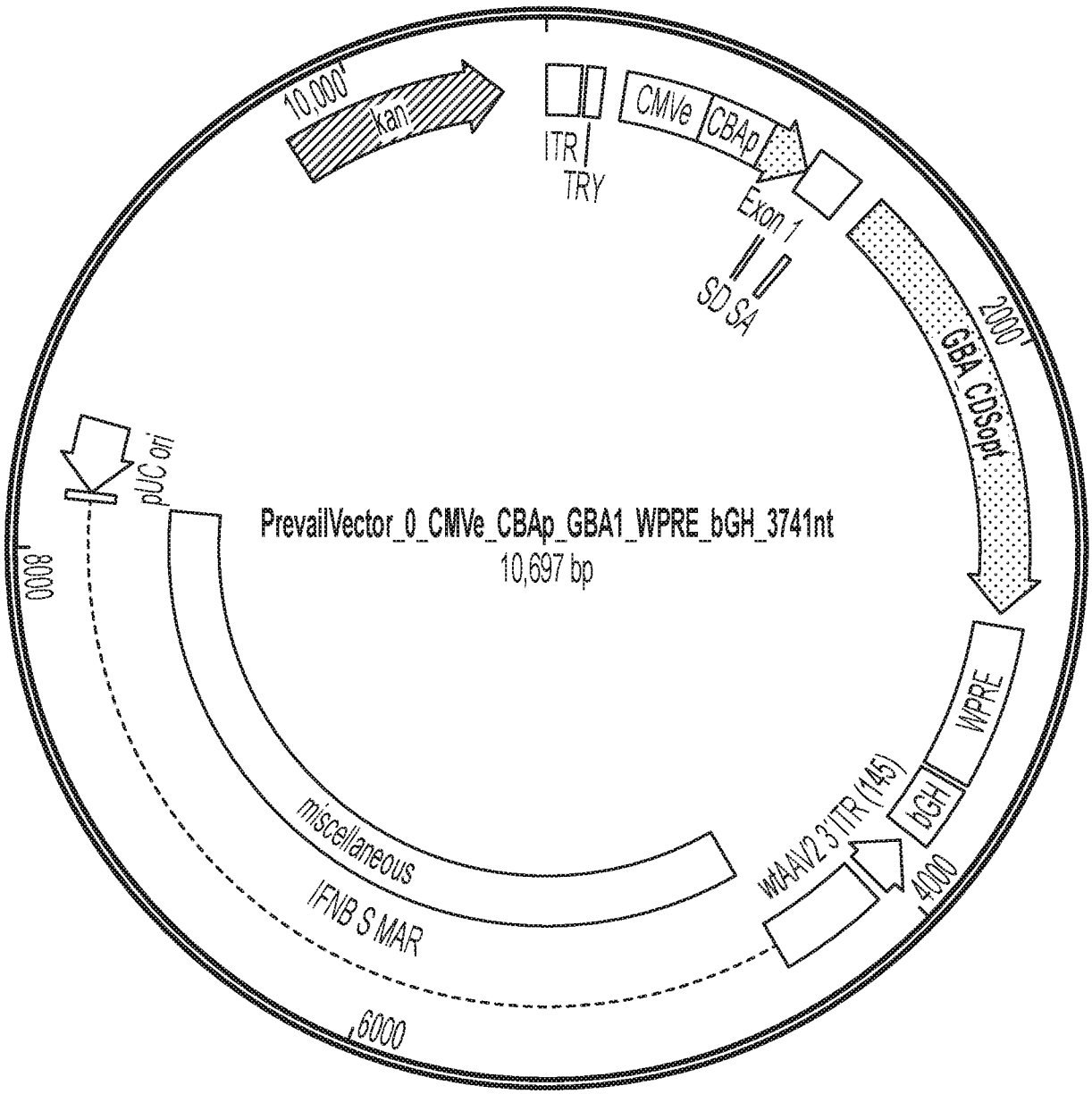

10,000
kan
ITR
TRY
CMVe
CBAp
Exon 1
SD
SA
2000
GBA CDSopt
WPRE
bGH
wtAAV2 3'ITR(145)
4000
6000
8000
pUC ori
miscellaneous
IFNB S MAR
PrevailVector_0_CMVe_CBAp_GBA1_WPRE_bGH_3741nt
10,697 bp

Figure 1

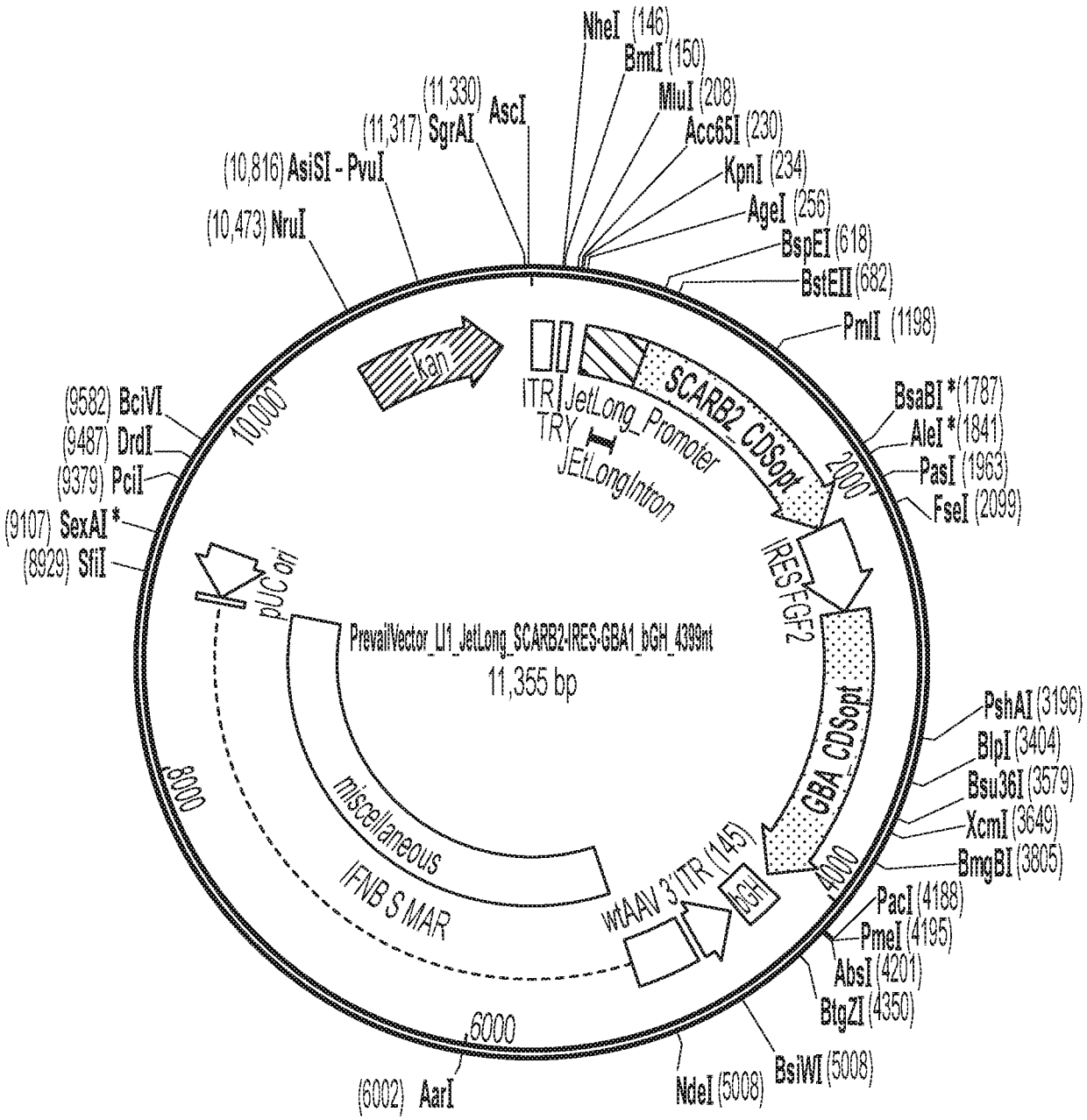
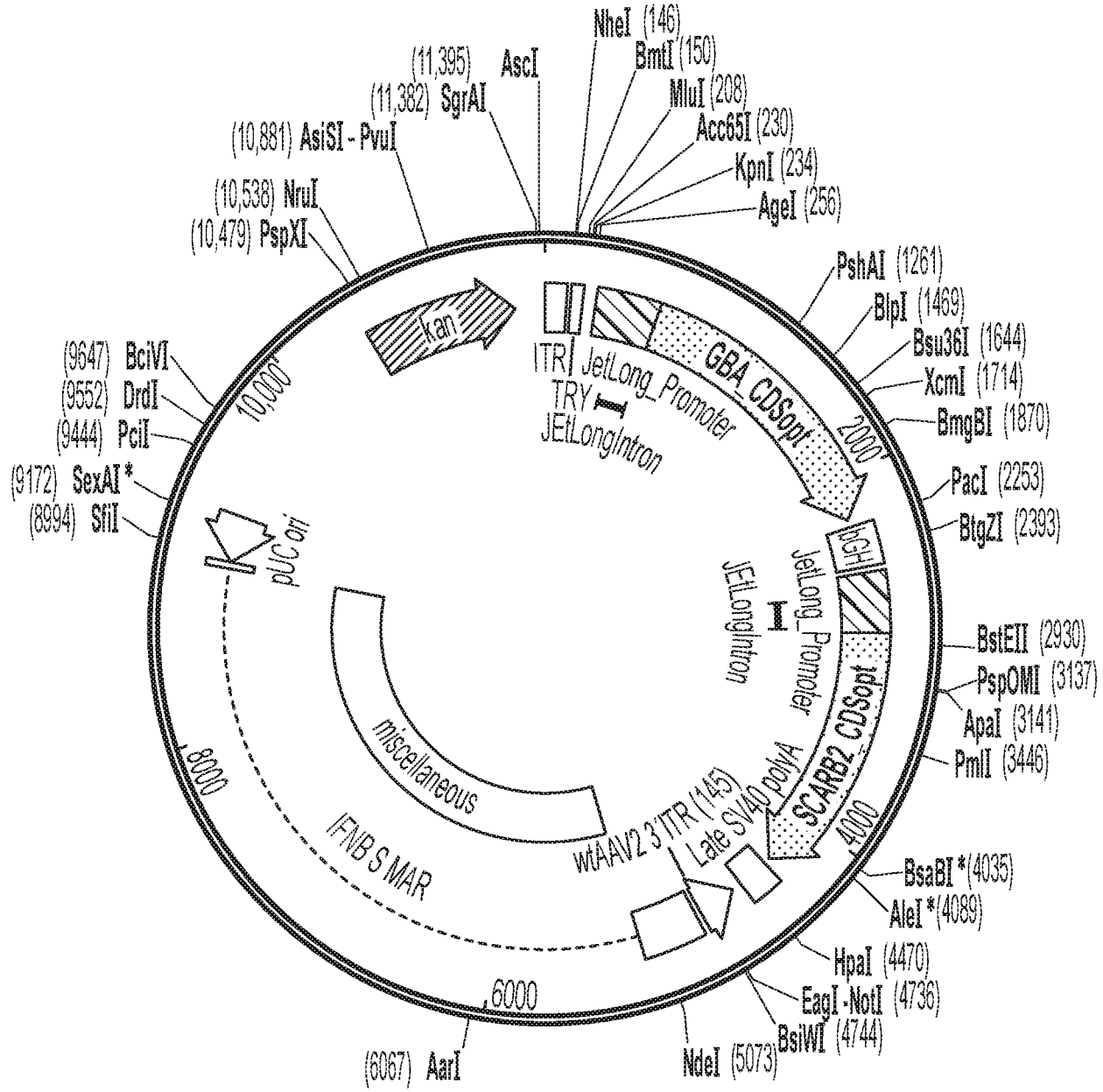

NheI (146)
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
BstEII (682)
PmlI (1198)
BsaBI *(1787)
AleI *(1841)
PasI (1963)
FseI (2099)
PshAI (3196)
BlpI (3404)
Bsu36I (3579)
XcmI (3649)
BmgBI (3805)
PacI (4188)
PmeI (4195)
AbsI (4201)
BtgZI (4350)
BsiWI (5008)
NdeI (5008)
(6002) 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
TRY
JetLong_Promoter
JETLongIntron
SCARB2_CDSopt
IRES
FGF2
GBA_CDSopt
bGH
wtAAV 3'ITR (145)
pUC ori
miscellaneous
IFNB S MAR
PrevailVector_LI1_JetLong_SCARB2-IRES-GBA1_bGH_4399nt
11,355 bp
2000
4000
6000
8000
10,000

Figure 2



PrevailVector_FP1_JetLong_GBA1_bGH_JetLong_SCARB2_SV40L_4464nt
11,420 bp

Figure 3

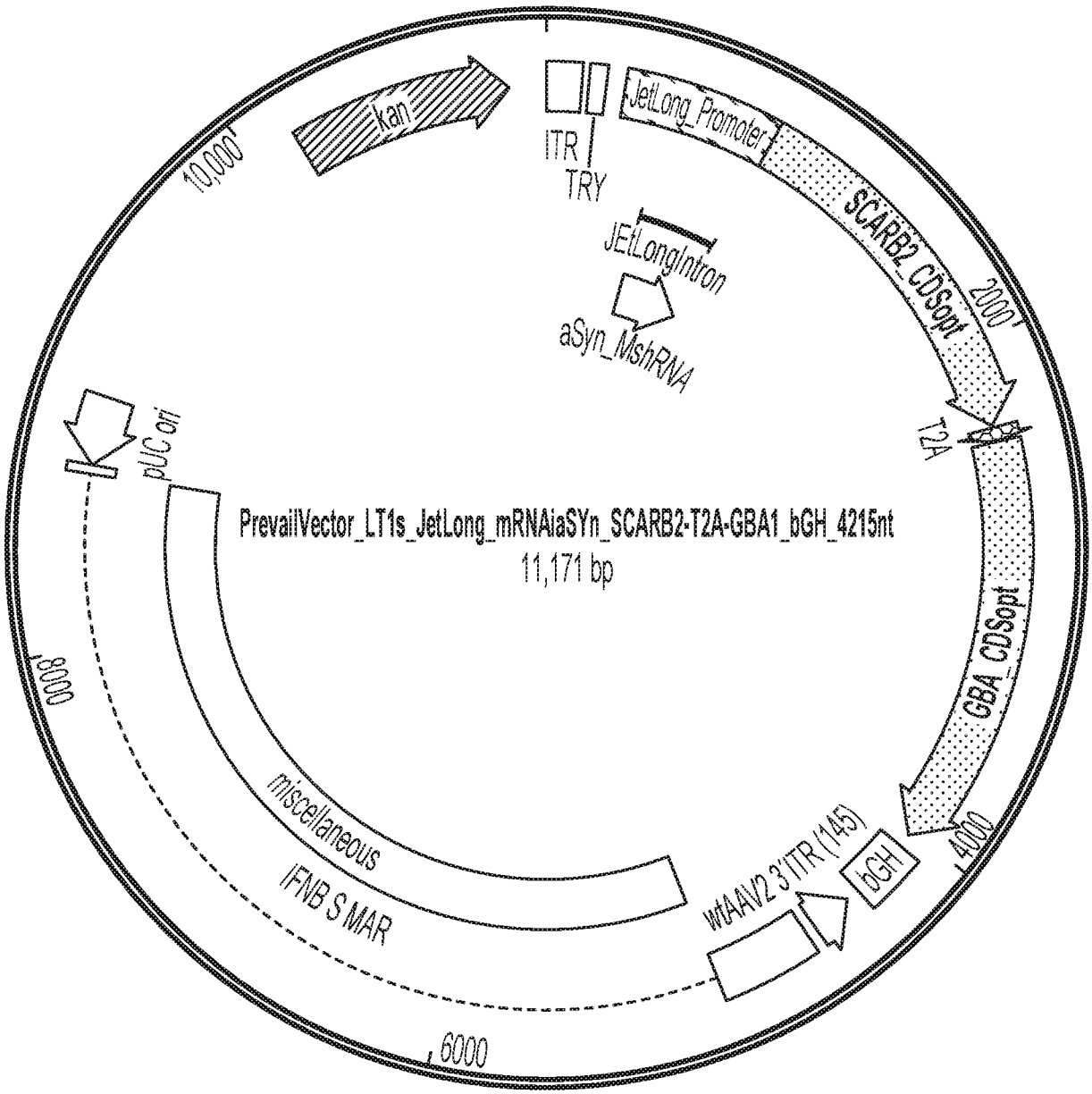

Kan
ITR
TRY
JetLong_Promoter
SCARB2_CDSopt
2000
T2A
GBA_CDSopt
4000
bGH
wtAAV2 3'ITR (145)
6000
IFNB S MAR
miscellaneous
8000
pUC ori
10,000
JEtLongIntron
aSyn_MshRNA
PrevailVector_LT1s_JetLong_mRNAiaSYn_SCARB2-T2A-GBA1_bGH_4215nt
11,171 bp

Figure 4

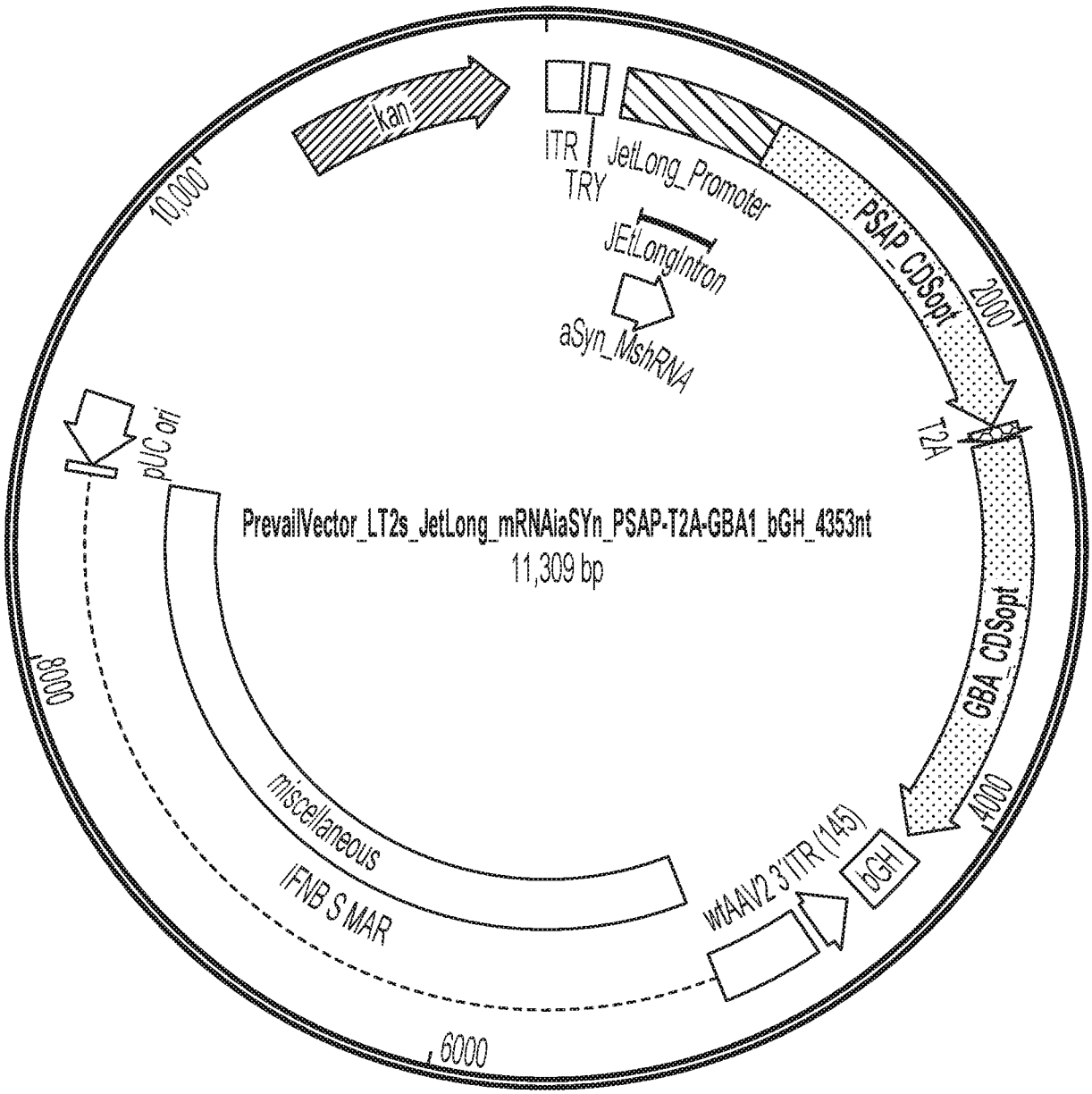

kan
ITR
TRY
JetLong_Promoter
JEtLongIntron
aSyn_MshRNA
PSAP_CDSopt
T2A
GBA_CDSopt
bGH
wtAAV2 3'ITR (145)
IFNB S MAR
miscellaneous
pUC ori
PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-T2A-GBA1_bGH_4353nt
11,309 bp
10,000
2000
4000
6000
8000

Figure 5

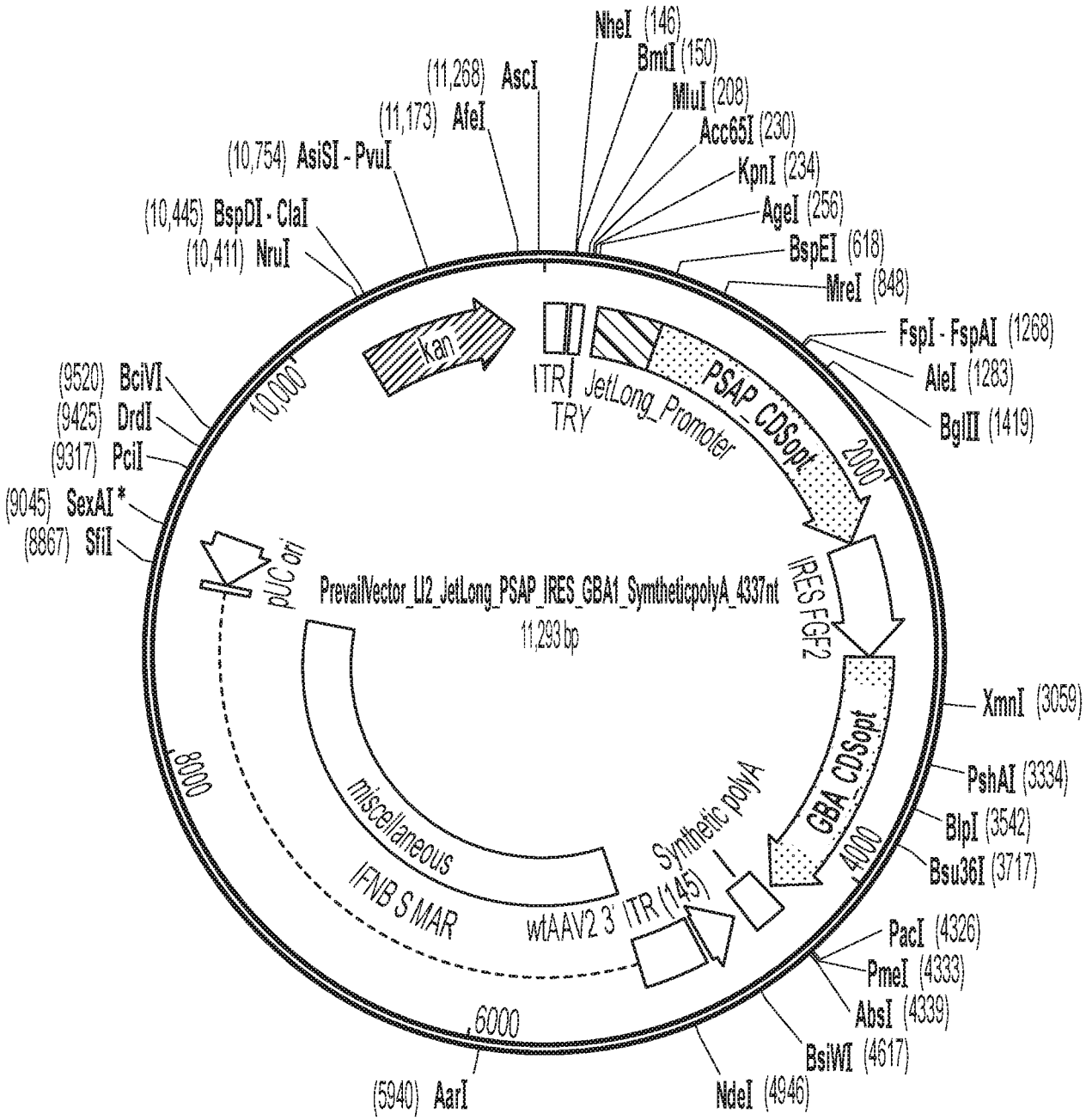

NheI (146)
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
MreI (848)
FspI - FspAI (1268)
AleI (1283)
BglII (1419)
XmnI (3059)
PshAI (3334)
BlpI (3542)
Bsu36I (3717)
PacI (4326)
PmeI (4333)
AbsI (4339)
BsiWI (4617)
NdeI (4946)
(5940) AarI
(9520) BciVI
(9425) DrdI
(9317) PciI
(9045) SexAI*
(8867) SfiI
(10,445) BspDI - ClaI
(10,411) NruI
(10,754) AsiSI - PvuI
(11,173) AfeI
(11,268) AscI
Kan
ITR
TRY
JetLong_Promoter
PSAP_CDSopt
IRES FGF2
GBA_CDSopt
Synthetic polyA
wtAAV2 3' ITR (145)
IFNB S MAR
miscellaneous
pUC ori
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1_SyntheticpolyA_4337nt
11,293 bp
2000
4000
6000
8000
10,000

