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(54) Title: RECOMBINANT REELIN GENE THERAPY

(57) Abstract: The disclosure describes novel Reelin repeat fusion proteins for use as a therapeutic in the treatment of a disease or disorder of the nervous system. Intracerebroventricular administration of AAV vectors expressing a Reelin repeat fusion protein is shown to rescue impairment of spine density in the hippocampus of reeler and attenuate hyperactivity of FMR-/- knockout mice in an open field assay.



RECOMBINANT REELIN GENE THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, pending U.S. Provisional Patent Application Serial No. 63/222,669, filed July 16th, 2021, the content of which is hereby expressly incorporated by reference into the present application in its entirety.

FIELD OF THE DISCLOSURE

[0002] The disclosure relates generally to the field of gene therapy of neurodegenerative diseases or disorders.

BACKGROUND

[0003] The autosomal recessive *reeler* mutation in mice causes disorganization of the cortical laminar layers and impaired development of the cerebellum which results in a characteristic “reeling gait” phenotype. The mutation was mapped to the Reelin gene which encodes a large, secreted extracellular matrix glycoprotein that is an important modulator of a neuronal signaling required for synaptic transmission and plasticity. At the cellular level, Reelin induces the clustering of apolipoprotein E receptor 2 (ApoER2) and very-low-density lipoprotein receptor (VLDLR), which triggers the phosphorylation of the cytosolic adaptor protein Disabled-1 (Dab1). Several pathways act downstream of Dab1 phosphorylation to regulate the cytoskeleton, adhesion molecules, ion channels, and neurotransmitter release. Deficiency of Reelin signaling is implicated in the etiology of several neurodevelopmental disorders. Delivery of Reelin to the brain may therefore be useful as a therapeutic for the treatment of several neurological diseases.

SUMMARY

[0004] The disclosure describes novel Reelin repeat fusion proteins for use as a therapeutic in the treatment of a disease or disorder of the nervous system. Intracerebroventricular administration of AAV vectors expressing a Reelin repeat fusion protein is shown to rescue impairment of spine density in the hippocampus of *reeler* and *FMR-/-* knockout mice and attenuate hyperactivity in an open field assay.

[0005] In one aspect, a viral vector composition for expressing a secreted recombinant Reelin fusion protein is disclosed comprising from one to three Reelin repeats consisting of a Reelin repeat R3, a Reelin repeat R3 and a Reelin repeat R5, a Reelin repeat R3 and a Reelin repeat R6 or a Reelin repeat R3, a Reelin repeat R5 and a Reelin repeat R6, wherein Reelin repeat R3 is amino terminal to Reelin repeats R5 and R6, Reelin repeat R5 if present, is amino terminal to Reelin repeat R6, and wherein the Reelin repeat R3 comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 4.

[0006] In one aspect, the polypeptide sequence of Reelin repeat R3 comprises a proteolytic cleavage site at Pro 1244.

[0007] In one aspect, the Reelin repeat R3 has at least 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 10.

[0008] In one aspect, the Reelin repeat R3 is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity to the nucleotide acid sequence of SEQ ID NO: 9.

[0009] In one aspect, the Reelin repeat R5 polypeptide comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 30.

[0010] In one aspect, the Reelin repeat R5 polypeptide comprises at least 80, 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 30.

[0011] In one aspect, the Reelin repeat R5 polypeptide is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity to the nucleotide acid sequence of SEQ ID NO: 29.

[0012] In one aspect, the Reelin repeat R6 polypeptide comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 12, 14, 16 or 18.

[0013] In one aspect, the Reelin repeat R6 polypeptide comprises at least 80, 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 20.

[0014] In one aspect, the Reelin repeat R6 polypeptide is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity to the nucleotide acid sequence of SEQ ID NO: 19.

[0015] In one aspect, the nucleotide sequence encoding the recombinant Reelin fusion protein is operably linked to a tissue-specific promoter.

[0016] In one aspect, the tissue-specific promoter comprises a synapsin or BM88 promoter.

[0017] In one aspect, the viral vector is an adeno-associated viral vector (AAV) selected from the group consisting of AAV-9, AAV-5, AAV-4, and AAV-1.

[0018] In a second aspect, a viral vector composition for expressing a recombinant Reelin fusion protein is disclosed that is encoded by a nucleotide sequence having from 80, 85, 90, 95 or 99% sequence identity to the nucleotide sequence of SEQ ID NO: 23.

[0019] In a second aspect, the recombinant Reelin fusion protein comprises at least 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 24.

[0020] In a second aspect, the viral vector composition expresses a therapeutically effective amount of the recombinant Reelin fusion protein effective at inducing Reelin-dependent ApoER2 receptor activation and signaling.

[0021] In a second aspect, the viral vector composition expresses a therapeutically effective amount of the recombinant Reelin fusion protein effective at increasing dendritic spine density, long-term potentiation (LTP), or synaptic plasticity.