Figure 6

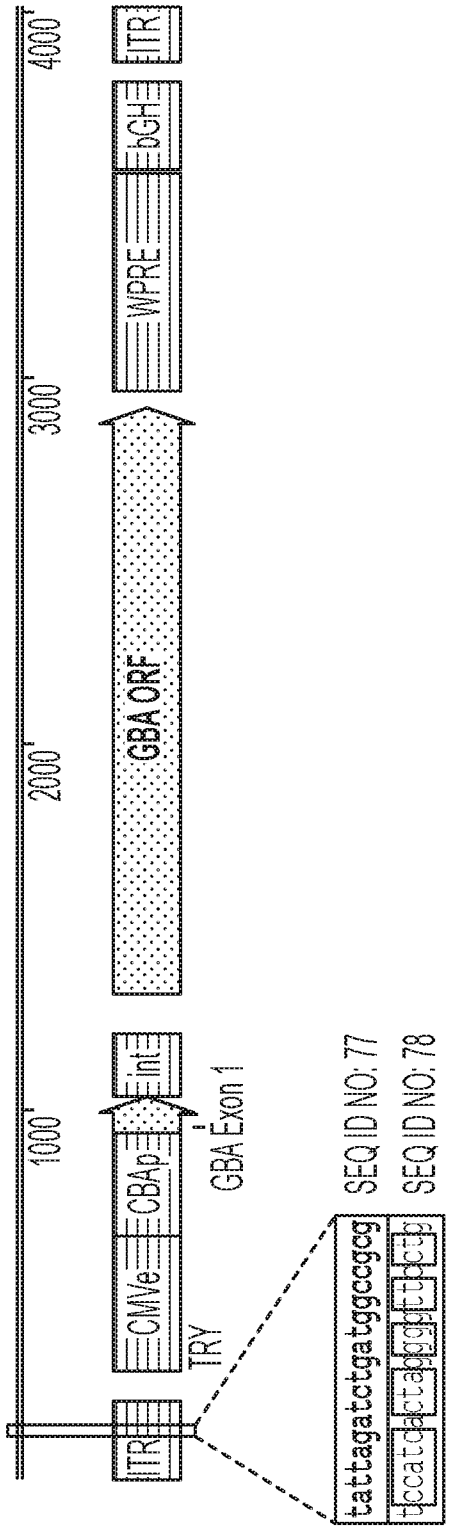

1000'
2000'
3000'
4000'
ITR
CMVe
CBAp
Int
TRY
GBA Exon 1
GBA ORF
WPRE
bGH
ITR
SEQ ID NO: 77
SEQ ID NO: 78

Figure 7

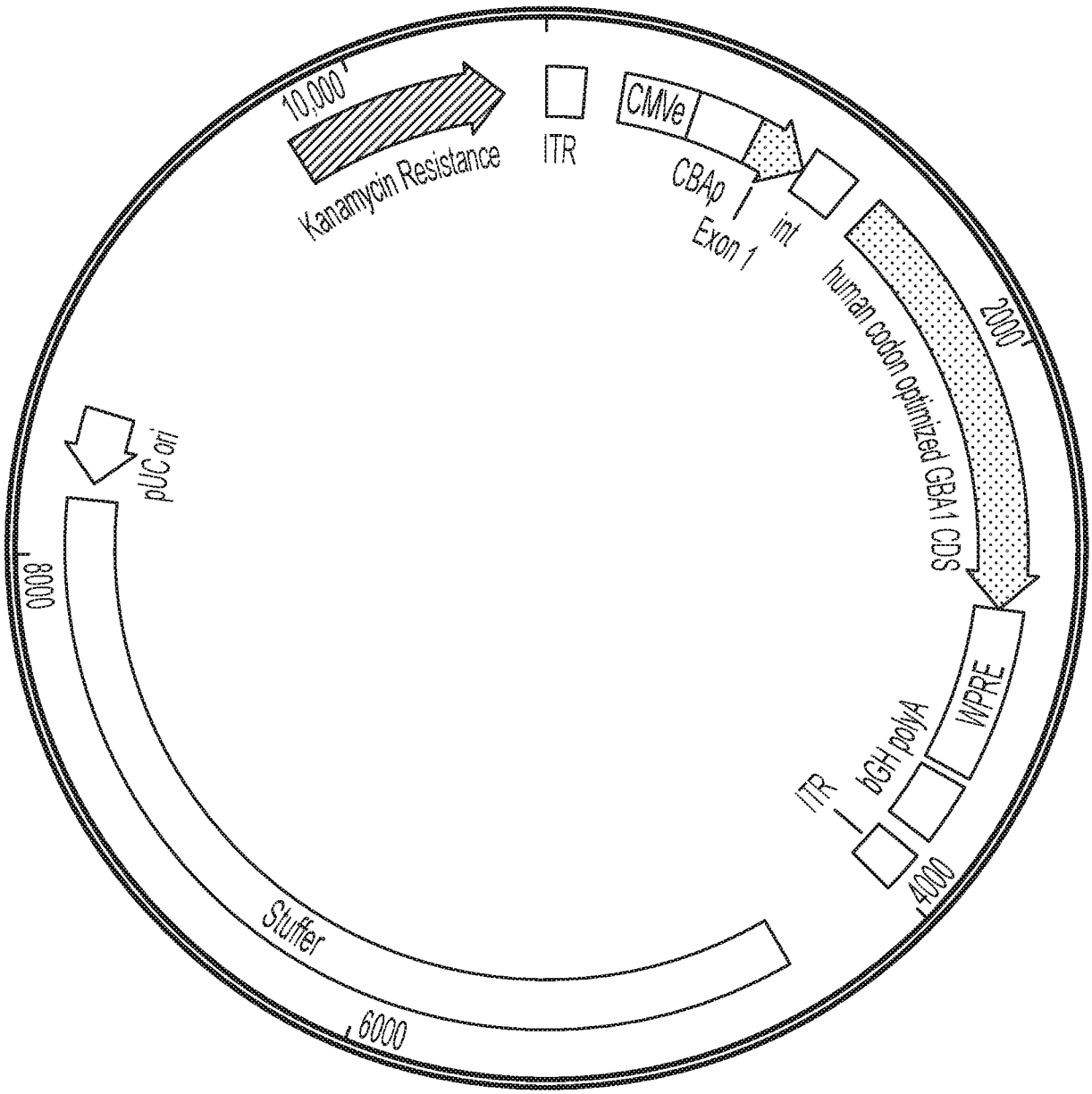

10,000
Kanamycin Resistance
ITR
CMVe
CBAp
Exon 1
int
human codon optimized GBA1 CDS
2000
WPRE
bGH polyA
ITR
4000
6000
Stuffer
8000
pUC ori

Figure 8

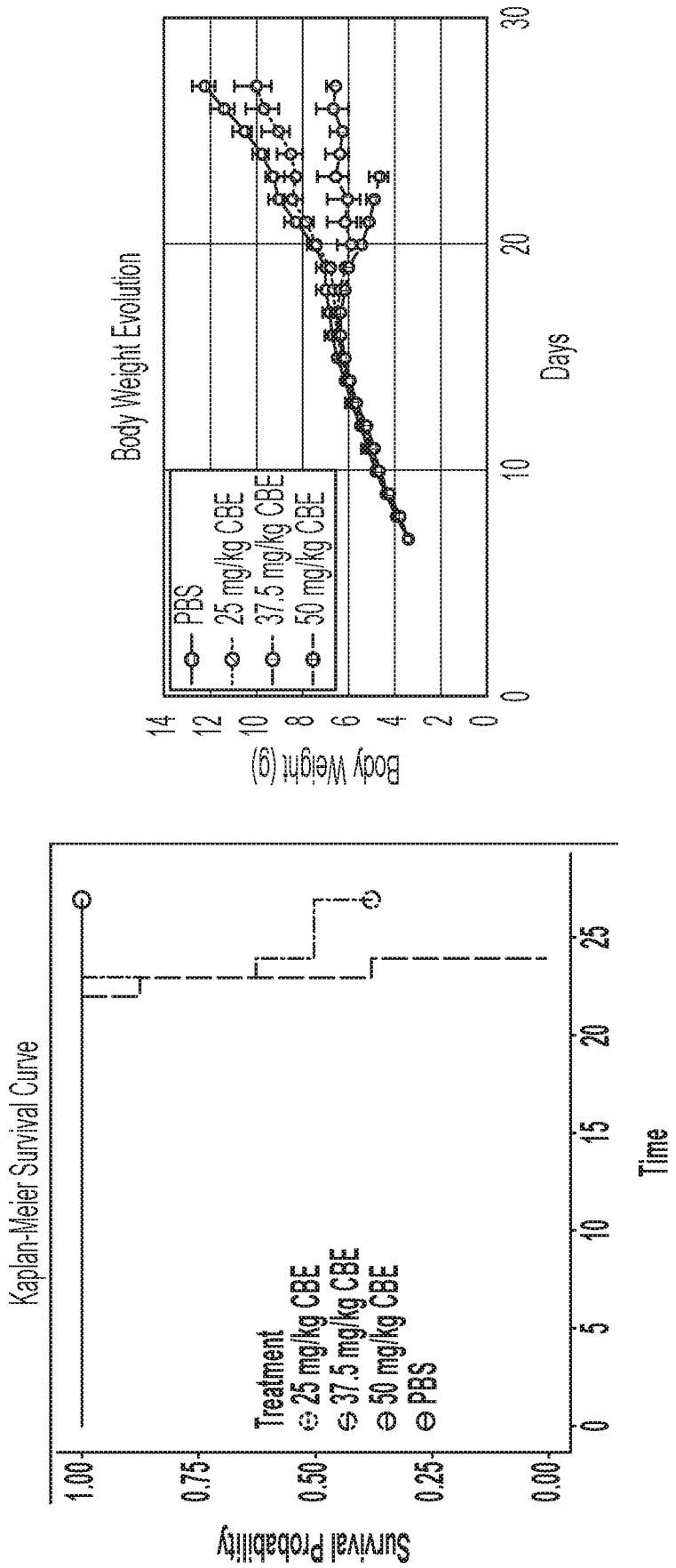

Body Weight Evolution
Body Weight (g)
Days
PBS
25 mg/kg CBE
37.5 mg/kg CBE
50 mg/kg CBE
Kaplan-Meier Survival Curve
Survival Probability
Time
Treatment
25 mg/kg CBE
37.5 mg/kg CBE
50 mg/kg CBE
PBS

Figure 9

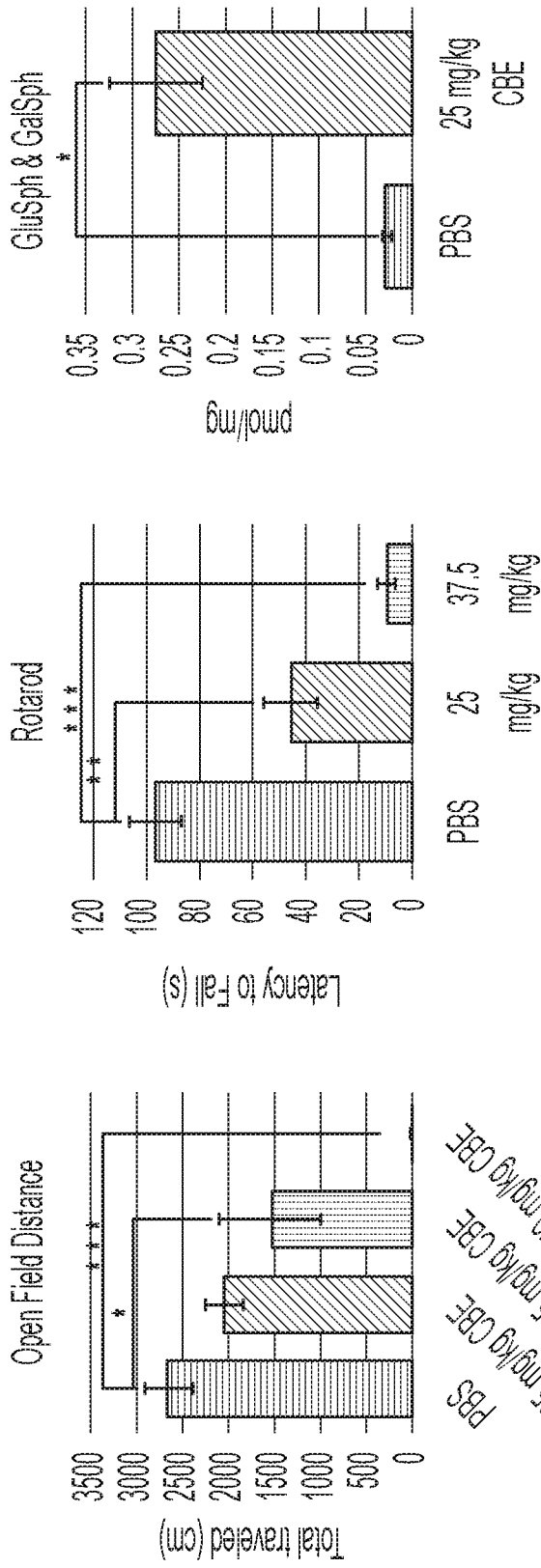
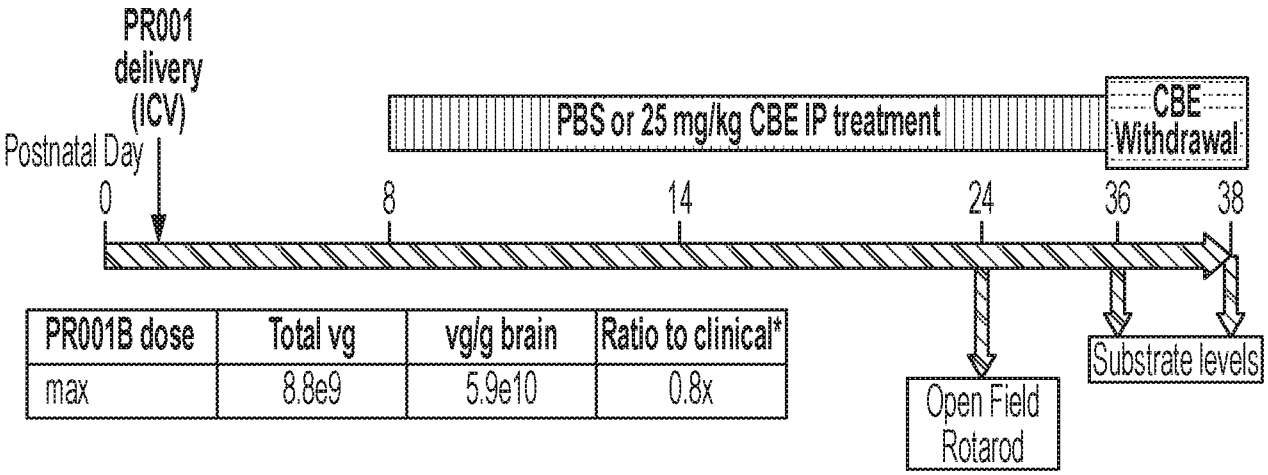


Figure 9 cont.



PR001B dose	Total vg	vg/g brain	Ratio to clinical*
max	8.8e9	5.9e10	0.8x

Figure 10

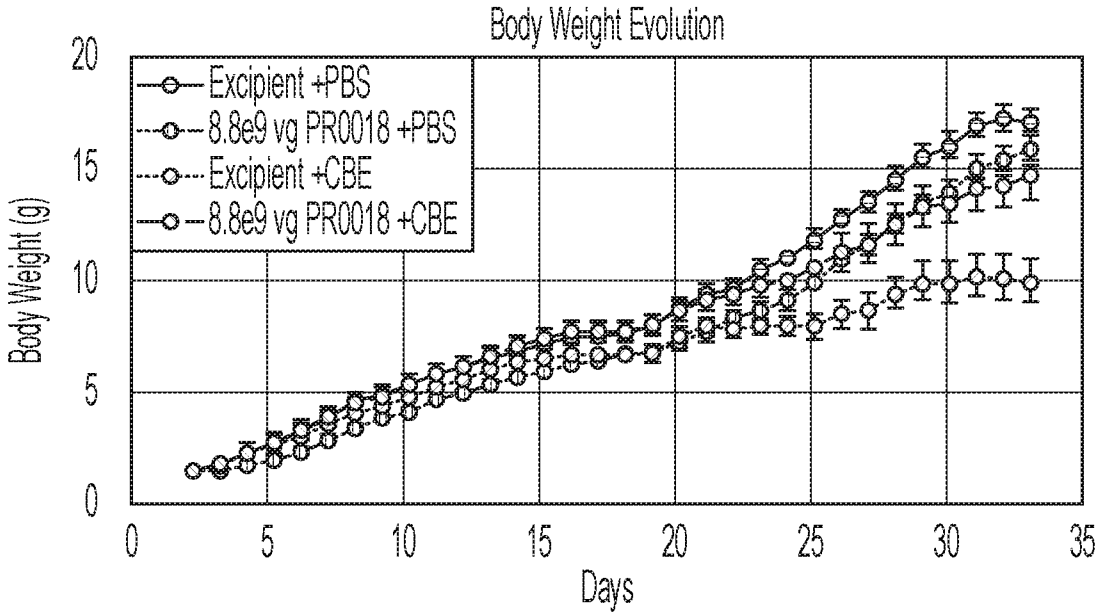
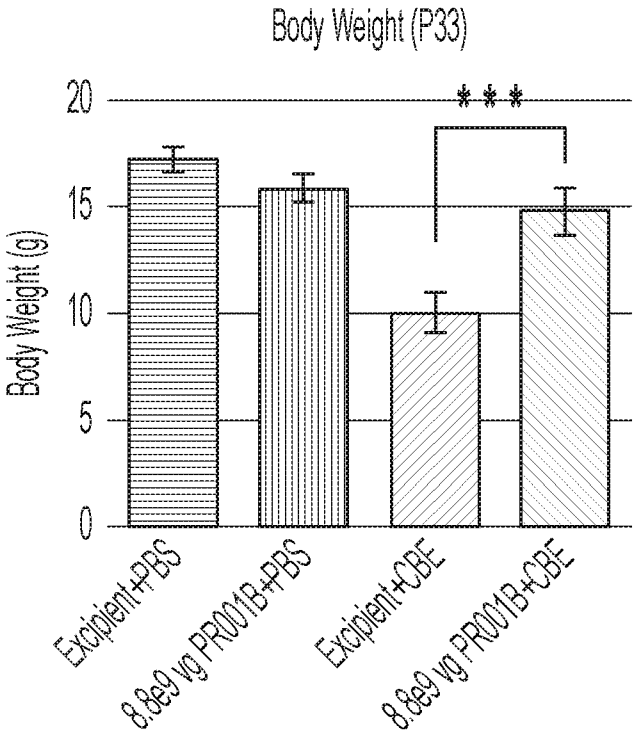


Figure 11



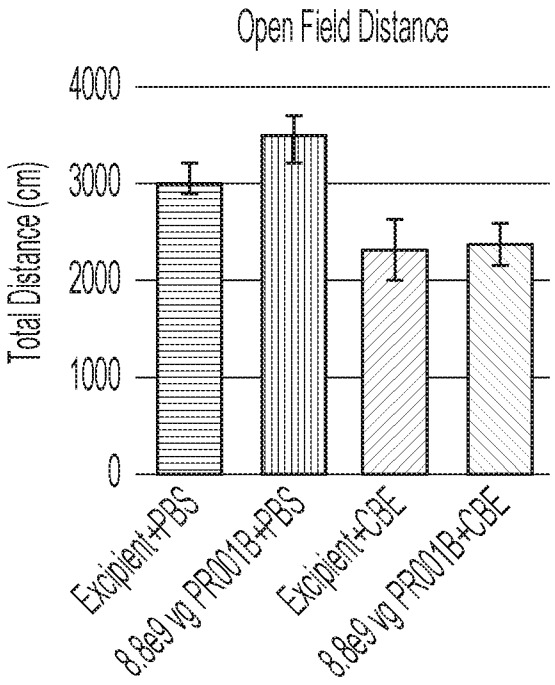


Figure 11 cont.

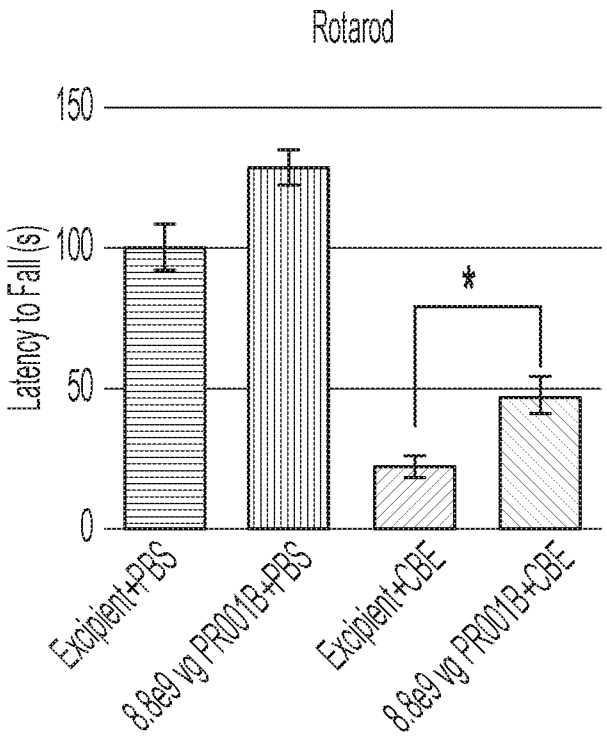


Figure 11 cont.