[0022] In a third aspect, a method of treating a disease or disorder of the nervous system, comprising administering the viral vector of claim 14 or 15 to a subject in need thereof.

[0023] In another aspect, the disease or disorder of the nervous system comprises Fragile X Syndrome.

[0024] In another aspect, the viral vector compositions are administered intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, or subcutaneously to a subject in need thereof.

[0025] In a fourth aspect, a kit is disclosed that comprises any one of the preceding viral vector compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1A shows an exemplary schema of the structure of Reelin and Reelin fragments generated by proteolytic cleavage. Reelin is a large extracellular matrix protein of 450 kDa that starts with a signal peptide followed by an F-spondin homology domain (F-sp) followed by eight Reelin-specific repeats: R1 to R8, each composed by two sub-repeats (A and B) flanking an EGF-like motif (EGF). The C terminus of Reelin ends with a basic stretch of 33 amino acids (++). After

its secretion, Reelin is cleaved at two major sites to produce 5 polypeptides named N-R6, R3-8, R3-6, N-R2 and R7-8. R3-6 is the smallest biologically active fragment. Interaction with Apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR) occurs through the first subdomain of R6. Proteases involved in the N-terminal (N-t) cleavage between Reelin repeat domains R2 and R3 and the C-terminal (C-t) cleavage between Reelin repeat domains R6 and R7 are shown. Figure reproduced from review by Jossin, Yves. 2020. "Reelin Functions, Mechanisms of Action and Signaling Pathways During Brain Development and Maturation." *Biomolecules* 10 (6) 964.

[0027] FIG. 1B depicts an exemplary modeling of the multiple functions of Reelin during dendritic growth and synaptic development and the corresponding signaling pathways (from review by Jossin, Yves. 2020 *supra*).

[0028] FIG. 2Ai shows the effect on secretion if the reelin signal peptide (SP) of a R3+R6v1 expression vector is replaced by a signal peptide from mouse IgK light chain, human serum albumin, human azurocidin or mouse IgK light chain. The different R3+R6v1 expression vectors were transiently transfected into HEK293, Hela or U87 cells. The expressed R3+R6v1 protein in cell lysates or the media was then probed by Western blot analysis.

[0029] FIG. 2Aii shows R3+R6v1 protein expression in U87 cells transiently transfected with plasmids in which R3+R6v1 expression was under the control of a CMV promoter, synapsin promoter, MECP2 promoter or CAG promoter.

[0030] FIG. 2Aiii shows a Western blot of lysates and media (supernatant) of HEK293 cells transfected with R3 or R3+R6v2 expression vectors (labeled in the figure as R3+6). Actin is used as a control.

[0031] FIG. 2Aiv shows a Western blot analysis of cell lysates and media of HEK293 cells transiently transfected with R3+R6v2 or R3+R6v1 expression vectors as described above. Comparable amounts of R3+R6v1 with R3+R6v2 protein expression was detected in cell lysates. However, only R3+R6v2 protein was secreted into the cell media.

[0032] FIG. 2B shows enhanced LTP after theta burst stimulation (TBS) of acute hippocampal slices from wild type (WT) animals treated with the R3+6 peptide (5nM) for 20 minutes.

[0033] FIG. 2C shows LTP is significantly enhanced in the final 20 minutes of recording in slices treated with R3+6 peptide. $* = p < 0.05$

[0034] Figure 3 shows R3+6 distribution *in vivo* in heterozygous reeler mice (A) AAV9_R3+6 injected ICV at postnatal day (pnd) 3 with a titer of 4.52×10^{10} vg/mouse into heterozygous reeler mice express high levels of R3+6 in the cortex and hippocampus (B) Western blots illustrating levels of endogenous Reelin (top), R3+6 in the cortex (middle), or R3+6 in the hippocampus (bottom). High: 4.52×10^{10} vg/mouse, Low: 4.52×10^9 vg/mouse (C) Bar graphs illustrating the quantification of Reelin or R3+6 levels from western blots in (FIG. 3B). WT: wildtype, KO: reeler heterozygous mouse, LD: low dose, HD: high dose. vg: viral genomes. R3+6 designates the R3+R6v2 Reelin adenoviral vector.

[0035] Figure 4 shows that R3+6 expression in heterozygous reeler mice rescues impairment of spine density in the hippocampus. (A) Representative images of Golgi stained mouse hippocampus at low and high magnification. (B) Representative images of 20 μ m long dendritic branches from vehicle injected wildtype, heterozygous reeler mice (HRM), or AAV9_R3+6 injected HRM. Injections were ICV at pnd 3 High: 4.52×10^{10} vg/mouse, Low: 4.52×10^9 vg/mouse (C) Quantification of number of spines per 20 μ m dendritic branch segments from apical hippocampal dendrites in area CA1. N = 40.

[0036] Figure 5 shows that treatment of FMR1 knock out (KO) animals with AAV9_R3+6 rescues hyperactivity in two open field assays. (A) FMR1 KO animals exhibit hyperactivity as measured by distance traveled over 30 min compared with WT animals. (B) FMR1 KO animals injected directly into the hippocampus with a low dose of AAV9_R3+6 at pnd28 demonstrate reduced hyperactivity compared with vehicle treated FMR1 KO animals over the course of 30 min. (C) Total distance travelled by mice in each of the groups in either the center or periphery of the open field arena. (D) A second study of FMR1 KO animals exhibit hyperactivity as measured by distance traveled over 30 min compared with WT animals. (E) FMR1 KO animals injected ICV with AAV9_R3+6 at pnd0 demonstrate reduced hyperactivity with both doses compared with vehicle treated FMR1 KO animals over the course of 30 min. (C) Total distance travelled by mice in each of the groups in either the center or periphery of the open field arena. High dose: 4.6×10^{10} vg/mouse, Low dose: 4.6×10^9 vg/mouse.

[0037] Figure 6 shows that AAV9_R3+6 rescues alterations in mGluR-LTD and spine density in FMR1 KO mice. (A) Representative western blot illustrating R3+6 protein levels in FMR1 treated animals. (B) Average fEPSP slope over time from acute hippocampal slices from experimental groups treated with DHPG to induce mGluR-LTD. (C) Graph illustrates average fEPSP slope for the final 20 minutes of recording after induction of mGluR-LTD. Aberrant mGluR-LTD is rescued in hippocampal slices from FMR1 KO animals treated with the low dose of AAV9_R3+6. (D) Representative images of golgi stained pyramidal neurons from area CA1 in the hippocampus. (E) Overall spine density of basal and apical dendrites from pyramidal neurons in area CA1 of all experimental groups. (F) Overall spine density from dendritic segments of all groups. Both high dose and low dose AAV9_R3+6 treatment rescues aberrant spine density observed in FMR1 KO animals. High dose: 4.6×10^{10} vg/mouse, Low dose: 4.6×10^9 vg/mouse. Data from E – F were generated by Neurodigitech.

[0038] FIG. 7 depicts exemplary recombinant Reelin AAV viral vectors comprising (A) R3+R5v2 and (B) R3+R5v2+R6 expression cassette.

[0039] In the Figures, R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0040] Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the materials, methods, and examples are illustrative only and not intended to be limiting. Other features of the disclosure are apparent from the following detailed description and the claims.

[0041] Titles or subtitles may be used in the specification for the sole convenience of the reader but are not intended to influence the scope of the present disclosure or to limit any aspect of the disclosure to any subsection, subtitle, or paragraph.

I. TERMINOLOGY

[0042] As used herein, the singular forms “a,” “an,” and “the,” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0043] The phrase “and/or,” as used herein and in the claims, is 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. 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 aspect, to A only (optionally including elements other than B); in another aspect, to B only (optionally including elements other than A); in yet another aspect, to both A and B (optionally including other elements); etc.

[0044] As used herein 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 aspect, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another aspect, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another aspect, 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.

[0045] As used herein, SEQ ID NO X/Y indicates SEQ ID NO: X is the nucleotide sequence and SEQ ID NO: Y is the corresponding amino acid sequence.

[0046] When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below those numerical values. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20%,

10%, 5%, or 1%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 10%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 5%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 1%.

[0047] As used herein, the term “substantial change” in the context of the amount of one or more RNA transcripts, an alternative splice variant thereof or an isoform thereof, or one or more proteins thereof, each expressed as the product of one or more of genes, means that the amount of such products changes by a statistically significant amount such as, in a nonlimiting example, a p value less than a value selected from 0.1, 0.01, 0.001, or 0.0001.

[0048] As used herein, the terms “subject” and “patient” are used interchangeably to refer to an animal or any living organism having sensation and the power of voluntary movement, and which requires for its existence oxygen and organic food. Non-limiting examples include members of the human, equine, porcine, bovine, rattus, murine, canine, and feline species. In some aspects, the subject is a mammal or a warm-blooded vertebrate animal. In certain aspects, the subject is a non-human animal. In specific aspects, the subject is a human.

[0049] When a range of values is listed herein, it is intended to encompass each value and sub-range within that range. For example, “1-5 ng” or a range of “1 ng to 5 ng” is intended to encompass 1 ng, 2 ng, 3 ng, 4 ng, 5 ng, 1-2 ng, 1-3 ng, 1-4 ng, 1-5 ng, 2-3 ng, 2-4 ng, 2-5 ng, 3-4 ng, 3-5 ng, and 4-5 ng.

[0050] It will be further understood that the terms “comprises,” “comprising,” “includes,” and/or “including,” when used herein, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0051] As used herein, the terms “treat,” “treatment,” “treating” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a disorder. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease, or disorder. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disorder is reduced or halted. That is, “treatment” includes not just the

improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment).