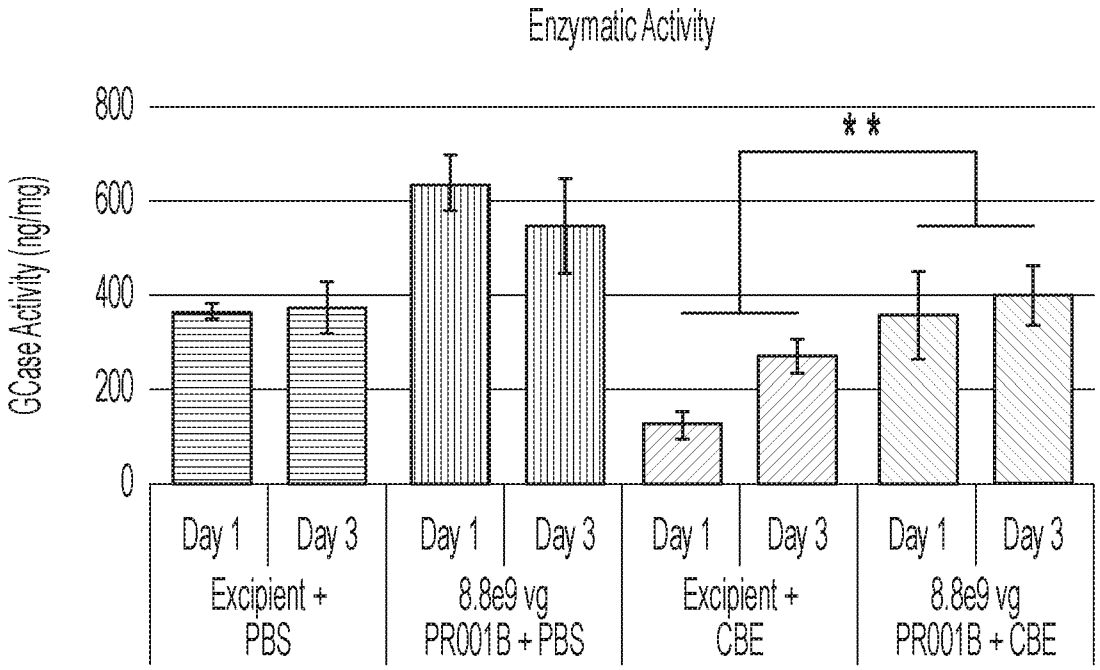


Figure 12

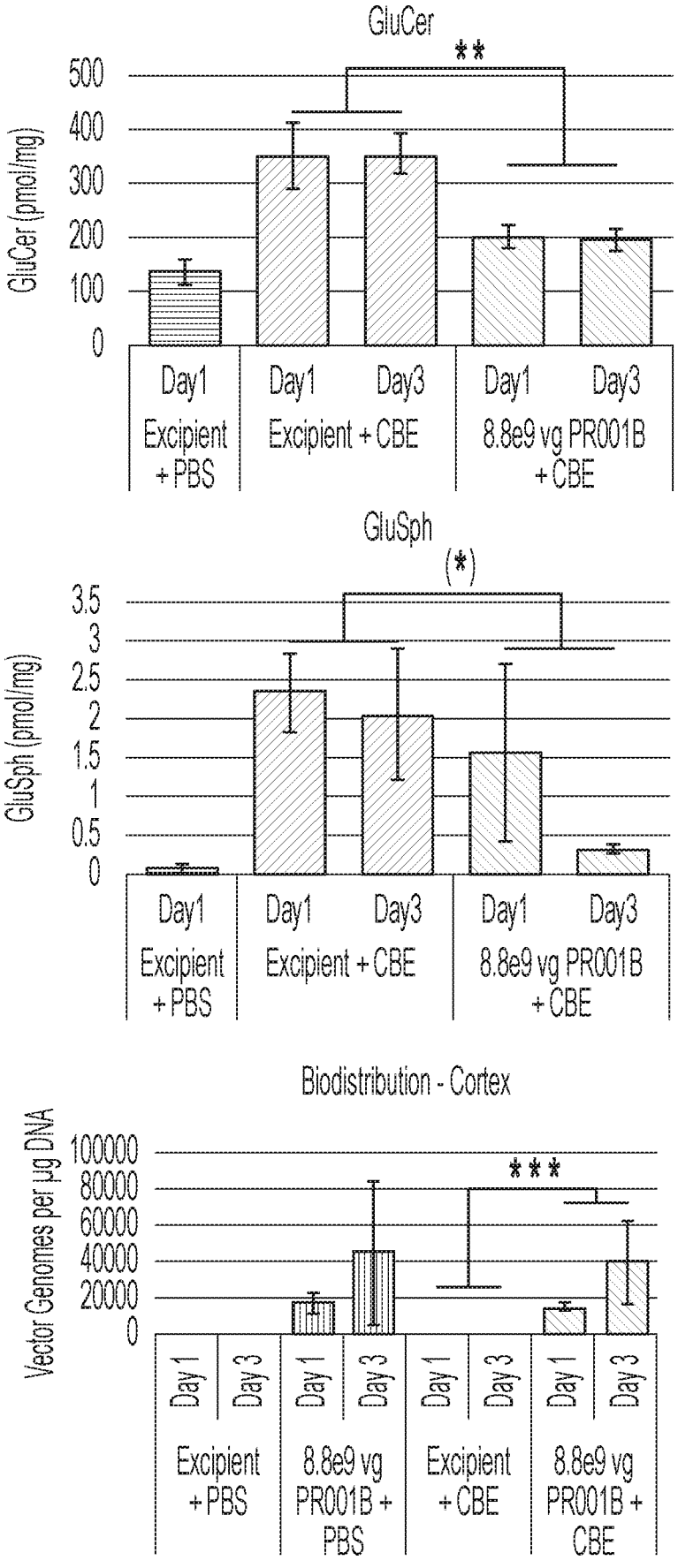


Figure 12 cont.

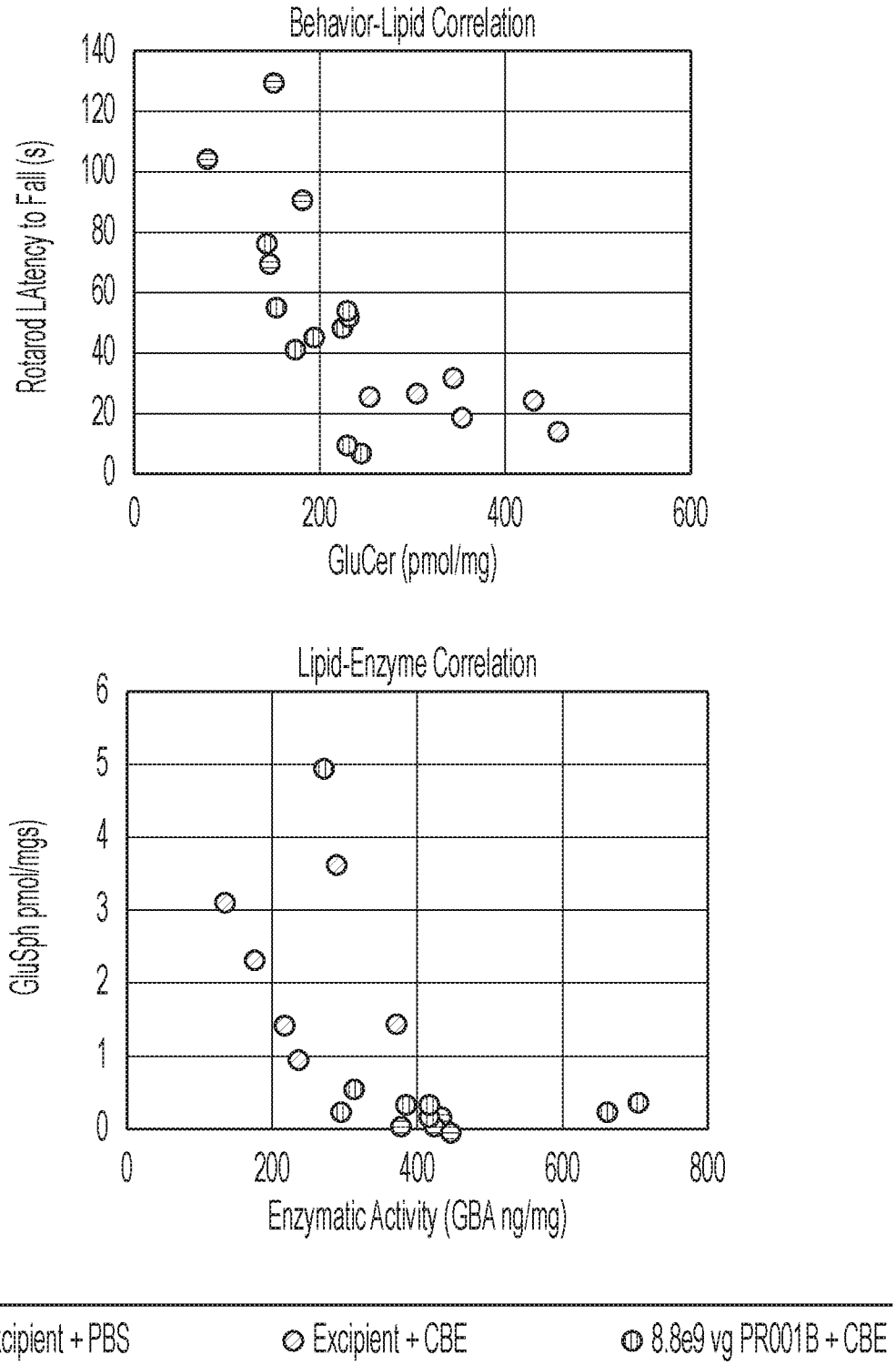

Behavior-Lipid Correlation
Rotarod LAtency to Fall (s)
140
120
100
80
60
40
20
0
0
200
400
600
GluCer (pmol/mg)
Lipid-Enzyme Correlation
GluSph pmol/mgs)
6
5
4
3
2
1
0
0
200
400
600
800
Enzymatic Activity (GBA ng/mg)
Excipient + PBS
Excipient + CBE
8.8e9 vg PR001B + CBE

Figure 13

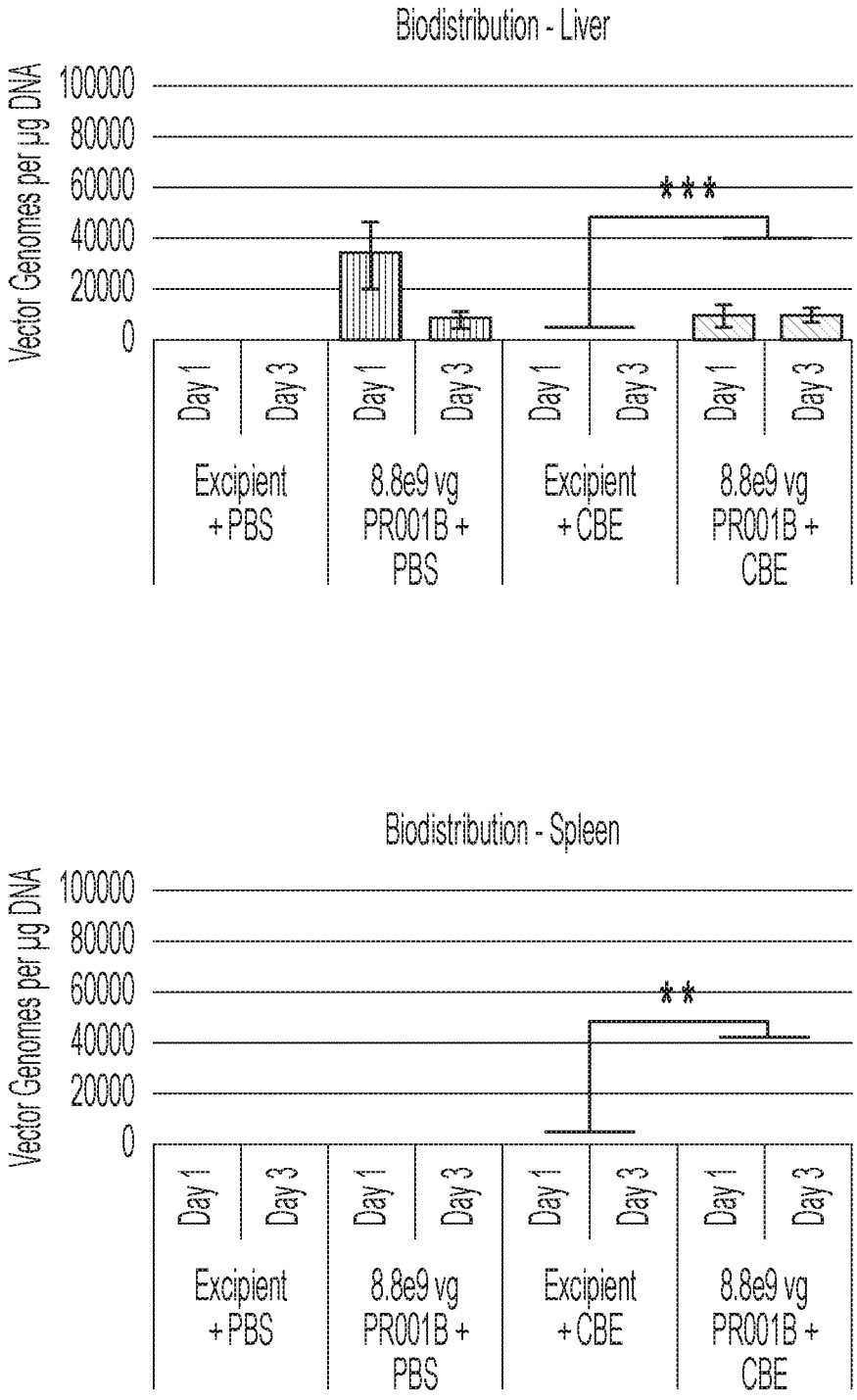
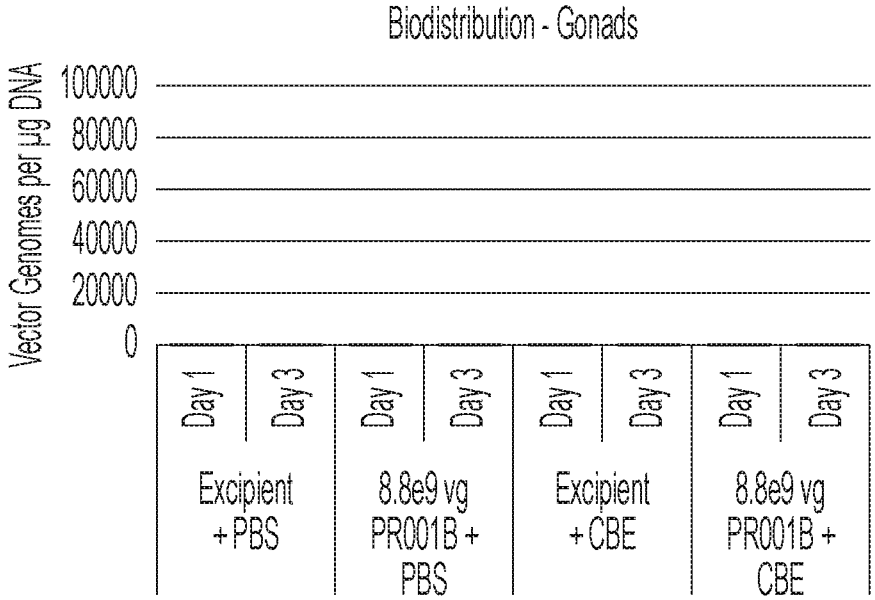

Biodistribution - Liver
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
***
Day 1
Day 3
Excipient + PBS
8.8e9 vg PR001B + PBS
Excipient + CBE
8.8e9 vg PR001B + CBE
Biodistribution - Spleen
**

Figure 14



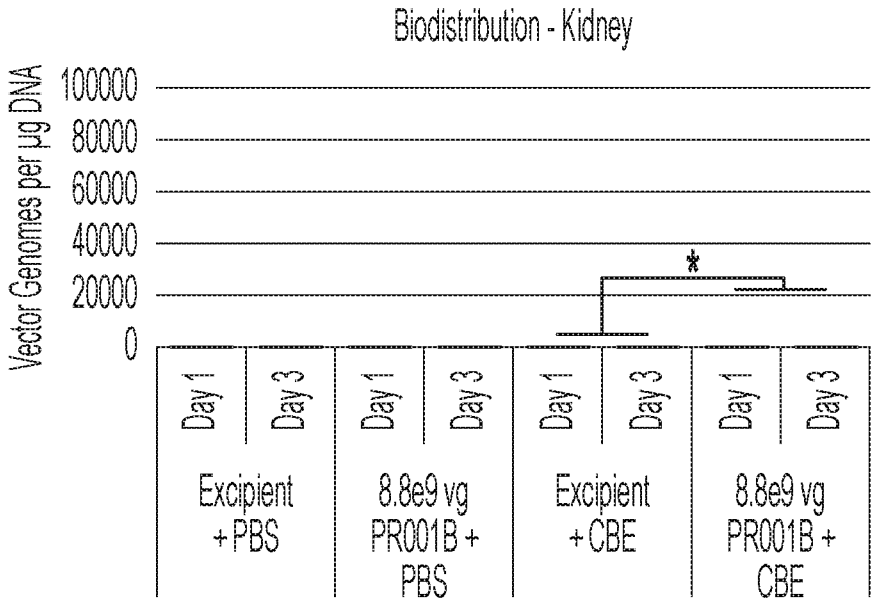
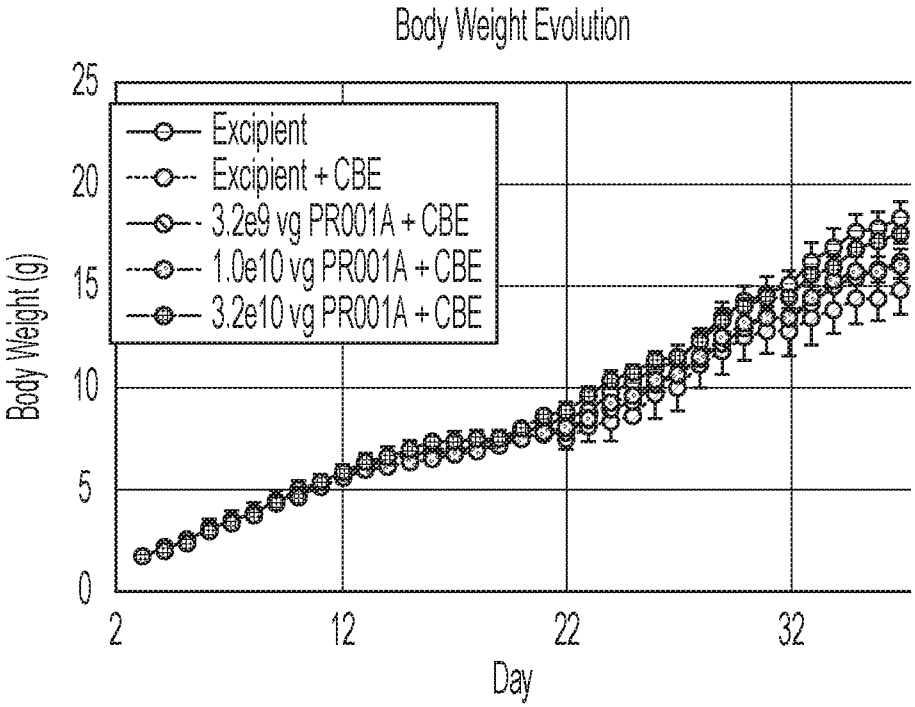


Figure 14 cont.


Body Weight Evolution
Body Weight (g)
25
20
15
10
5
0
2
12
22
32
Day
Excipient
Excipient + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

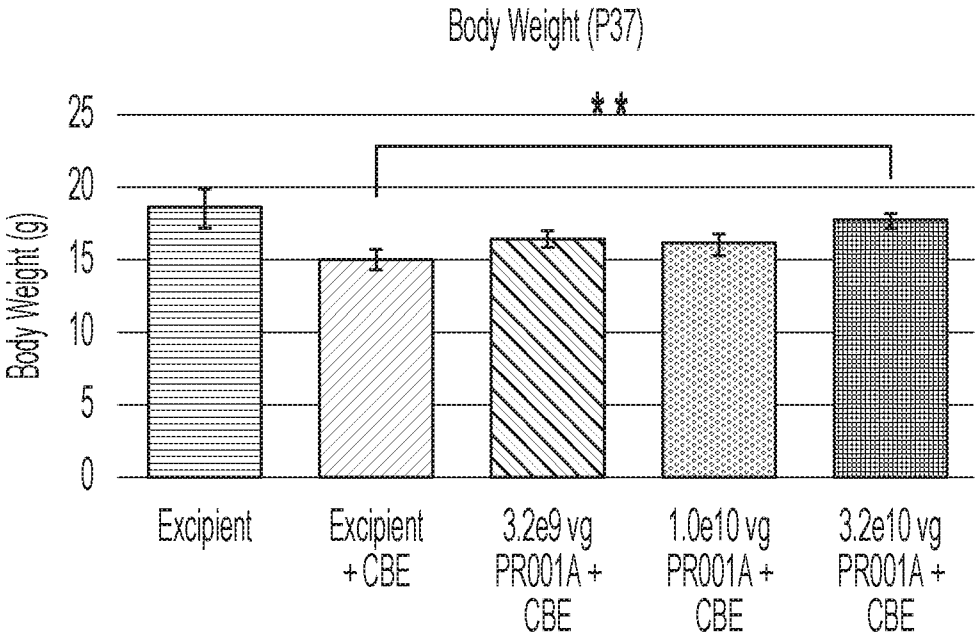

Body Weight (P37)
Body Weight (g)
25
20
15
10
5
0
**
Excipient
Excipient + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE

Figure 15

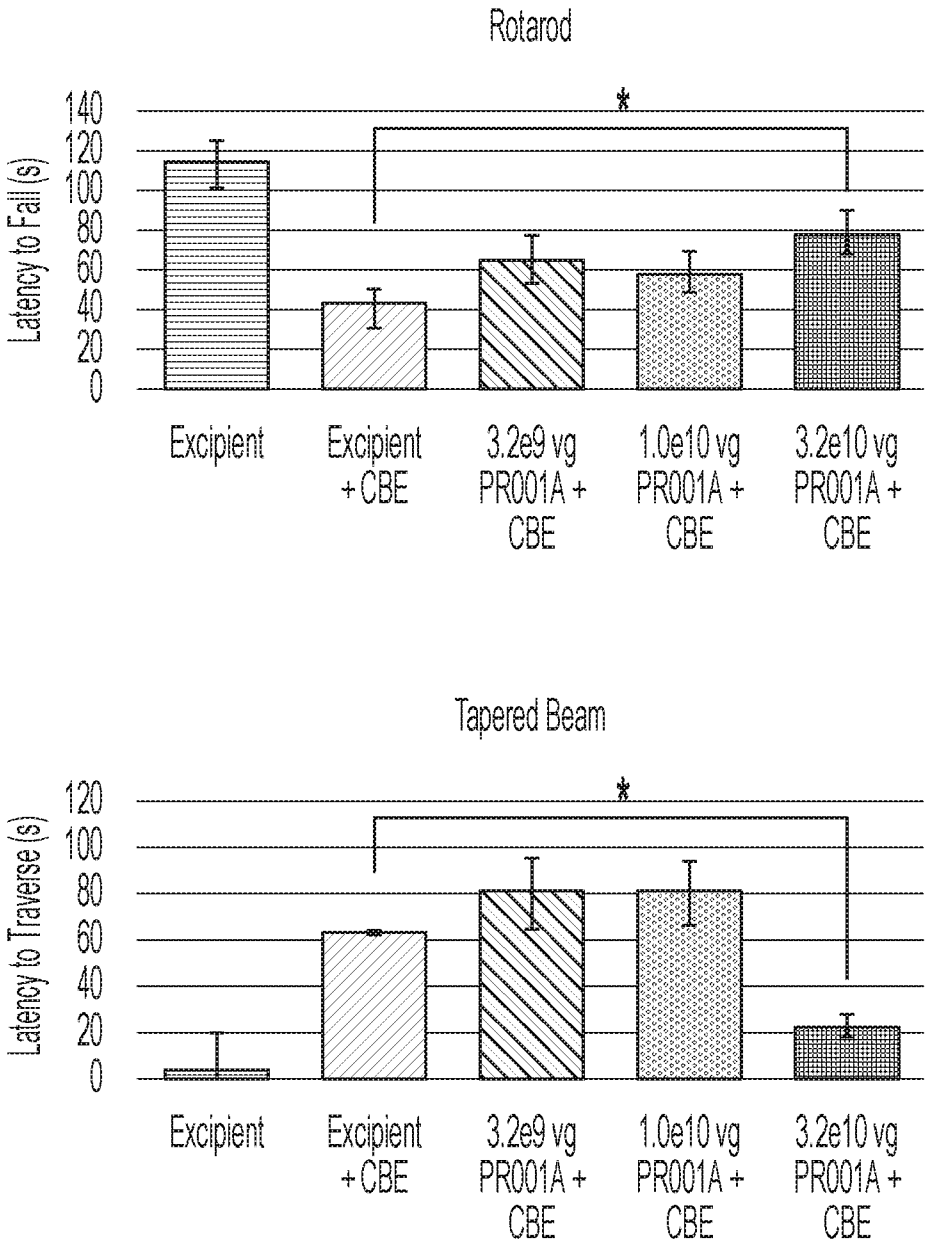


Figure 15 cont.

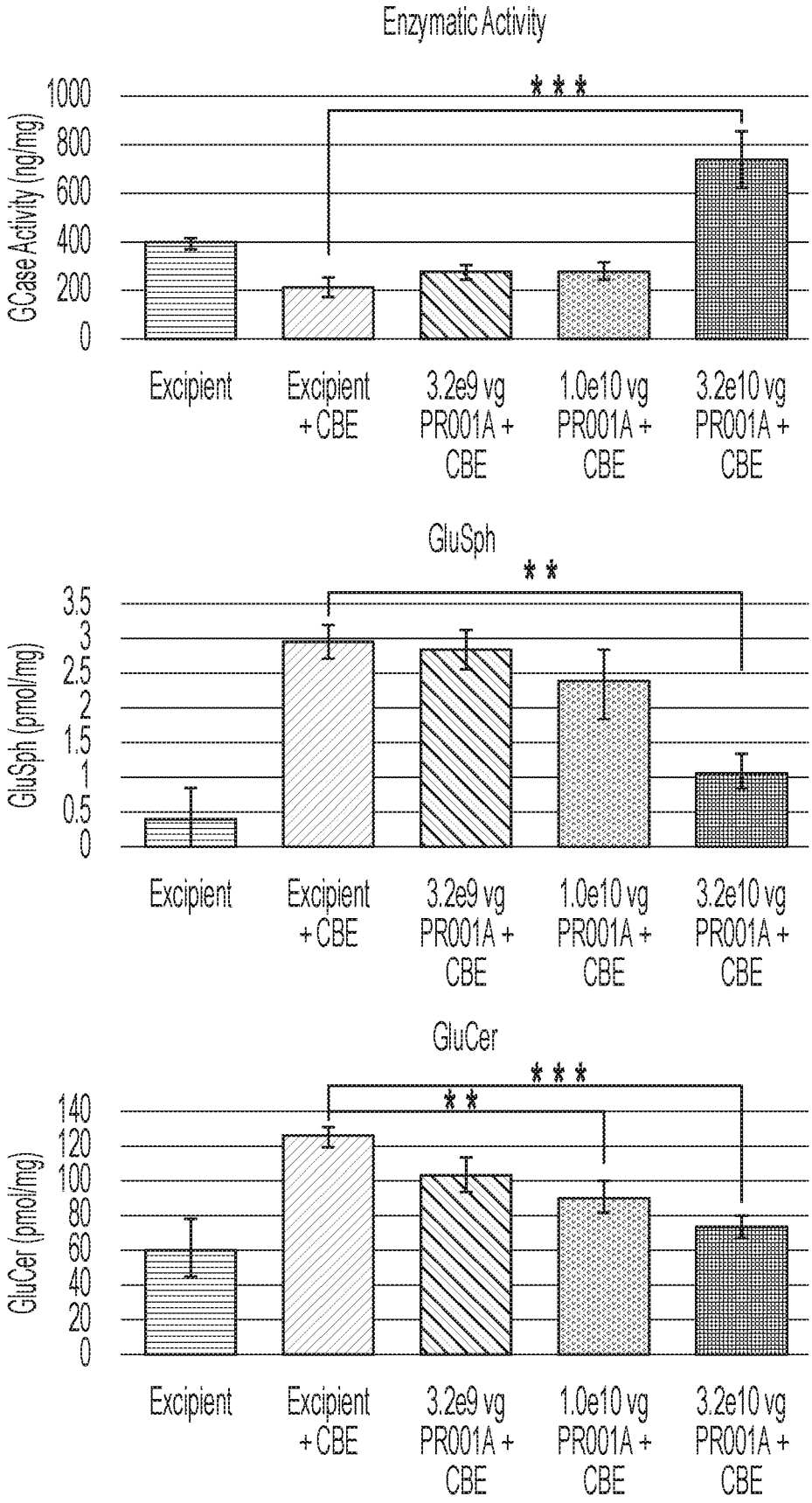

Enzymatic Activity
GCase Activity (ng/mg)
1000
800
600
400
200
0
***
Excipient
Excipient + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A + CBE
GluSph
GluSph (pmol/mg)
3.5
3
2.5
2
1.5
1
0.5
0
**
Excipient
Excipient + CBE
3.2e9 vg PR001A + CBE
1.0e10 vg PR001A + CBE
3.2e10 vg PR001A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

Figure 16

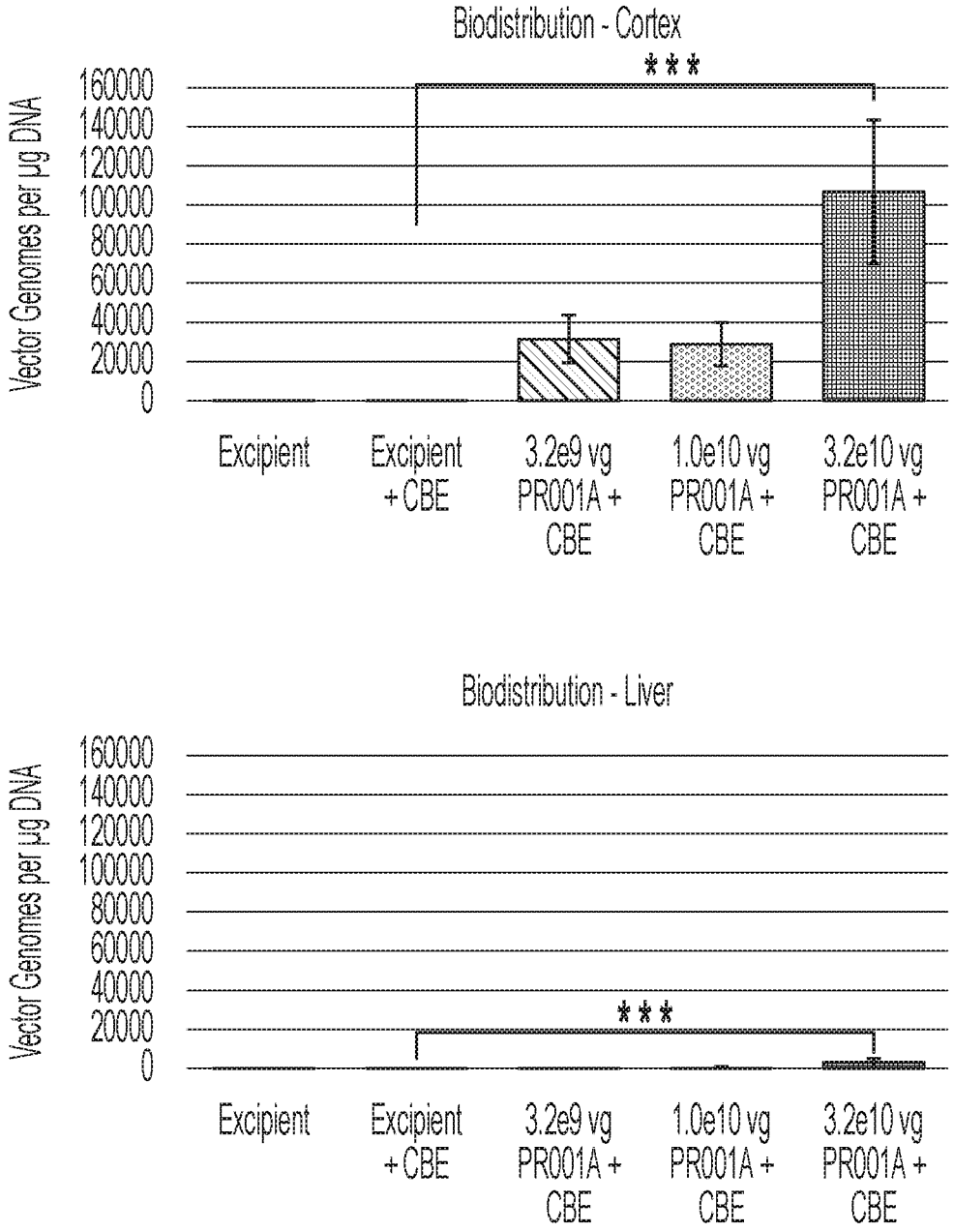


Figure 16 cont.

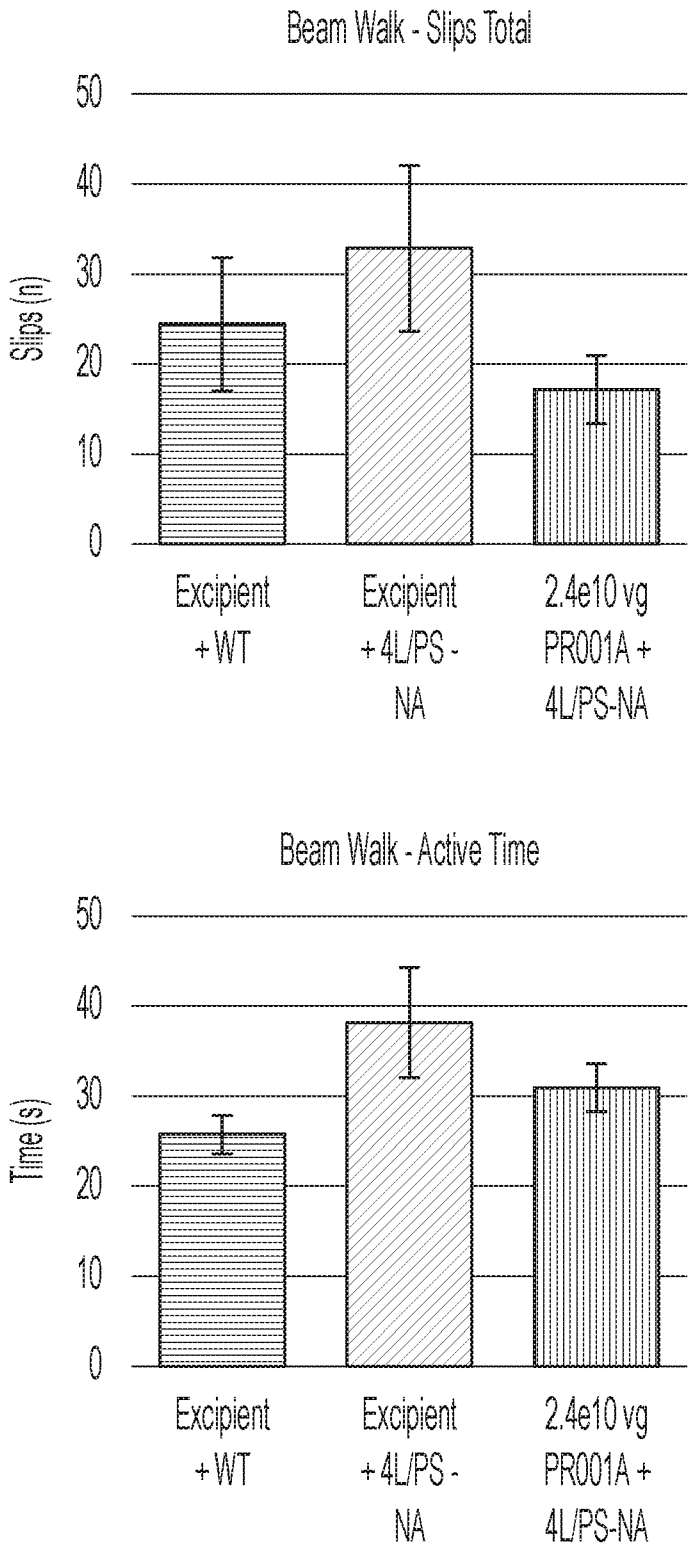

Beam Walk - Slips Total
Slips (n)
50
40
30
20
10
0
Excipient + WT
Excipient + 4L/PS - NA
2.4e10 vg PR001A + 4L/PS-NA
Beam Walk - Active Time
Time (s)
50
40
30
20
10
0
Excipient + WT
Excipient + 4L/PS - NA
2.4e10 vg PR001A + 4L/PS-NA

Figure 17

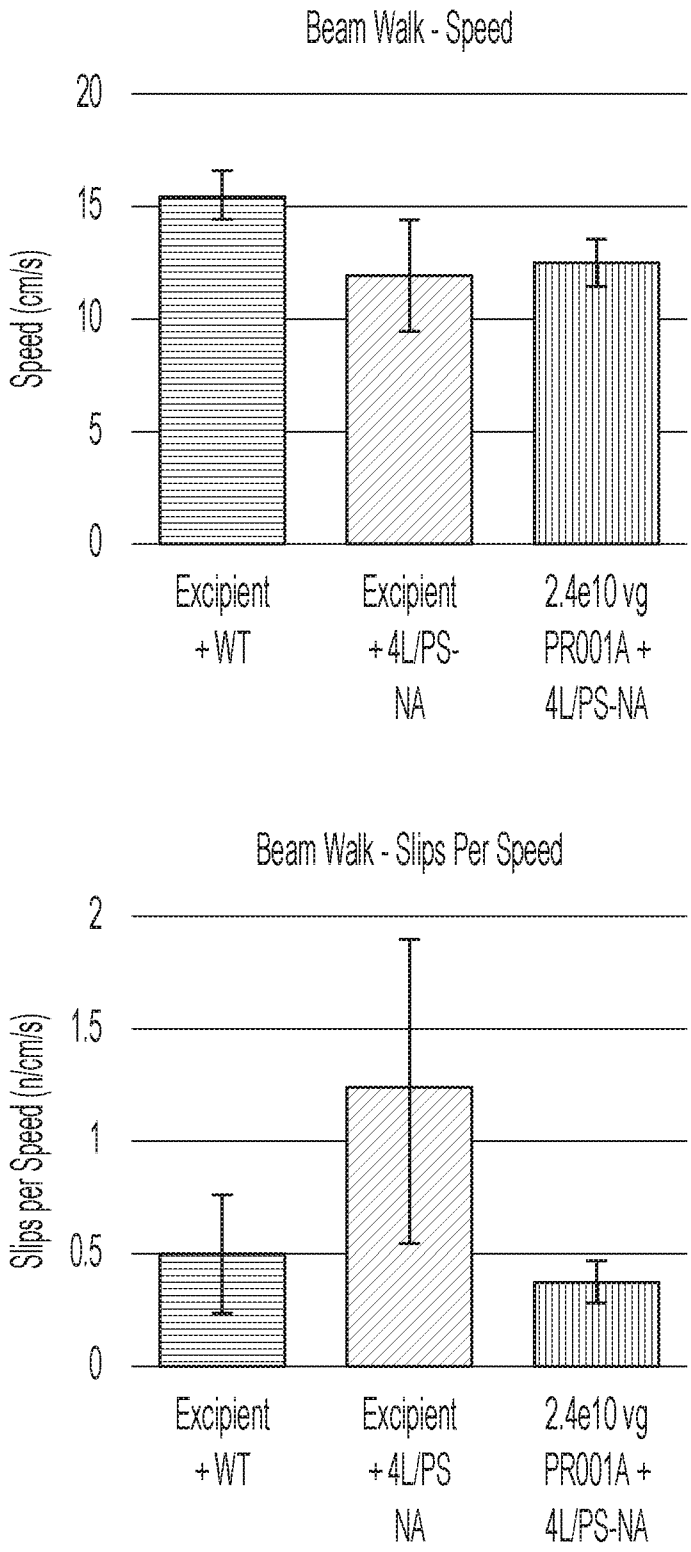
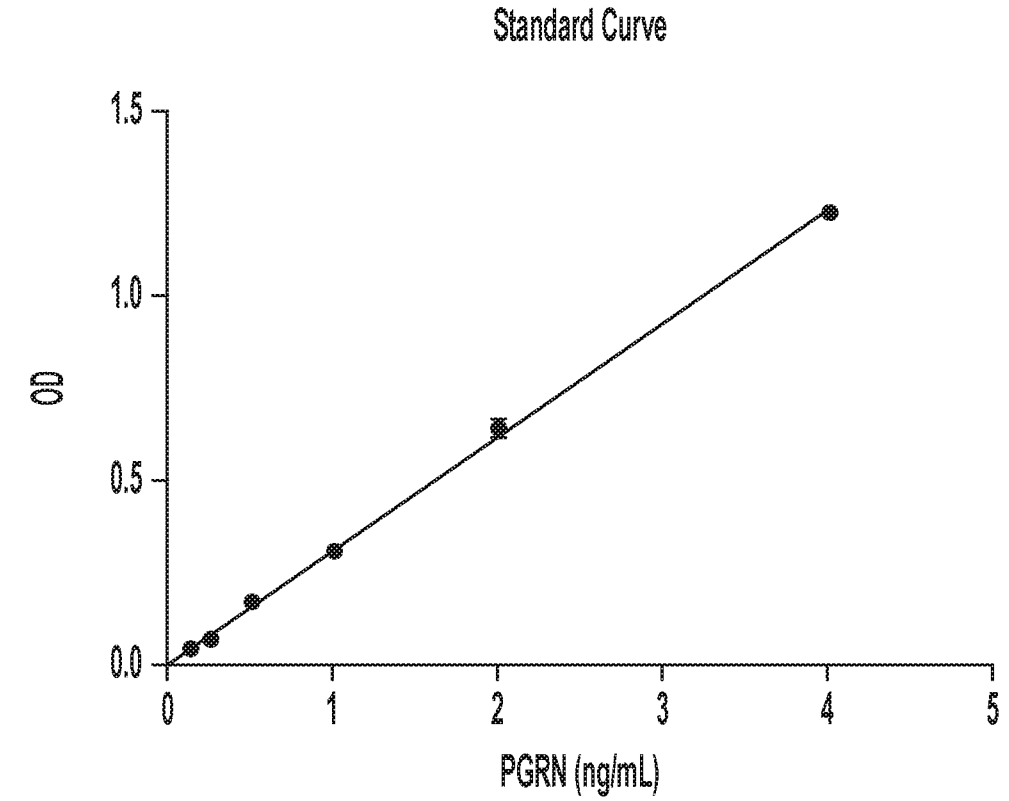


Figure 17 cont.


Standard Curve
OD
1.5
1.0
0.5
0.0
0
1
2
3
4
5
PGRN (ng/mL)

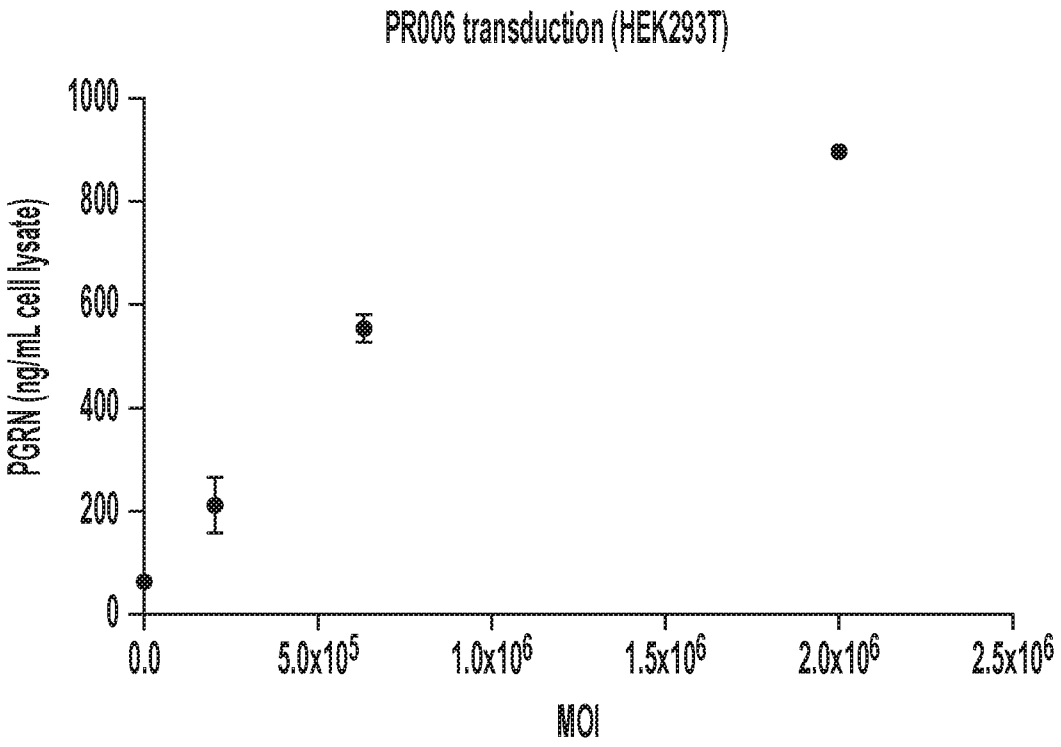

PR006 transduction (HEK293T)
PGRN (ng/mL cell lysate)
1000
800
600
400
200
0
0.0
5.0x10^5
1.0x10^6
1.5x10^6
2.0x10^6
2.5x10^6
MOI

Figure 18

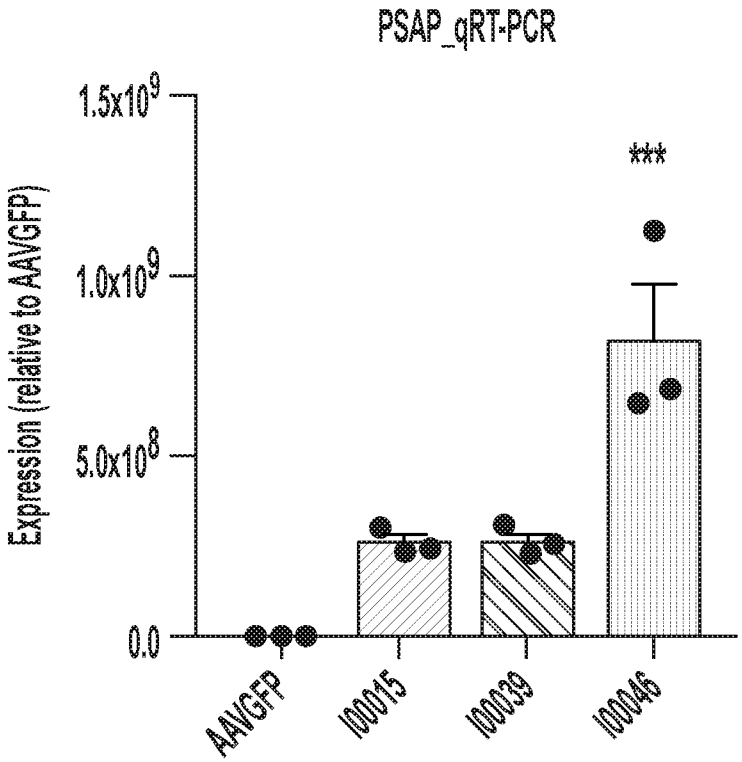

PSAP_qRT-PCR
Expression (relative to AAVGFP)
1.5x10^9
1.0x10^9
5.0x10^8
0.0
***
AAVGFP
I00015
I00039
I00046