[0052] As used herein, the term “effective amount” of a substance is an amount sufficient to produce a desired effect. In some embodiments, an effective amount of a vector is an amount sufficient to infect a sufficient number of target cells of a target tissue of a subject. In some embodiments, a target tissue is neuronal tissue (e.g., brain tissue, such as, cerebral cortex (CCTX), hippocampus (HIPPO), thalamus (TH), inferior colliculus (IC), olfactory bulb (OB), anterior olfactory nucleus (AON), hypothalamus (HT), cerebellum (CBL), etc.). In some embodiments, an effective amount of a vector or composition may be an amount sufficient to have a therapeutic benefit in a subject, e.g., to increase or replace the expression of a gene or protein (e.g., Reelin), to extend the lifespan of a subject, to improve in the subject one or more symptoms of a neurologic disorder, e.g. Fragile X Syndrome. The effective amount will depend on a variety of factors such as, for example, the species, age, weight, health of the subject, and the mode or site of administration, and may thus vary among subjects and administrations.

[0053] As used herein, the terms “protein” and “polypeptide” are used interchangeably and thus the term polypeptide may be used to refer to a full-length protein and may also be used to refer to a fragment of a full-length protein, and/or functional variants thereof. As used herein, the terms “polynucleotide” and “nucleic acid sequence” may be used interchangeably and may comprise genetic material including, but not limited to: RNA, DNA, mRNA, cDNA, etc., which may include full length sequences, functional variants, and/or fragments thereof.

[0054] The term “sample,” as used herein, generally refers to a biological sample. A sample may be a fluid or tissue sample. The sample may include proteins and nucleic acid molecules, such as deoxyribonucleic acid (DNA) molecules, ribonucleic acid (RNA) molecules, or both. The RNA molecules may be messenger RNA (mRNA) molecules. The sample may be a tissue sample. The

sample may be a cellular sample, such as a sample comprising one or more cells. The sample may be plasma, serum or blood (e.g., whole blood sample). The sample may be a cell-free sample (e.g., cell-free DNA, or cfDNA).

[0055] As used herein, the term “tissue” refers to an aggregation of morphologically similar cells and associated intercellular matter, i.e., extracellular matrix, acting together to perform one or more specific functions in the body. In some embodiments, tissues fall into one of four basic types: muscle, nerve, epidermal, and connective. In some embodiments, a tissue is substantially solid, e.g., cells within the tissue are strongly associated with one another to form a multicellular solid tissue. In some embodiments, a tissue is substantially non-solid, e.g., cells within the tissue are loosely associated with one another, or not at all physically associated with one another, but may be found in the same space, bodily fluid, etc. For example, blood cells are considered a tissue in non-solid form.

[0056] The term “expression vector”, as used herein, refers to a nucleic acid construct comprising nucleic acid elements sufficient for the expression of a transgene product in a cell or in an in vitro assay. For example, a transgene expression vector, disclosed herein, comprises a promoter operatively linked to a transgene transcription unit comprising a transcription initiation site, a 5' untranslated region (UTR), a transgene nucleotide sequence and a 3' untranslated region (UTR) comprising one or more post-transcriptional regulatory elements, e.g., a polyadenylation sequence.

[0057] The term “operably linked” refers to a functional relationship between two or more polynucleotide (e.g., DNA) segments. Typically, the term refers to the functional relationship of a transcriptional regulatory sequence and a transgene to be transcribed. For example, a promoter or enhancer sequence is operably linked to a transgene if it, e.g., stimulates or modulates the transgene transcription in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a sequence are contiguous to that sequence or are separated by short spacer sequences, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0058] As used herein, “recombinant” refers to nucleic acids or polypeptides that are genetically engineered.

[0059] A "transgene" is a polynucleotide sequence that may encode an RNA (mRNA) that is translated into protein, or a polynucleotide may encode an RNA that is not translated into protein (e.g. guide RNAs, ribozymes, aptamers, antisense RNAs, piwi-interacting RNAs (piRNAs), short interfering RNAs (siRNAs), microRNAs (miRNAs), shRNAs or recombinant U RNAs). In some aspects, a transgene sequence encodes a therapeutic protein or a recombinant protein, e.g., Reelin protein or Reelin protein fragment, or recombinant Reelin fusion protein comprising one or more Reelin repeats as disclosed herein. In some aspects, a transgene nucleotide sequence is a cDNA sequence. In some aspects, the transgene nucleotide sequence may comprise one or more introns. In other aspects, the transgene can be polycistronic (e.g., two coding regions separated by internal ribosome entry site (IRES)). In some aspects, a transgene may encode more than one protein.

[0060] In one aspect, a transgene comprises a "protein coding sequence" or a sequence that encodes a particular protein or polypeptide, i.e., a nucleic acid sequence that is capable of being transcribed into mRNA and translated into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence may be determined by a start codon at the 5' terminus (N-terminus) and a translation stop nonsense codon at the 3' terminus (C-terminus). A coding sequence can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic nucleic acids. A transcription termination sequence will usually be located 3' to the coding sequence.