Figure 19

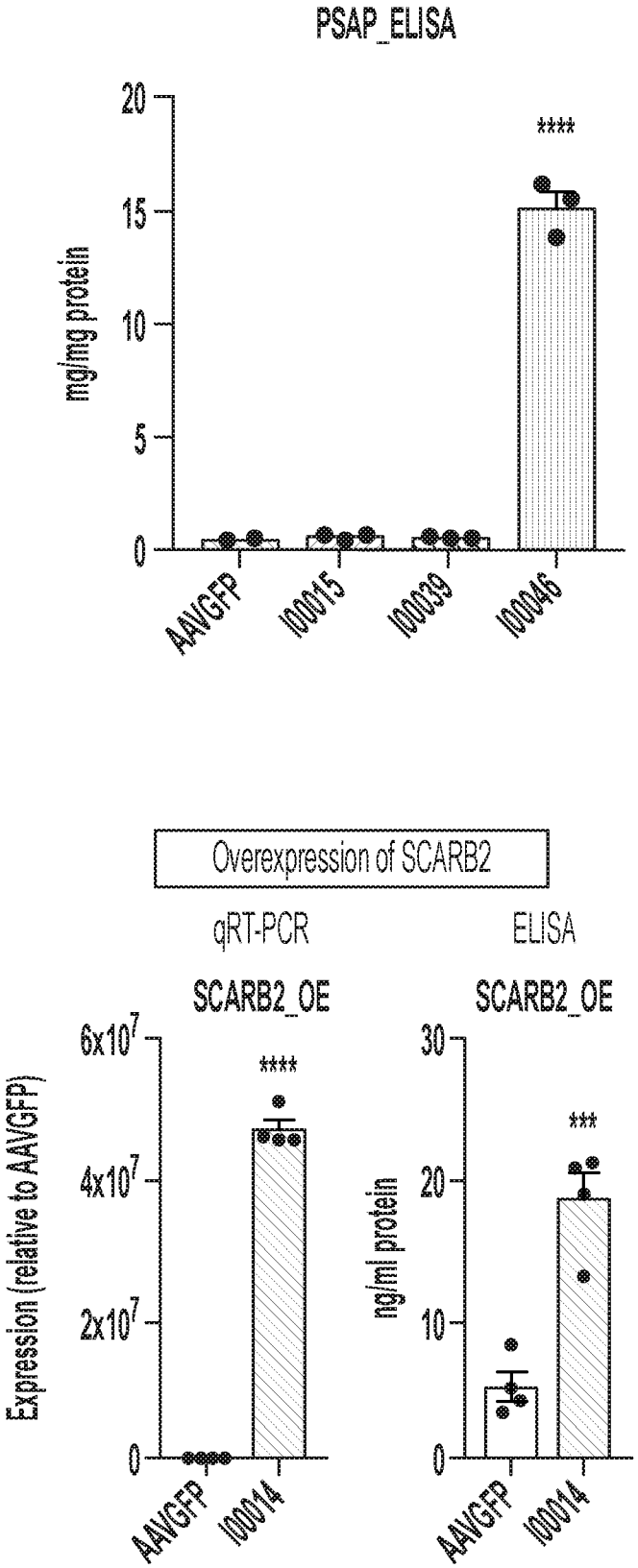


Figure 19 cont.

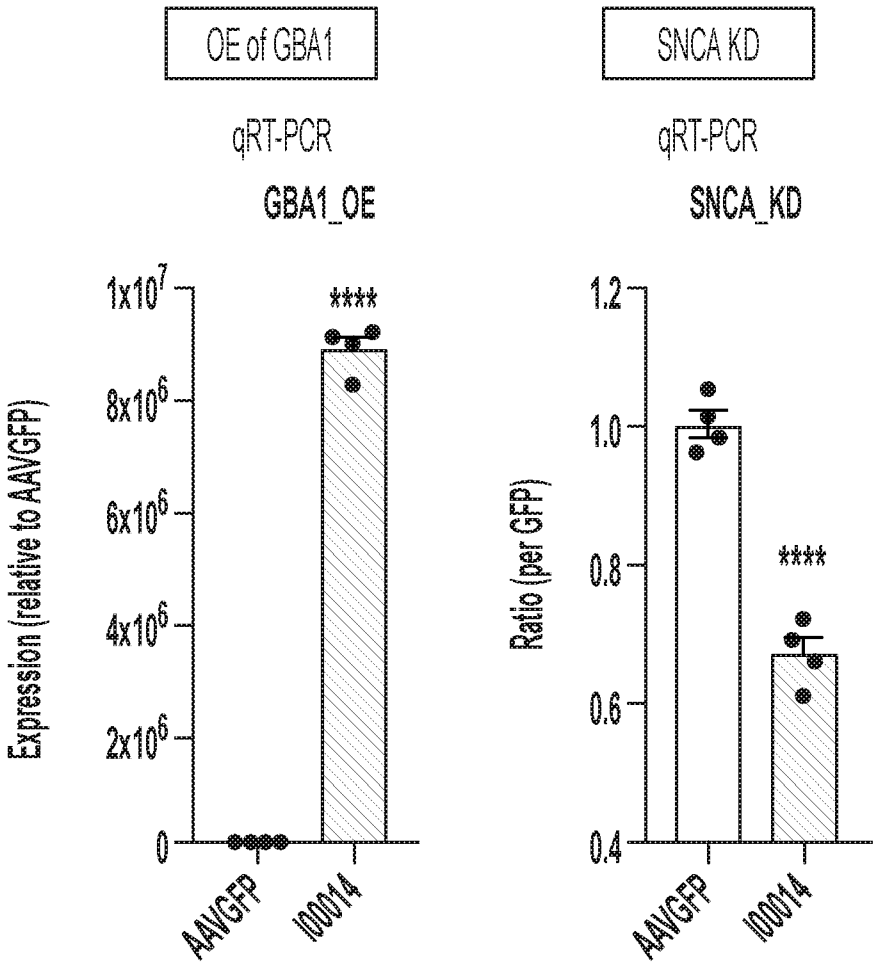


Figure 19 cont.

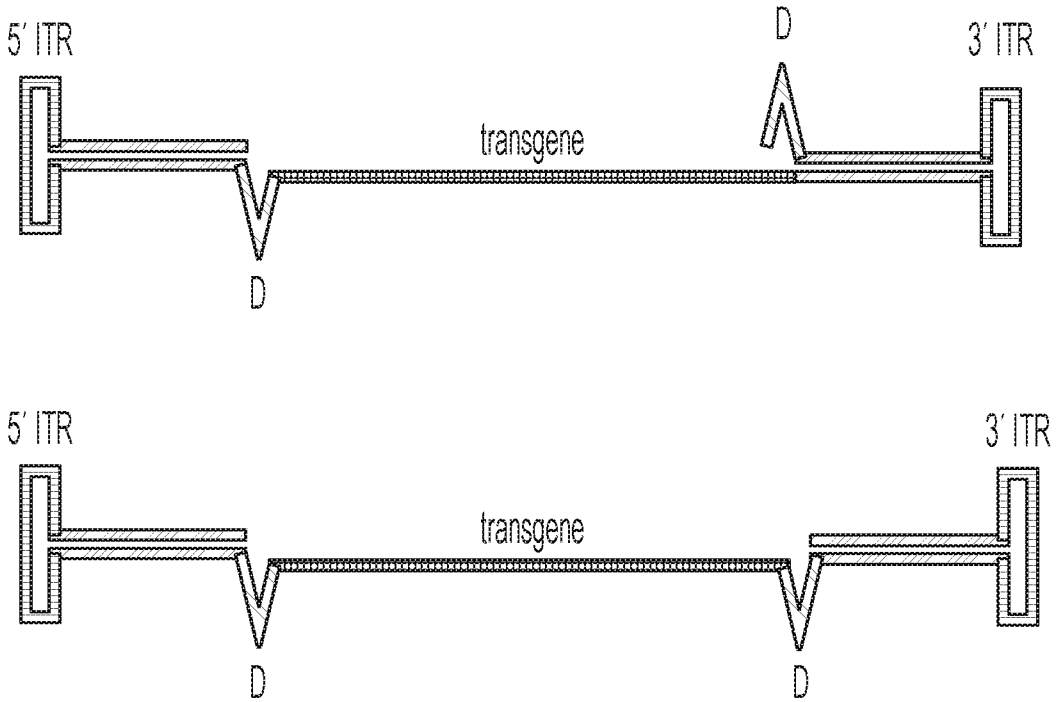


Figure 20

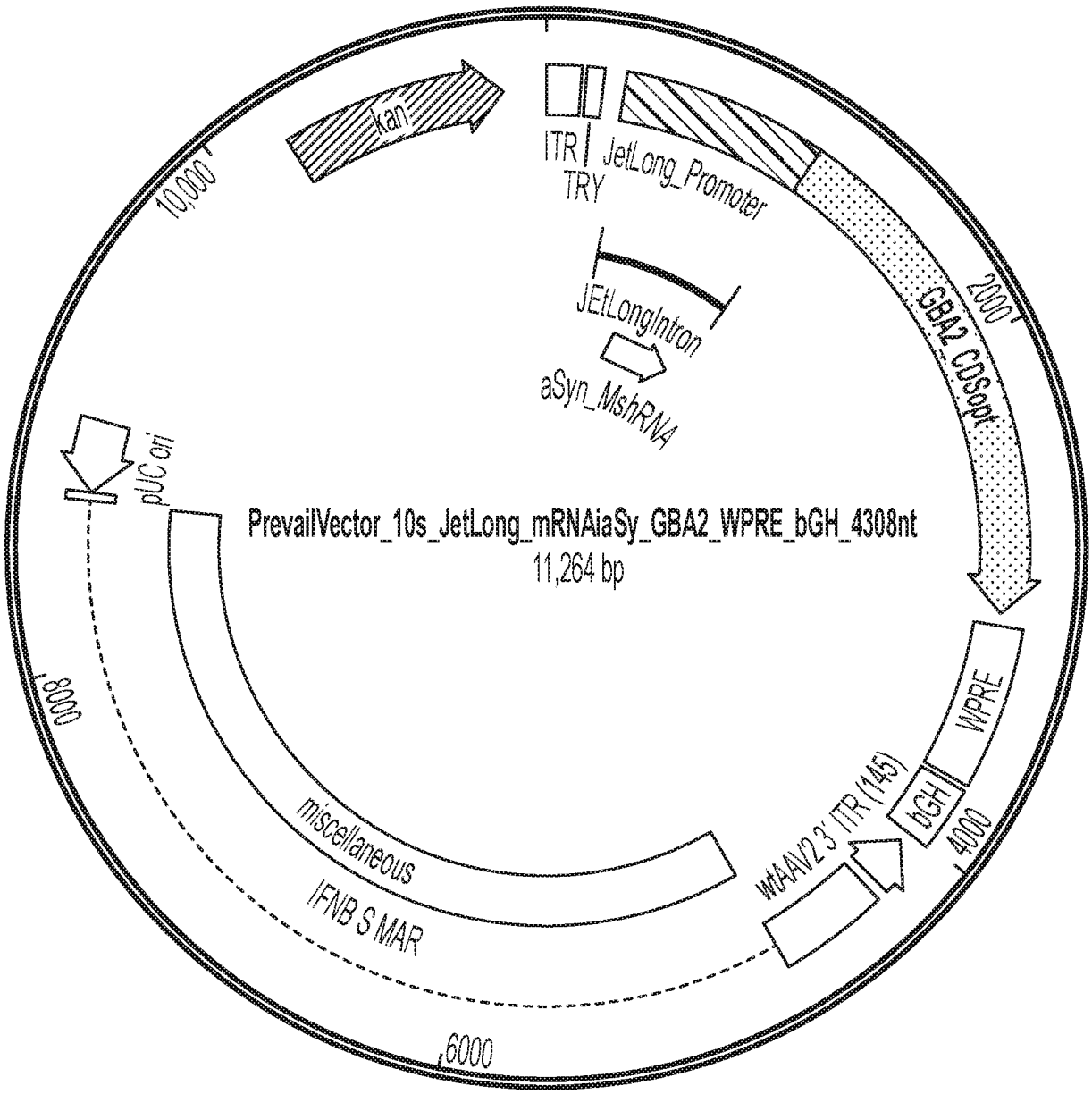

kan
ITR
TRY
JetLong_Promoter
JETLongIntron
aSyn_MshRNA
GBA2 CDSopt
WPRE
bGH
wtAAV2 3' ITR (145)
miscellaneous
IFNB S MAR
pUC ori
PrevailVector_10s_JetLong_mRNAiaSy_GBA2_WPRE_bGH_4308nt
11,264 bp
10,000
2000
4000
6000
8000

Figure 21

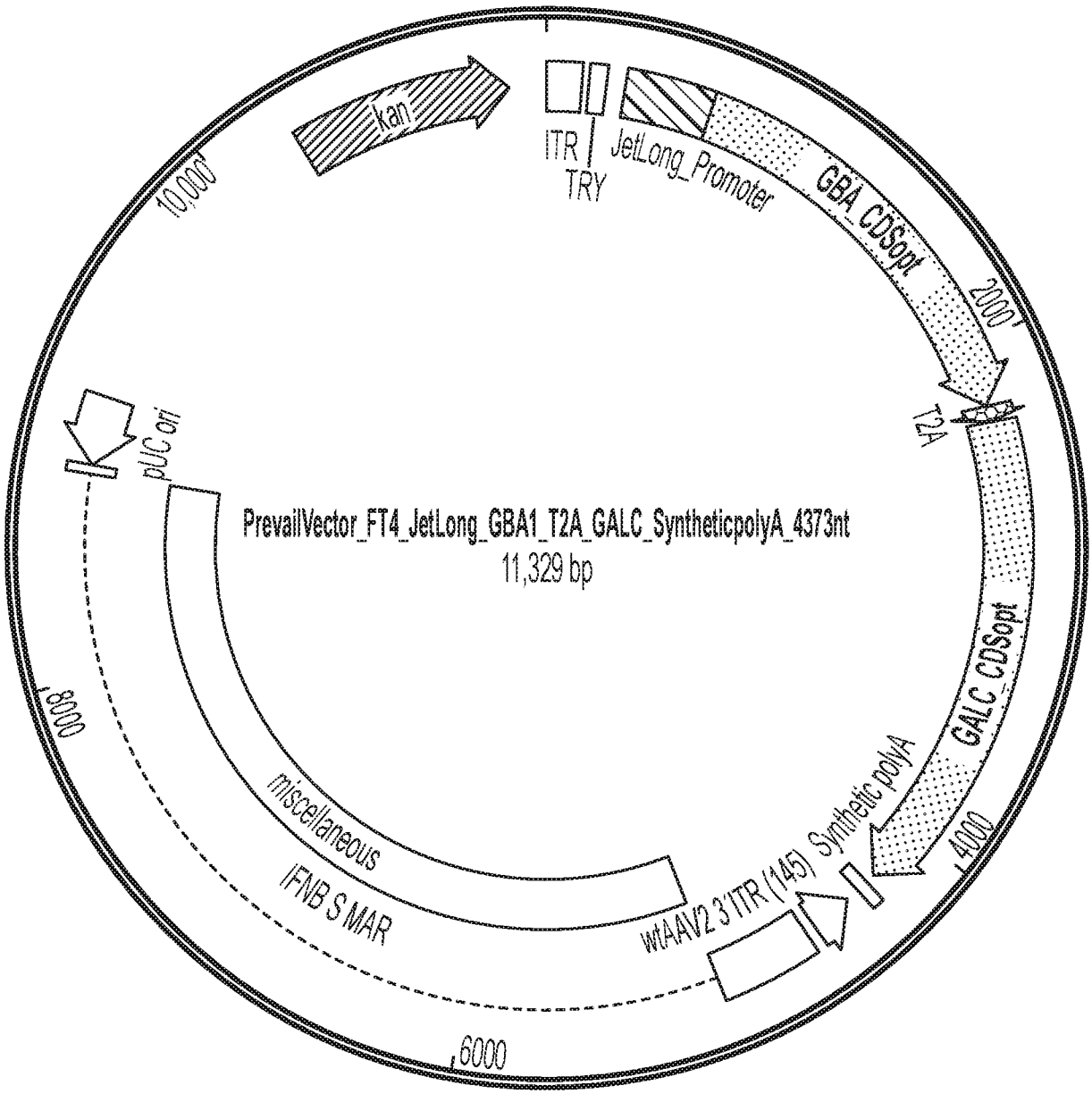

kan
ITR
TRY
JetLong_Promoter
GBA_CDSopt
2000
T2A
GALC_CDSopt
4000
Synthetic polyA
wtAAV2 3'ITR (145)
6000
IFNB S MAR
miscellaneous
8000
pUC ori
10,000
PrevailVector_FT4_JetLong_GBA1_T2A_GALC_SyntheticpolyA_4373nt
11,329 bp

Figure 22

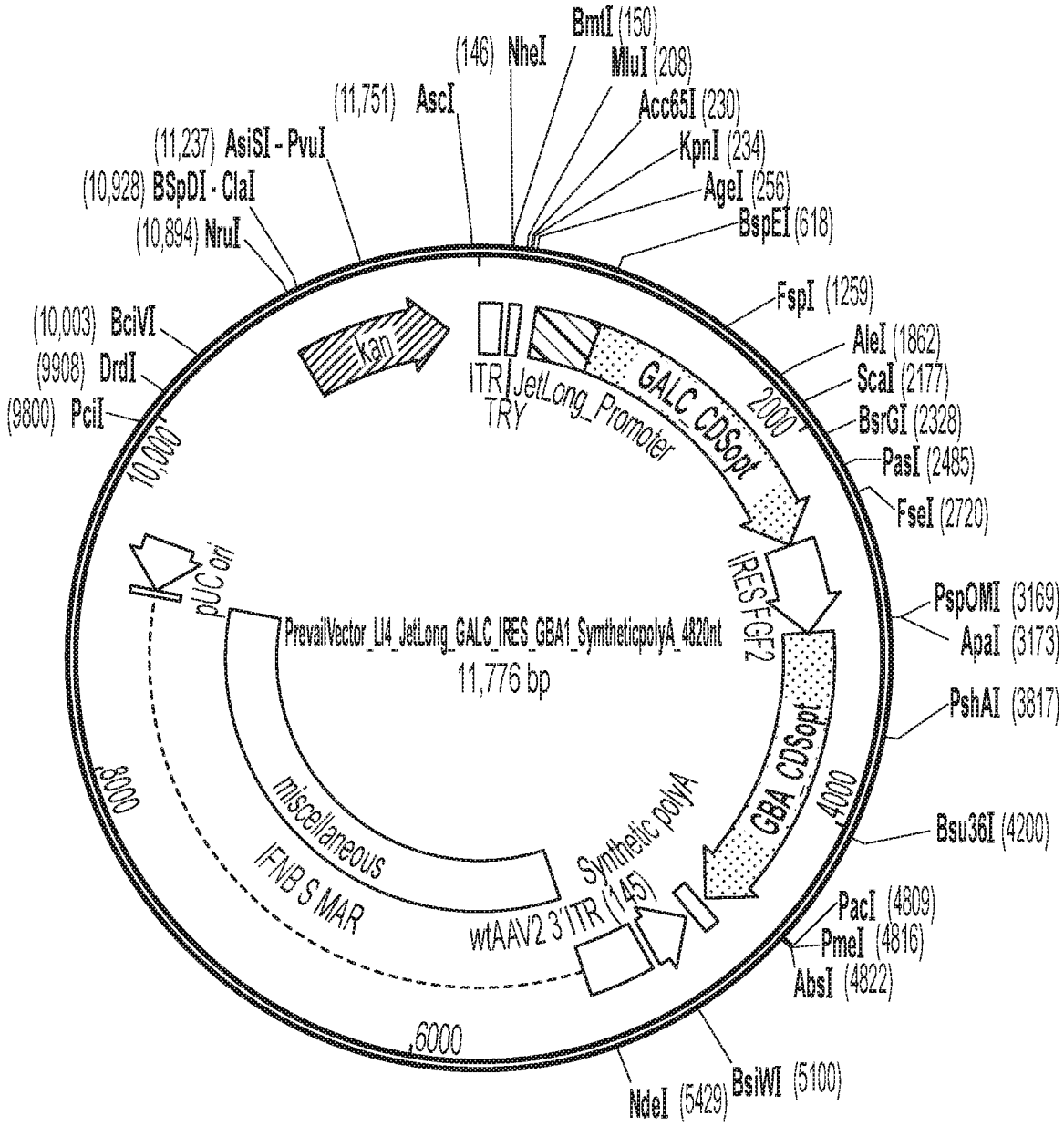
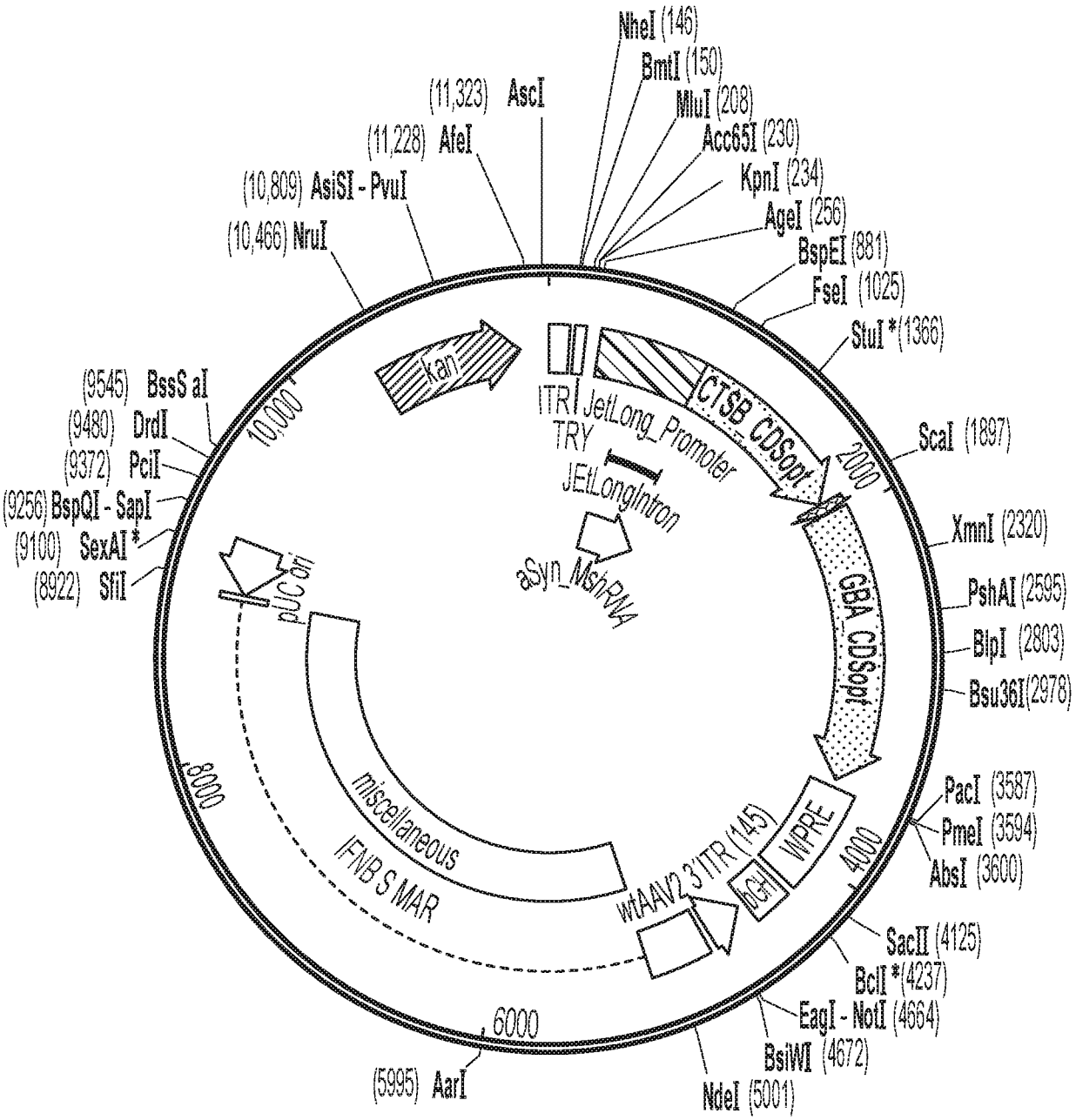

BmtI (150)
(146) NheI
MluI (208)
(11,751) AscI
Acc65I (230)
KpnI (234)
(11,237) AsiSI - PvuI
AgeI (256)
(10,928) BSpDI - ClaI
BspEI (618)
(10,894) NruI
FspI (1259)
(10,003) BciVI
AleI (1862)
(9908) DrdI
ScaI (2177)
(9800) PciI
BsrGI (2328)
PasI (2485)
FseI (2720)
Kan
ITR
TRY
JetLong_Promoter
GALC_CDSopt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

Figure 23



PrevailVector_LT5s_JetLong_mRNAiaSyn_CTSB-T2A-GBA1_WPRE_bGH_4392nt
11,348 bp

Figure 24

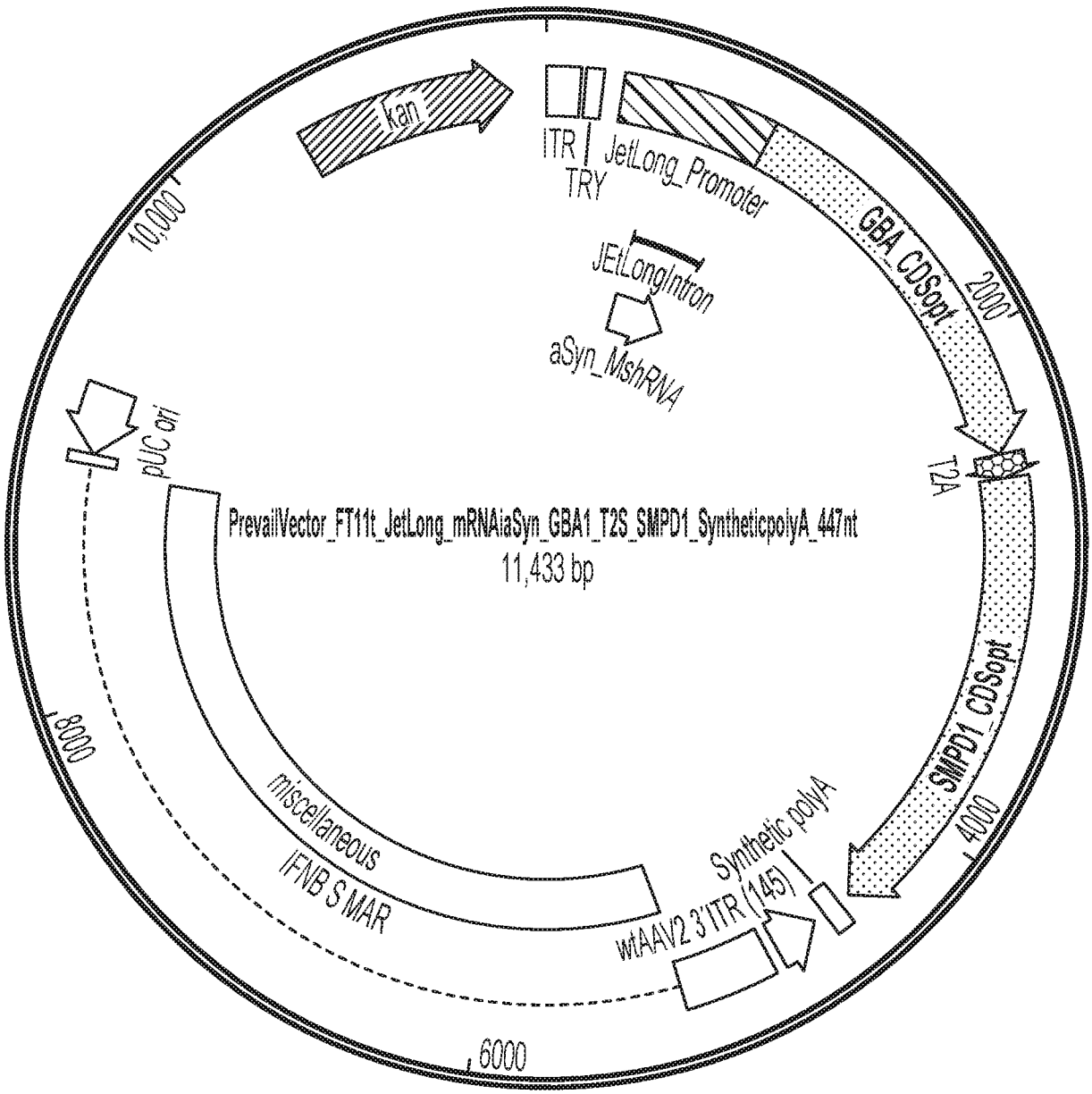

Kan
ITR
TRY
JetLong_Promoter
GBA_CDSopt
2000
T2A
SMPD1_CDSopt
4000
Synthetic polyA
wtAAV2 3'ITR (145)
6000
miscellaneous
IFNB S MAR
8000
pUC ori
10,000
JEtLongIntron
aSyn_MshRV4
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_447nt
11,433 bp

Figure 25

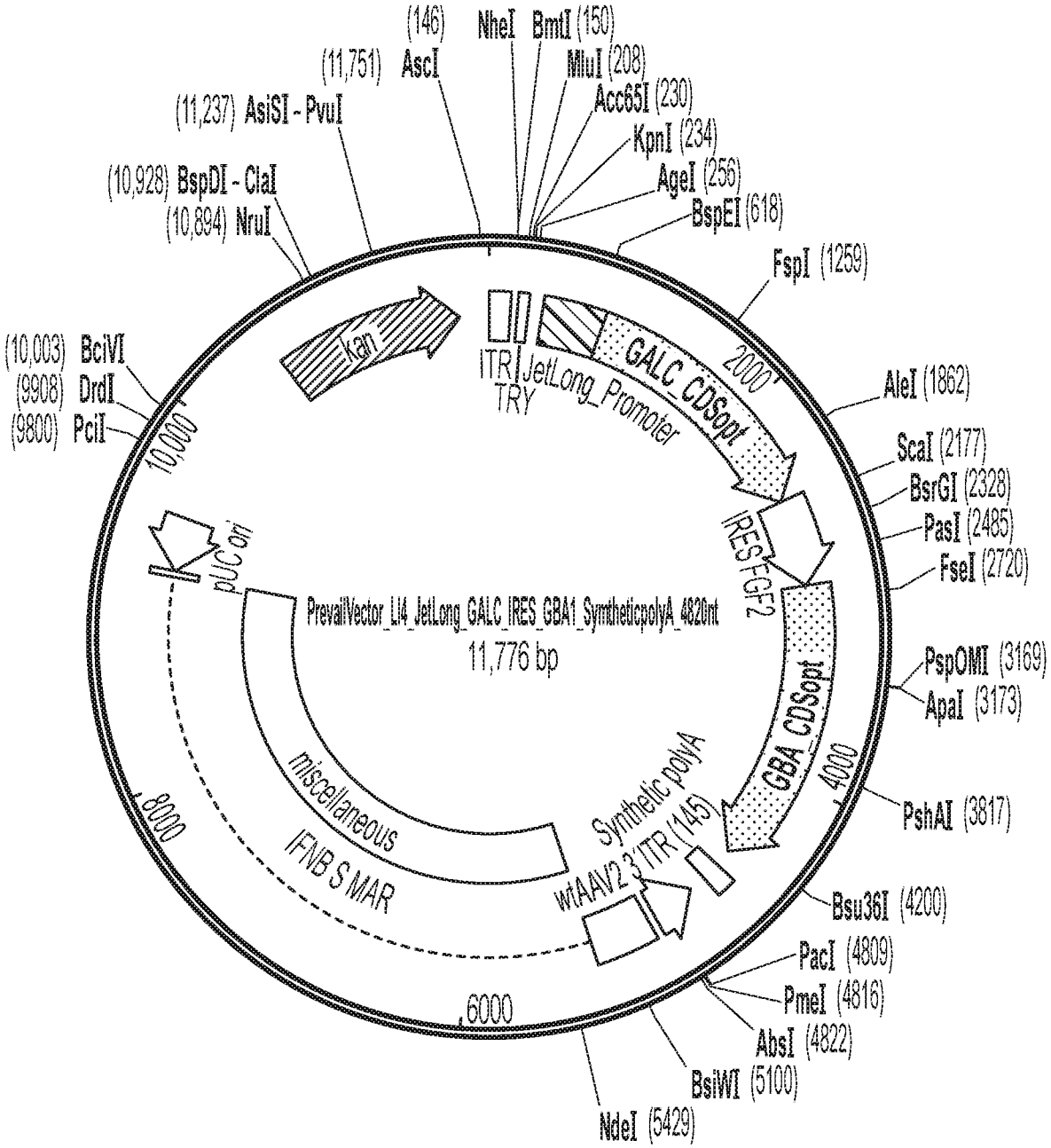
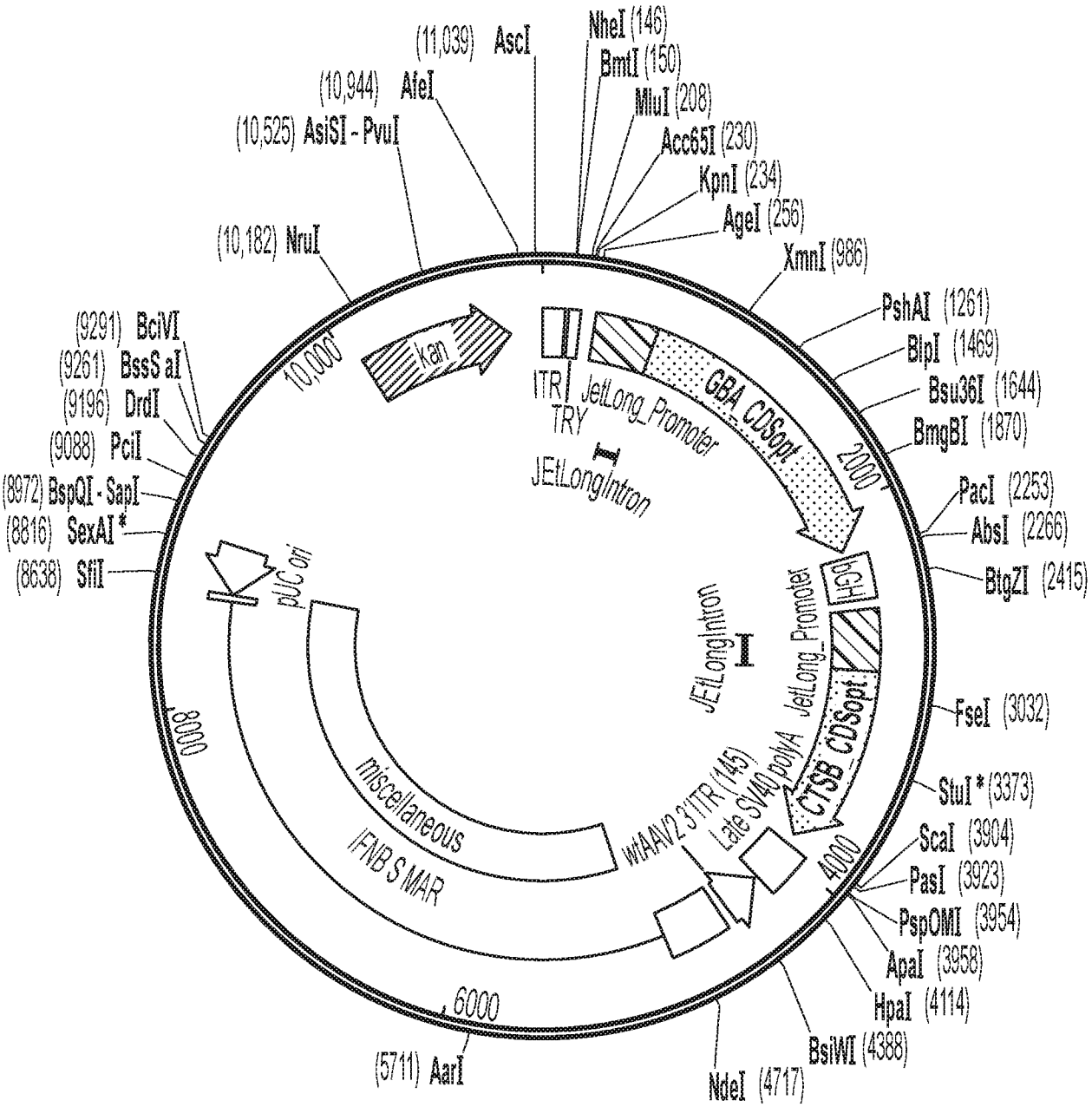

(146) NheI BmtI (150)
(11,751) AscI MluI (208)
Acc65I (230)
(11,237) AsiSI - PvuI
KpnI (234)
AgeI (256)
(10,928) BspDI - ClaI
(10,894) NruI
BspEI (618)
FspI (1259)
(10,003) BciVI
(9908) DrdI
(9800) PciI
kan
ITR
TRY
JetLong_Promoter
GALC_CDSopt
2000
AleI (1862)
ScaI (2177)
BsrGI (2328)
PasI (2485)
FseI (2720)
10,000
pUC ori
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SyntheticpolyA_4620int
11,776 bp
IRES FGF2
PspOMI (3169)
ApaI (3173)
GBA_CDSopt
4000
PshAI (3817)
miscellaneous
Synthetic polyA
wtAAV2 3'ITR (145)
8000
IFNB S MAR
Bsu36I (4200)
PacI (4809)
PmeI (4816)
6000
AbsI (4822)
BsiWI (5100)
NdeI (5429)

Figure 26



PrevailVector_FP5_JetLong_GBA1_bGH_JetLong_CTSB_SV40I_4108nt
11,064 bp

Figure 27

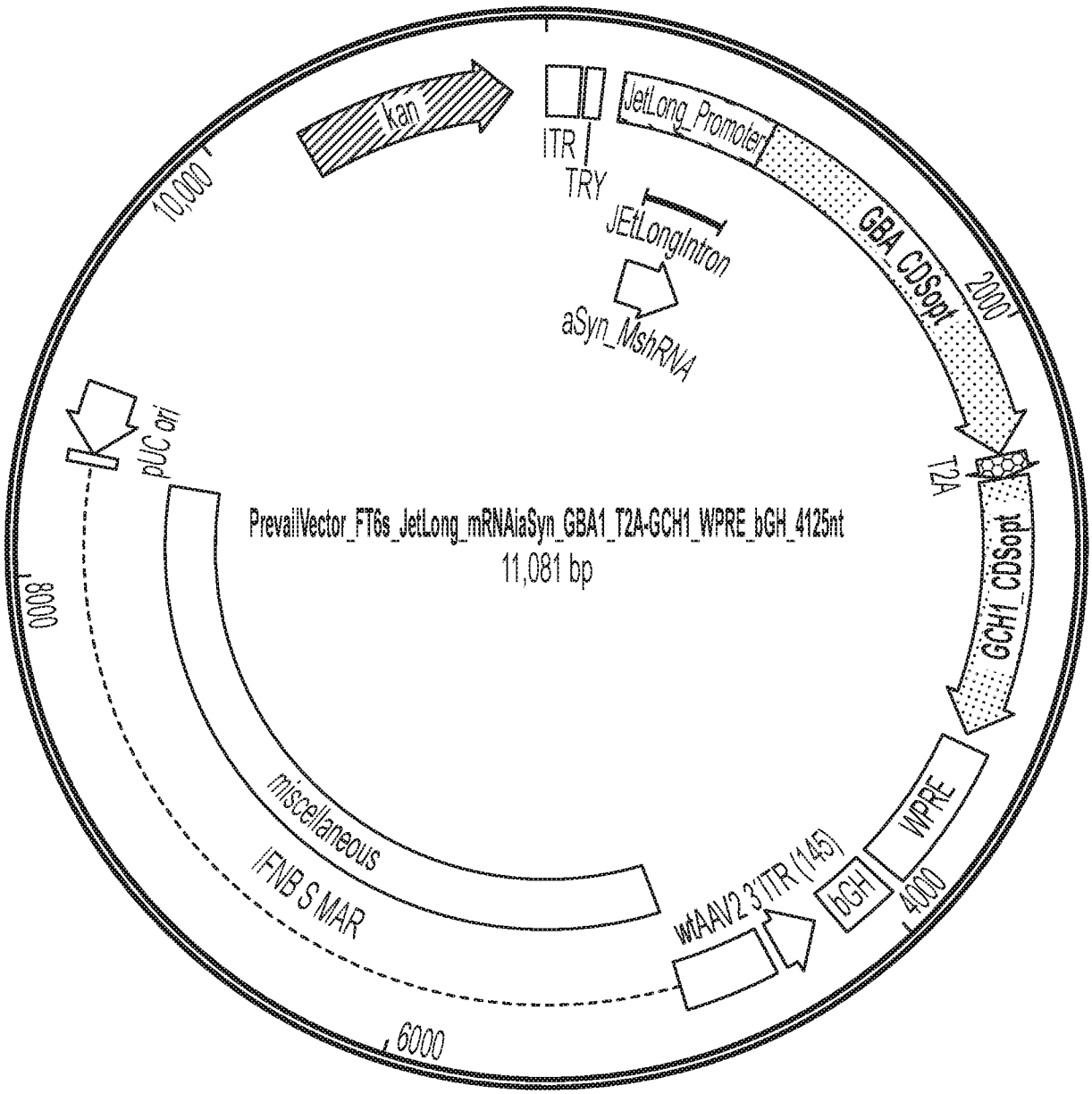
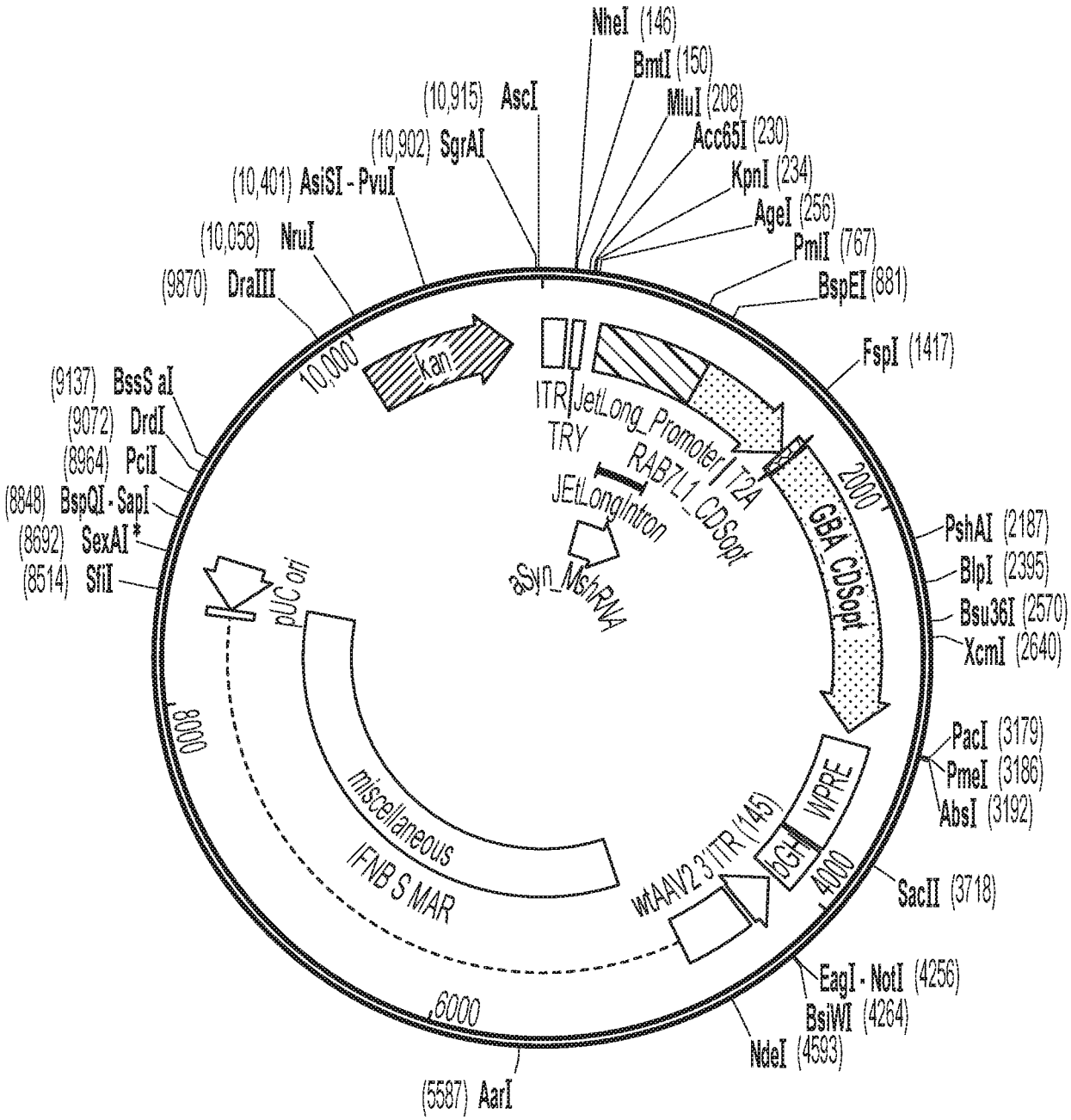

kan
ITR
TRY
JetLong_Promoter
GBA_CDSopt
2000
T2A
GCH1_CDSopt
WPRE
4000
bGH
wtAAV2 3'ITR (145)
6000
IFNB S MAR
miscellaneous
pUC ori
8000
10,000
JEtLongIntron
aSyn_MshRNA
PrevailVector_FT6s_JetLong_mRNAiaSyn_GBA1_T2A-GCH1_WPRE_bGH_4125nt
11,081 bp