II. RECOMBINANT REELIN NUCLEOTIDE AND PROTEIN SEQUENCES

[0061] The Reelin gene is also known as the PRO1598, ETL7, RL or LIS2. Located on chromosome 7 at 7q22.1 in humans, the Reelin gene (HGNC: 9957; Entrez Gene: 5649; Ensembl: ENSG00000189056; OMIM: 600514; RefSeq DNA sequence NC_000007.14 is approximately 518 kb in length and consists of 67 exons that encode a 388kD Reelin protein (UniProtKB: P78509-1).

[0062] Reelin encodes a large secreted extracellular matrix protein thought to control cell-cell interactions critical for cell positioning and neuronal migration during brain development. Mutations of this gene are associated with autosomal recessive lissencephaly with cerebellar

hypoplasia. Reelin is abundantly produced during brain ontogenesis by the Cajal-Retzius cells and other pioneer neurons located in the telencephalic marginal zone and by granule cells of the external granular layer of the cerebellum. In adult brain, Reelin is preferentially expressed in GABAergic interneurons of prefrontal cortices, temporal cortex, hippocampus and glutamatergic granule cells of cerebellum. Reelin is also expressed in fetal and adult liver.

[0063] Reelin may be involved in schizophrenia, autism, bipolar disorder, major depression and in migration defects associated with temporal lobe epilepsy.

[0064] In certain aspects, diseases associated with Reelin deficiency include Fragile X Syndrome.

[0065] Transcription of the Reelin gene generates two transcript variants encoding distinct isoforms have been identified. Transcript Variant 2 lacks an in-frame, 6 nt microexon in the coding region compared to the variant 1, resulting in an isoform (b) that is 2 aa shorter than isoform a.

[0066] Homo sapiens reelin (RELN), transcript variant 1, mRNA (Accession No.: NM_005045) is 11708 nucleotides in length which encodes a Reelin isoform 'a' precursor having an amino acid sequence of 3460 residues (SEQ ID NO: 44, NCBI Reference Sequence: NP_005036.2, see below).

[0067] Homo sapiens reelin (RELN), transcript variant 2, mRNA Accession No. NM_173054 is 11702 bp in length that encodes a Reelin isoform 'b' precursor having an amino acid sequence of 3458 residues (NCBI Reference Sequence: NP_774959.1). This isoform has a deletion of two amino acid deletion at the C terminus (valine and serine) corresponding to residues 3428 and 3429 of SEQ ID NO: 44.

[0068] As used herein, the term "Reelin" encompasses Reelin protein isoforms and proteolytic cleaved Reelin protein fragments. In certain aspect, Reelin protein includes any Reelin protein variant or mutant.

[0069] In one aspect, Reelin gene may comprise a polymorphic GGC triplet repeat located in the 5'-UTR region of RELN gene, which harbors in the normal population 8 to 10 repeats, but is significantly increased in autistic patients to carry 4 to 23 additional repeats (RELN_HUMAN-P78509).