Figure 28



PrevailVector_LT7s_JetLong_mRNAiaSyn_RAB7L1-T2A-GBA1_WPRE_bGH_3984nt
10,940 bp

Figure 29

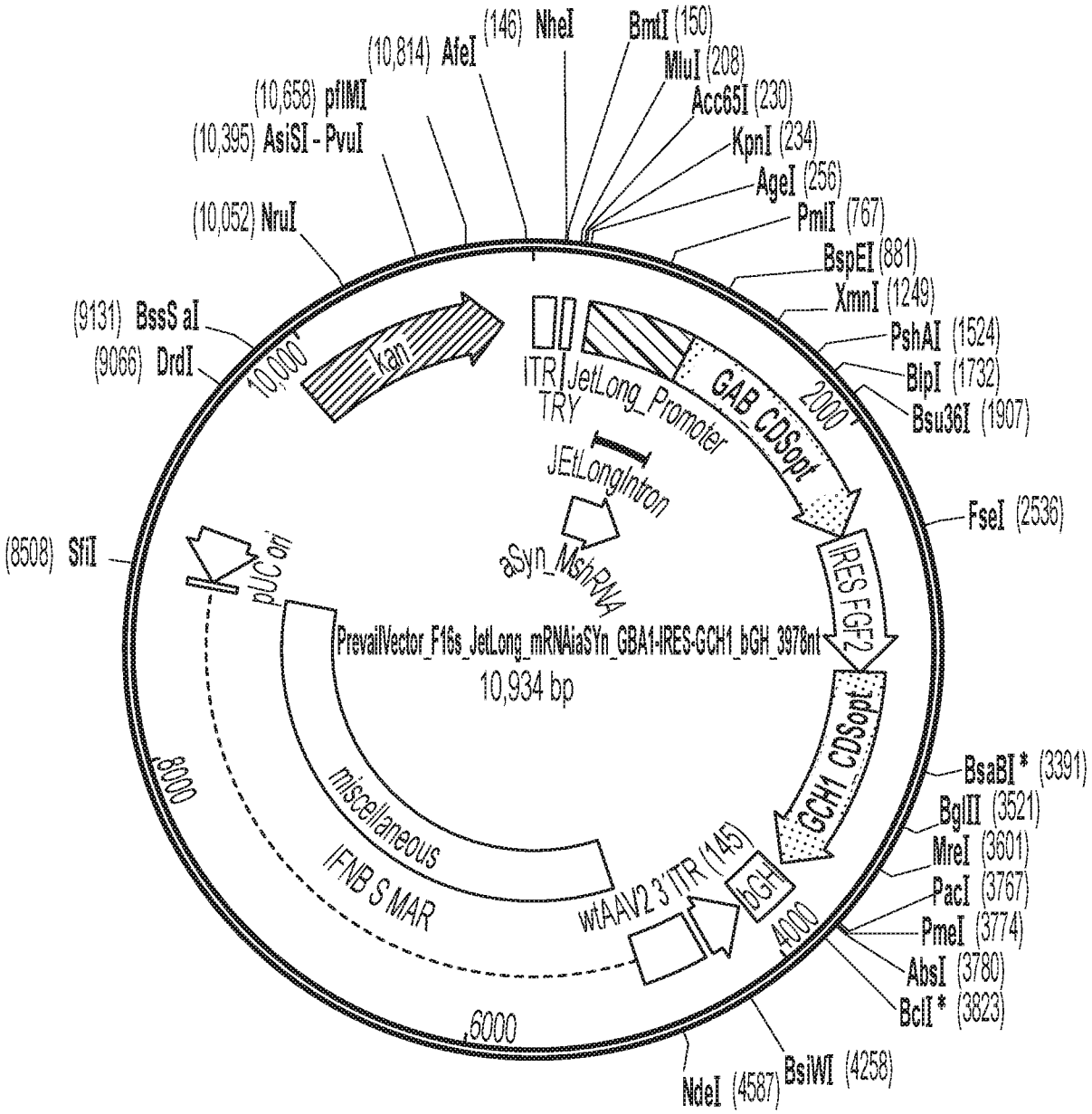

(146) NheI
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
PmlI (767)
BspEI (881)
XmnI (1249)
PshAI (1524)
BlpI (1732)
Bsu36I (1907)
FseI (2536)
BsaBI* (3391)
BglII (3521)
MreI (3601)
PacI (3767)
PmeI (3774)
AbsI (3780)
BclI* (3823)
BsiWI (4258)
NdeI (4587)
(10,814) AfeI
(10,658) pflMI
(10,395) AsiSI - PvuI
(10,052) NruI
(9131) BssS aI
(9066) DrdI
(8508) SfiI
10,000
2000
4000
6000
8000
kan
ITR
TRY
JetLong_Promoter
GAB_CDSopt
JETLongIntron
aSyn_MshRNA
IRES FGF2
GCH1_CDSopt
pUC ori
PrevailVector_F16s_JetLong_mRNAiaSYn_GBA1-IRES-GCH1_bGH_3978nt
10,934 bp
miscellaneous
IFNB S MAR
wtAAV2 3'ITR (145)
bGH

Figure 30

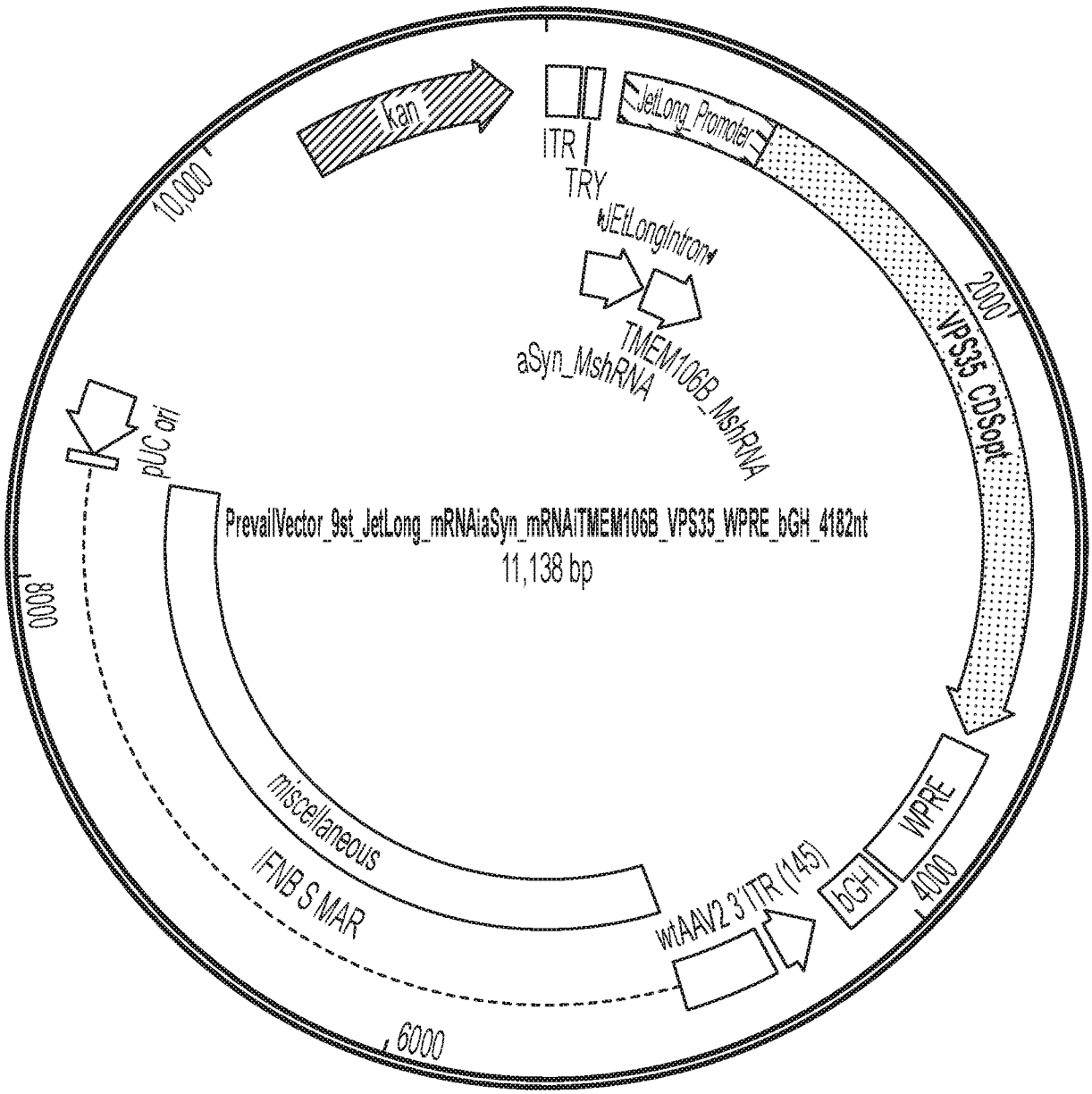

Kan
ITR
TRY
JetLong_Promoter
JEtLongIntron
TMEM106B_MshRNA
aSyn_MshRNA
VPS35_CDSopt
WPRE
bGH
wtAAV2 3'ITR (145)
miscellaneous
IFNB S MAR
pUC ori
PrevailVector_9st_JetLong_mRNAiaSyn_mRNAiTMEM106B_VPS35_WPRE_bGH_4182nt
11,138 bp
2000
4000
6000
8000
10,000

Figure 31

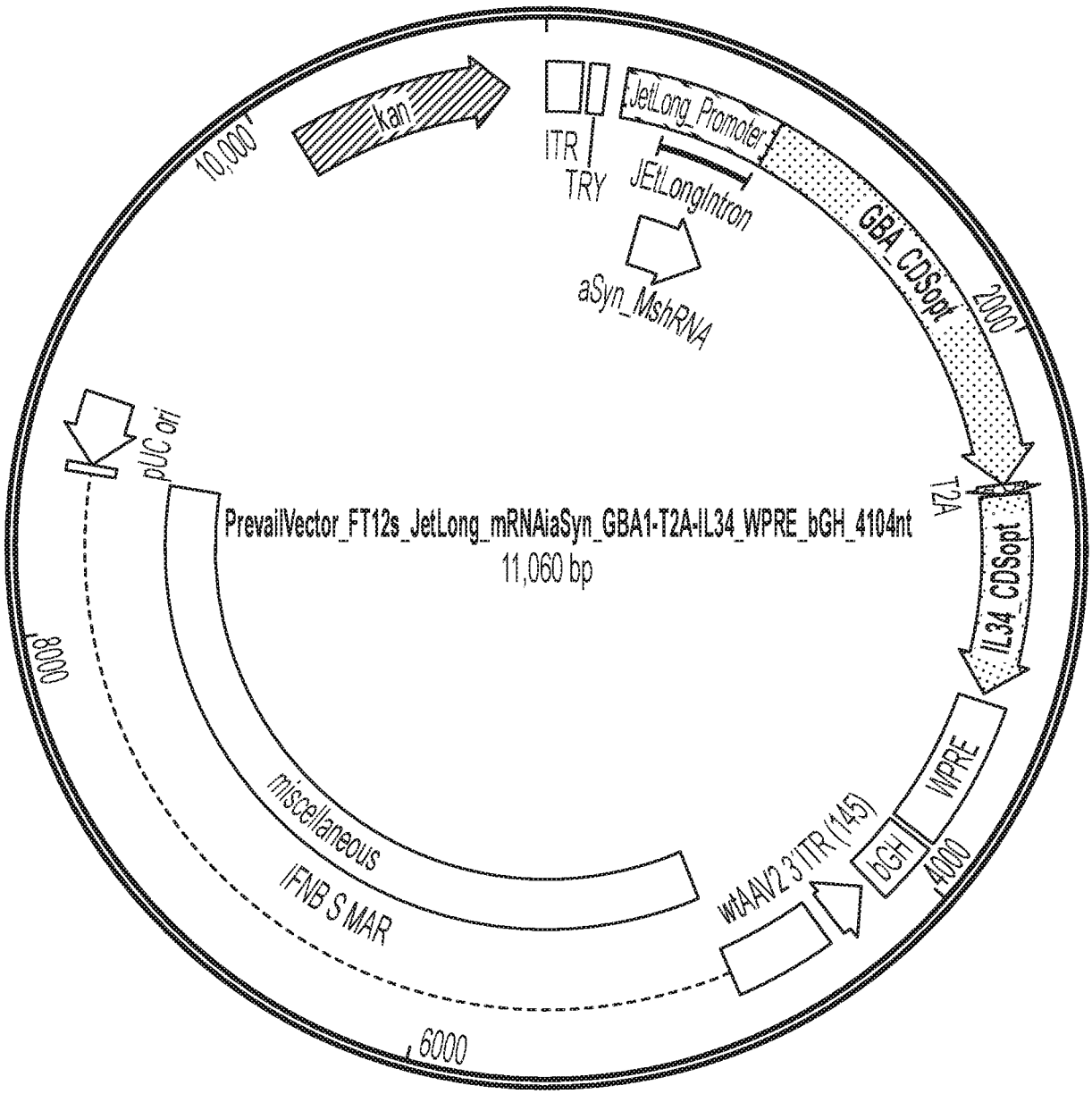
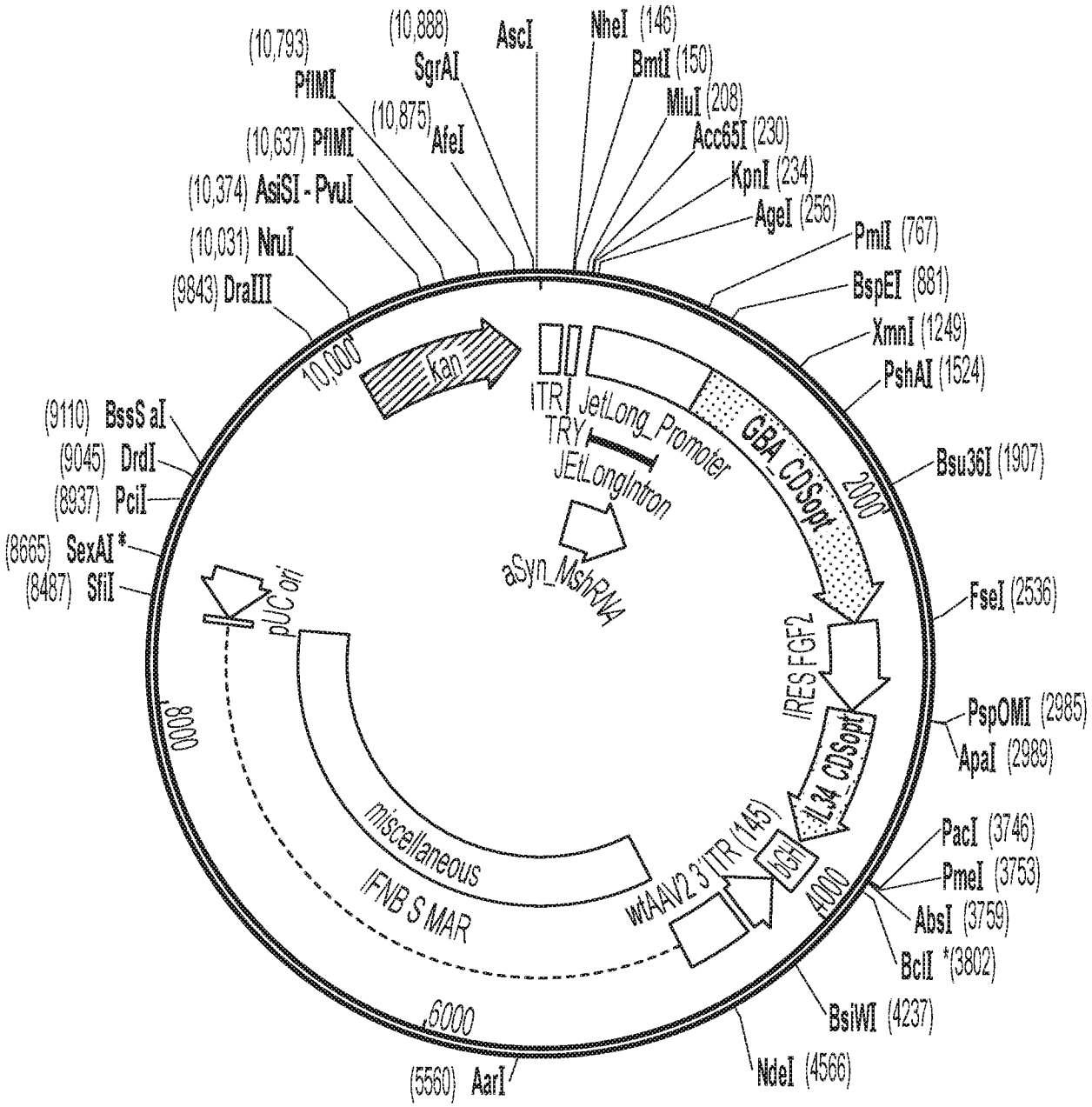

10,000
kan
ITR
TRY
JetLong_Promoter
JETLongIntron
aSyn_MshRNA
GBA_CDSopt
2000
T2A
IL34_CDSopt
4000
WPRE
bGH
wtAAV2 3'ITR (145)
6000
IFNB S MAR
miscellaneous
8000
pUC ori
PrevailVector_FT12s_JetLong_mRNAiaSyn_GBA1-T2A-IL34_WPRE_bGH_4104nt
11,060 bp

Figure 32



PrevailVector_FI12s_JetLong_mRNAiaSYn_GBA1-IRES-IL34_bGH_3957nt

Figure 33

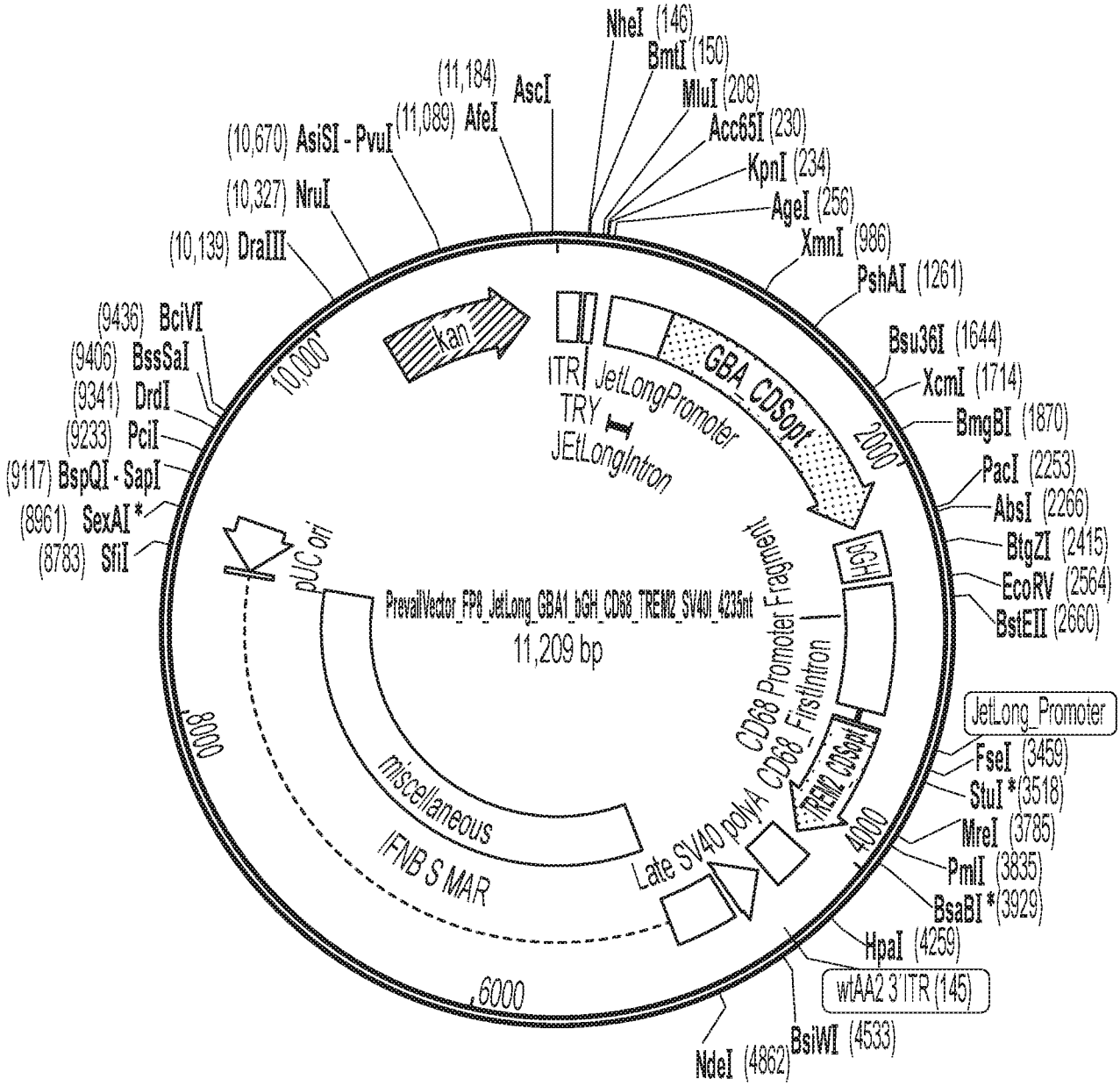
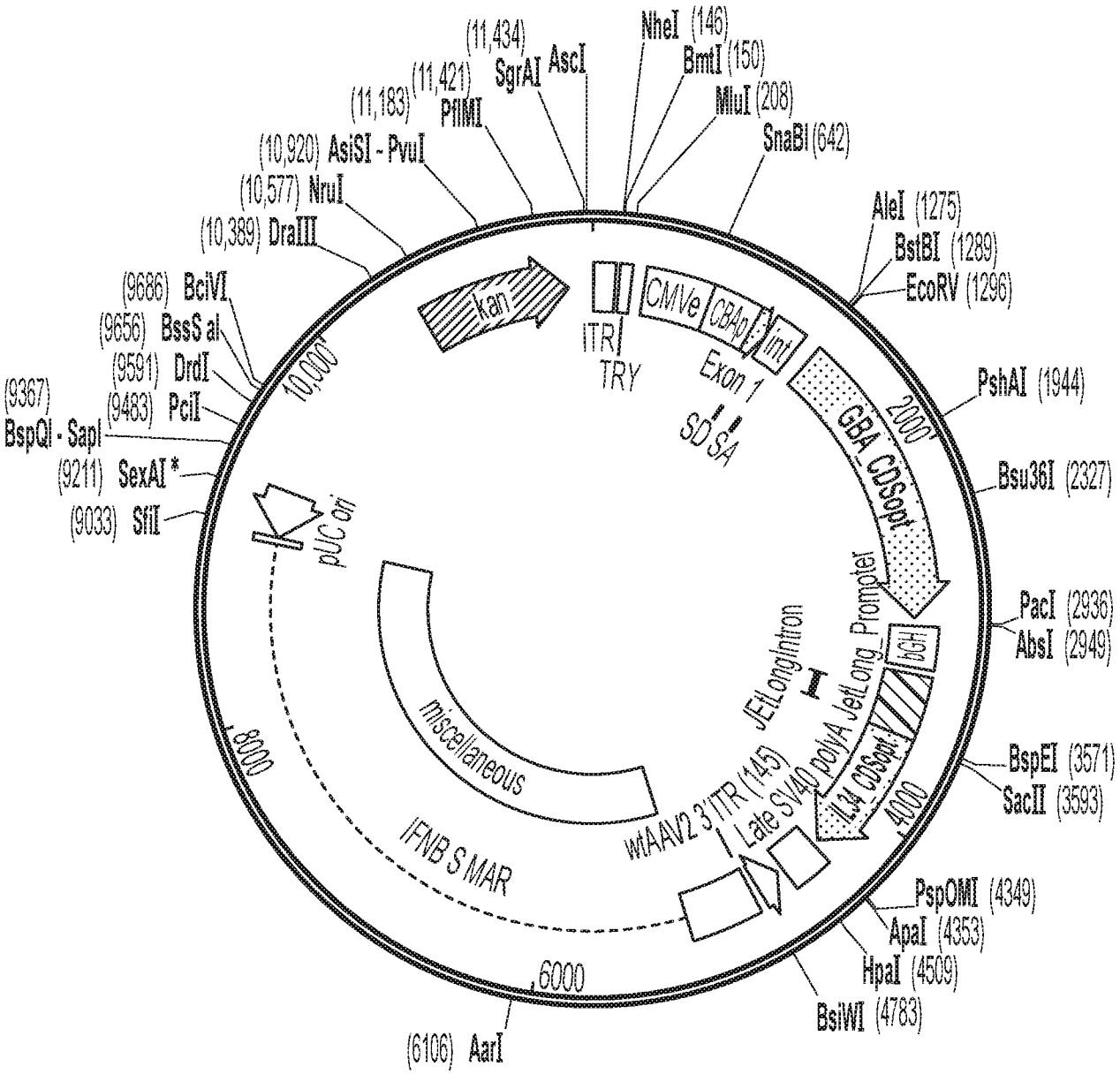

NheI (146)
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
XmnI (986)
PshAI (1261)
Bsu36I (1644)
XcmI (1714)
BmgBI (1870)
PacI (2253)
AbsI (2266)
BtgZI (2415)
EcoRV (2564)
BstEII (2660)
JetLong_Promoter
FseI (3459)
StuI *(3518)
MreI (3785)
PmlI (3835)
BsaBI *(3929)
HpaI (4259)
wtAA2 3'ITR (145)
BsiWI (4533)
NdeI (4862)
6000
8000
pUC ori
PrevailVector_FP9_JetLong_GBA1_bGH_CD68_TREM2_SV40I_4235nt
11,209 bp
miscellaneous
IFNB S MAR
Late SV40 polyA
CD68 Promoter Fragment
CD68_FirstIntron
TREM2 CDSopt
bGH
ITR
TRY
JETLongIntron
JetLongPromoter
GBA_CDSopt
2000
4000
Kan
10,000
(9436) BciVI
(9406) BssSaI
(9341) DrdI
(9233) PciI
(9117) BspQI - SapI
(8961) SexAI *
(8783) SfiI
(10,139) DraIII
(10,327) NruI
(10,670) AsiSI - PvuI
(11,089) AfeI
(11,184) AscI

Figure 34



PrevailVector_FP12_CMVe_CBA_GBA1_bGH_JetLong_IL34_SV40I_4503nt
11,459 bp

Figure 35

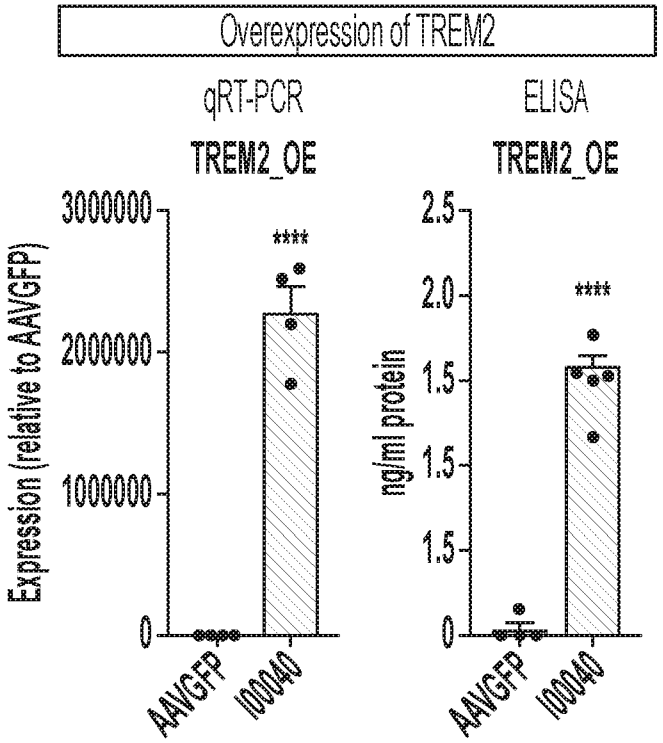


Figure 36A

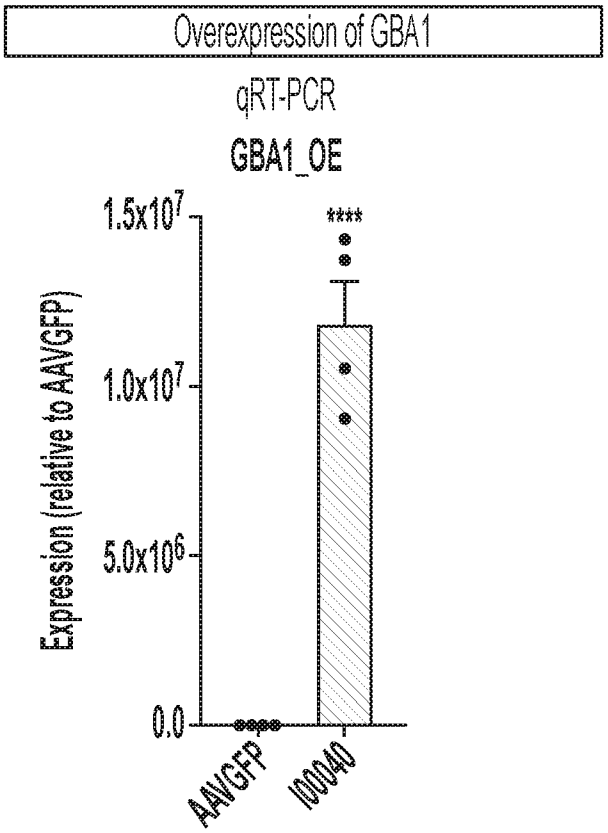


Figure 36B

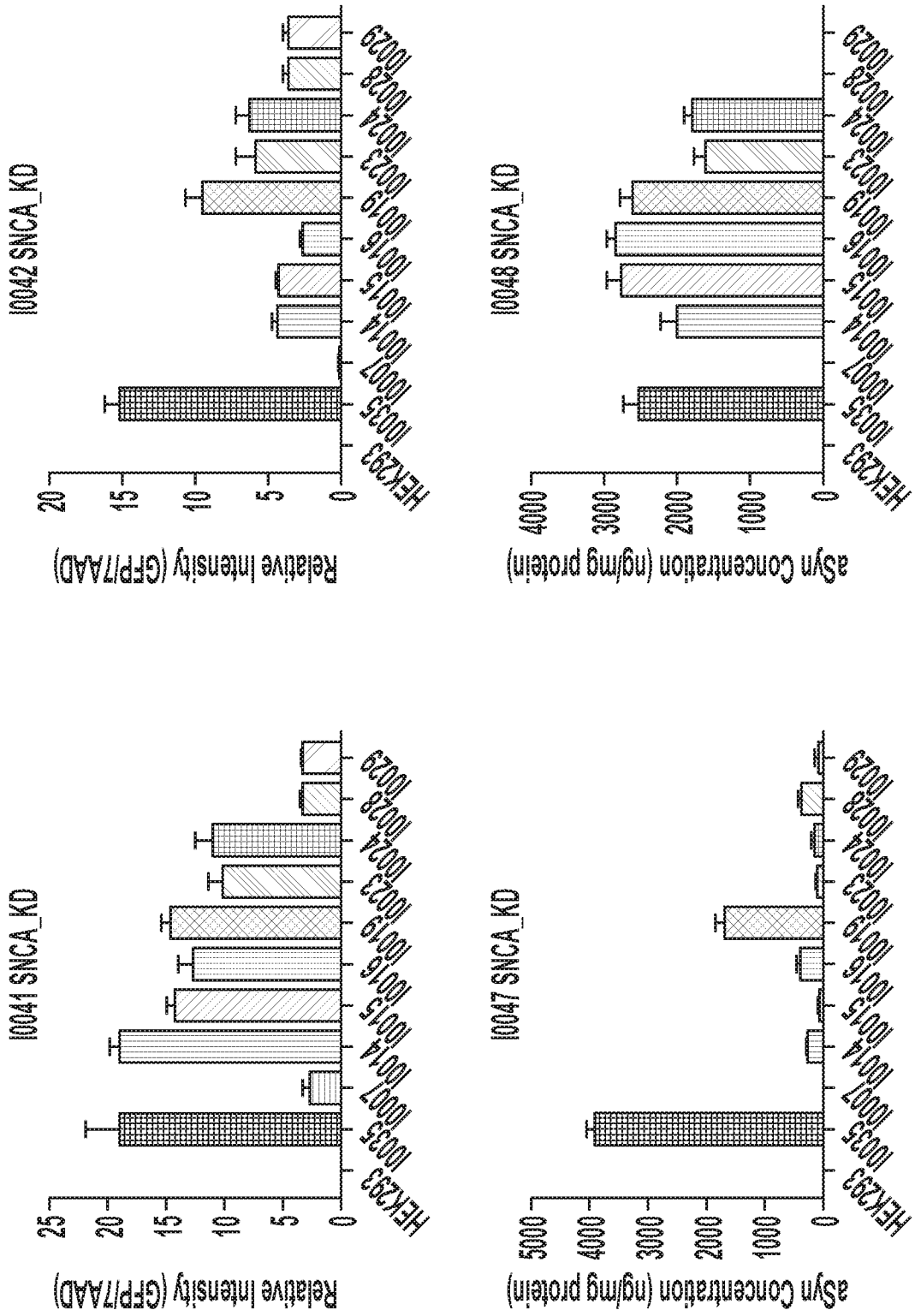

I0042 SNCA_KD
Relative Intensity (GFP/7AAD)
20
15
10
5
0
HEK293
I0035
I0007
I0014
I0015
I0016
I0019
I0023
I0024
I0028
I0029
I0048 SNCA_KD
aSyn Concentration (ng/mg protein)
4000
3000
2000
1000
0
I0041 SNCA_KD
Relative Intensity (GFP/7AAD)
25
20
15
10
5
0
I0047 SNCA_KD
aSyn Concentration (ng/mg protein)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

Figure 37

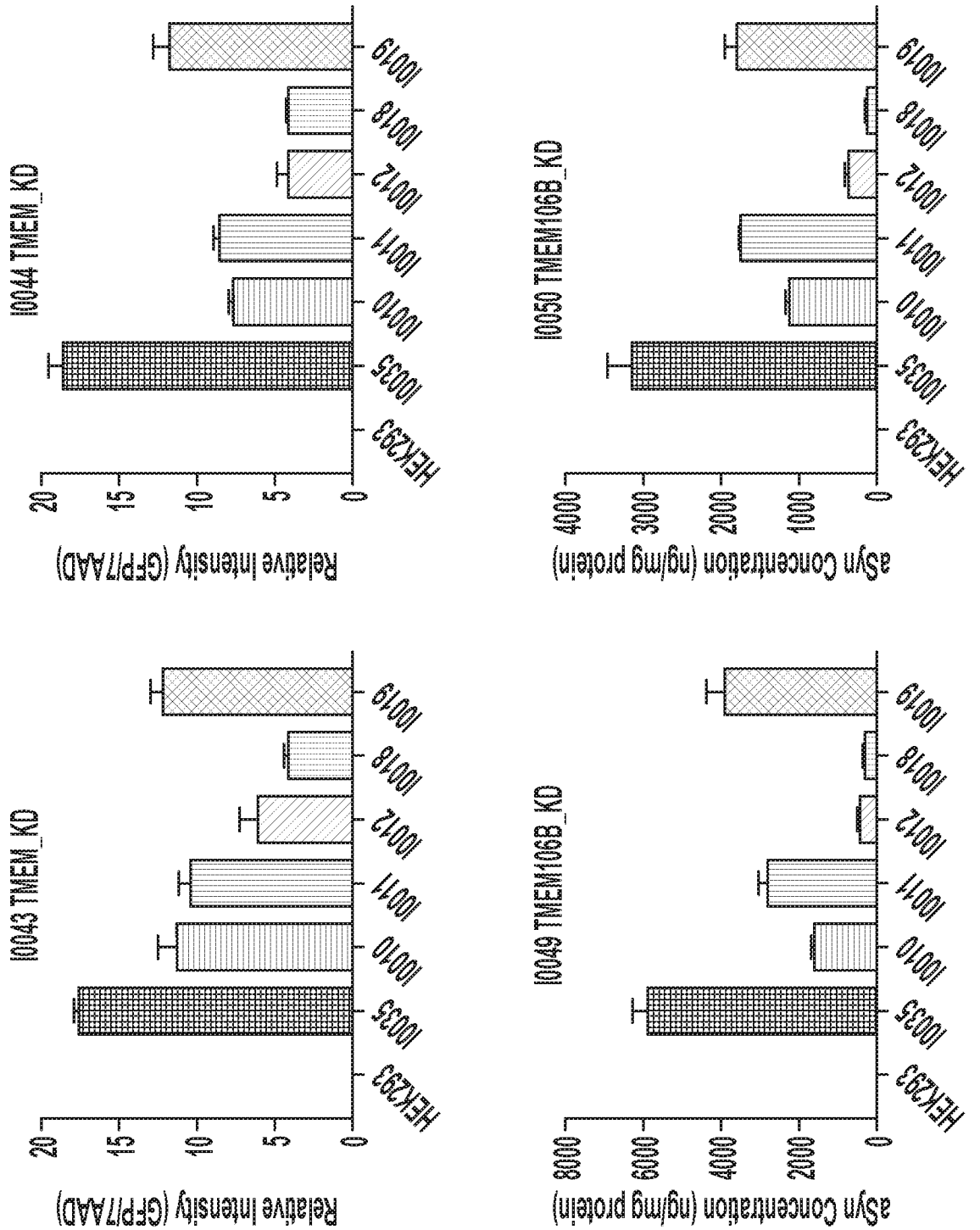

I0044 TMEM_KD
Relative Intensity (GFP/7AAD)
20
15
10
5
0
HEK293
I0035
I0010
I0011
I0012
I0018
I0019
I0050 TMEM106B_KD
aSyn Concentration (ng/mg protein)
4000
3000
2000
1000
0
I0043 TMEM_KD
I0049 TMEM106B_KD
8000
6000
4000
2000

Figure 38

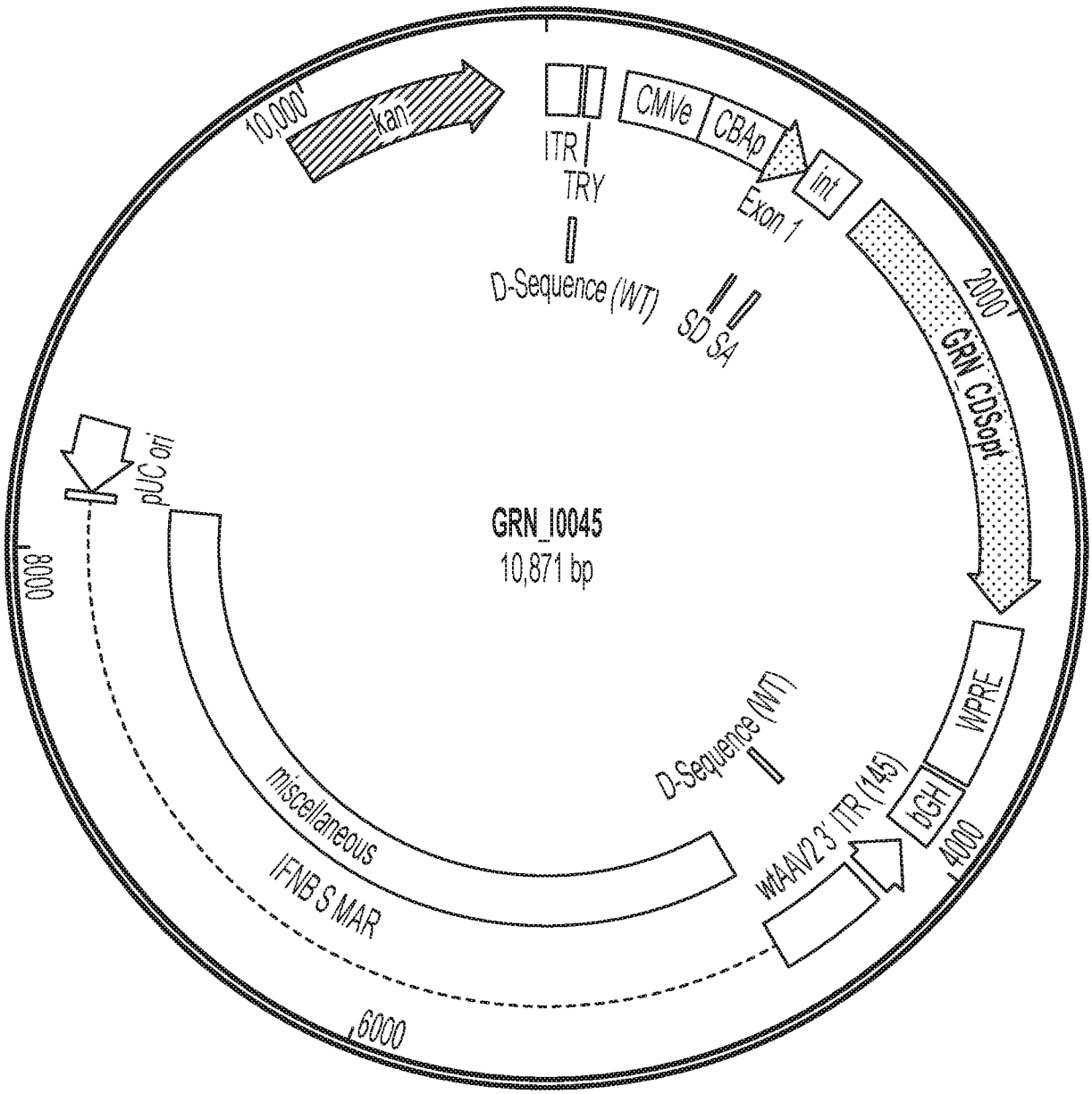

10,000
kan
ITR
TRY
CMVe
CBAp
Exon 1
int
2000
GRN CDSopt
D-Sequence (WT)
SD
SA
GRN_I0045
10,871 bp
pUC ori
8000
D-Sequence (WT)
WPRE
bGH
4000
wtAAV2 3' ITR (145)
miscellaneous
IFNB S MAR
6000

Figure 39

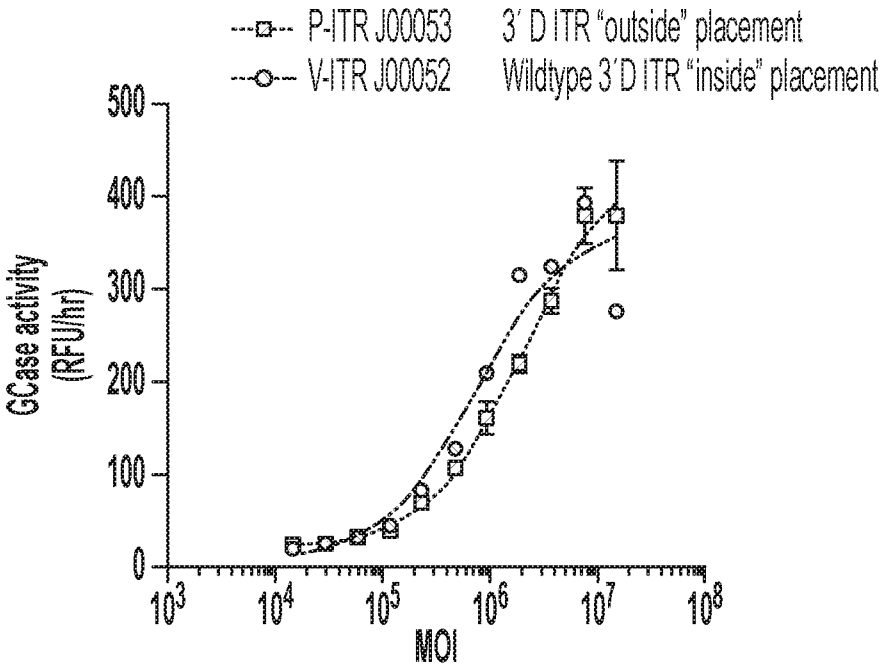

P-ITR J00053 3′ D ITR "outside" placement
V-ITR J00052 Wildtype 3′D ITR "inside" placement
GCase activity (RFU/hr)
500
400
300
200
100
0
10^3
10^4
10^5
10^6
10^7
10^8
MOI

Figure 40

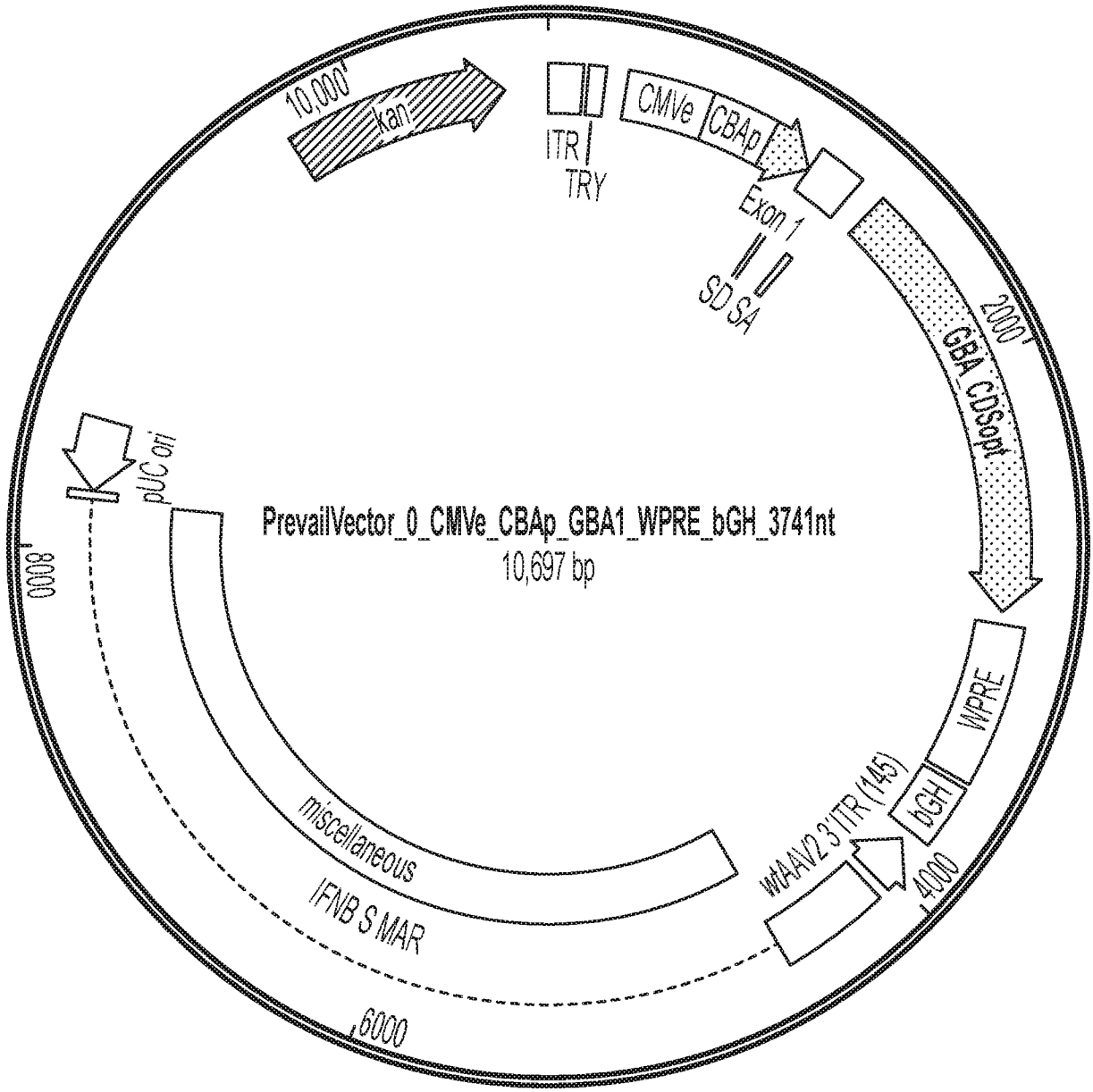

10,000
Kan
ITR
TRY
CMVe
CBAp
Exon 1
SD SA
2000
GBA_CDSopt
PrevailVector_0_CMVe_CBAp_GBA1_WPRE_bGH_3741nt
10,697 bp
pUC ori
8000
miscellaneous
IFNB S MAR
6000
wtAAV2 3'ITR (145)
bGH
WPRE
4000

Figure 1