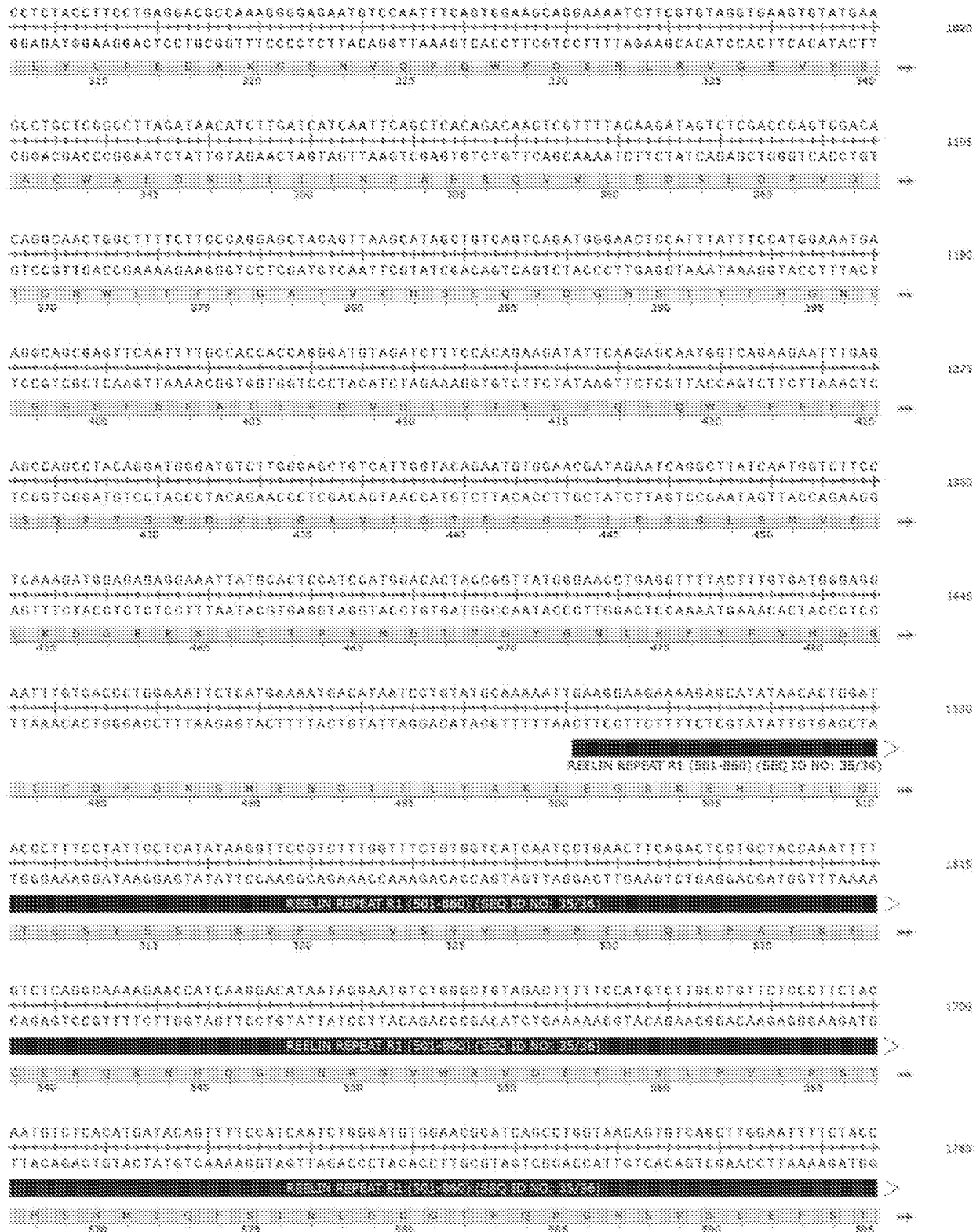
[0070] In certain aspects, a Reelin cDNA may comprise one or more single nucleotide polymorphisms (SNPs).

[0071] In certain aspects, Reelin protein encompasses recombinant Reelin fusion proteins comprising one or more Reelin repeats as described, for example, below.

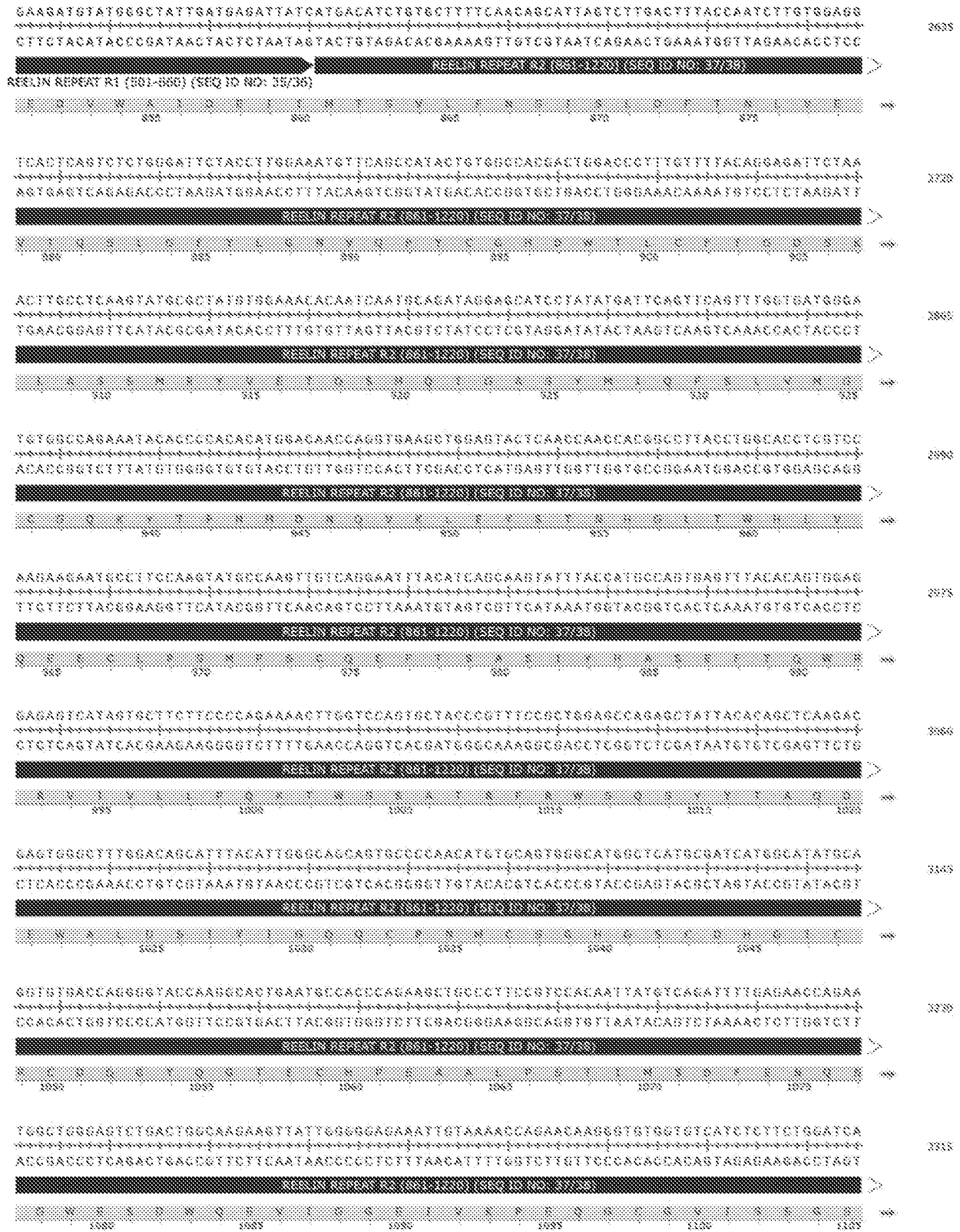
[0072] The nucleotide and amino acid sequence of full length Reelin below shows the location of the Reelin repeats 1-8. The arrow indicates the location of a proteolytic cleavage site at Pro 1244. The site of cysteine 2101 involved in covalent dimerization is highlighted with a black box.

Step 22

14



16

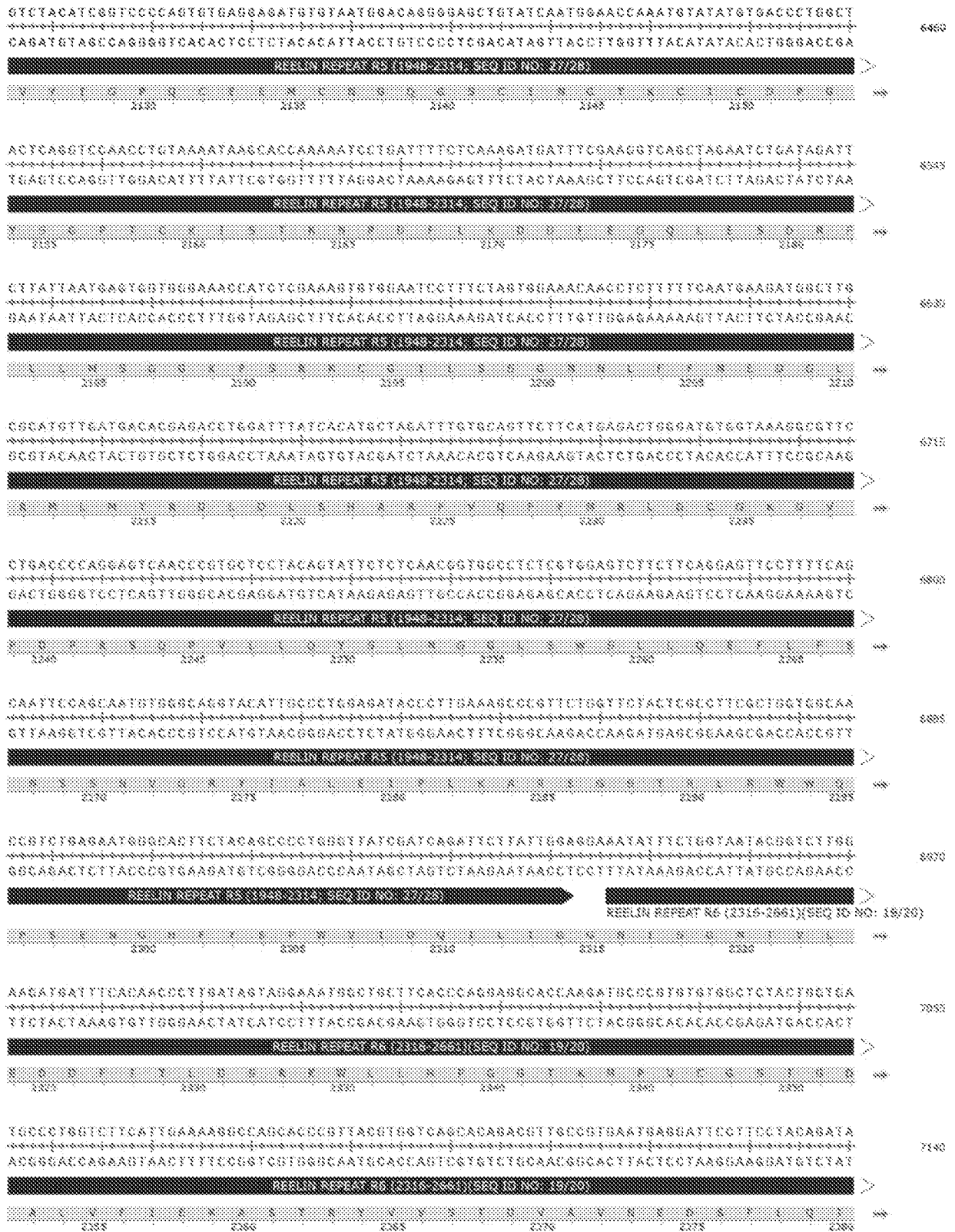


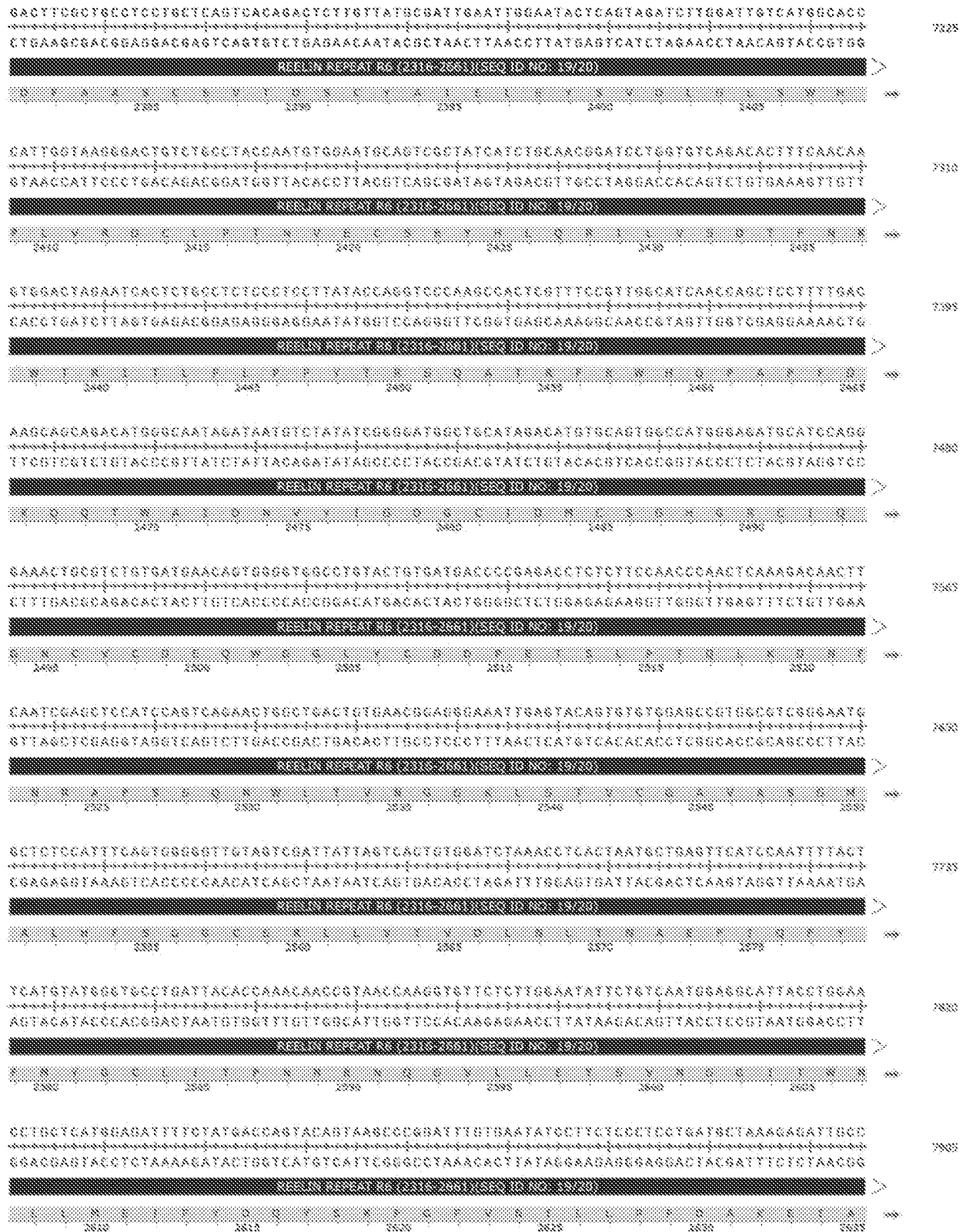
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GTGTGTCCCTGAAACTTCTTACCTGTTCTTASTGTAACAATAAGGTTCCAGAGAAAGTAAGGTCGTTCTGCTCTAAGGCTACCT
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
H T G D T E E W T R I T I V I P S S L A S S K T E F R N
1365 1370 1375 1380 1385
TCCAGGAGAGCAGCTCACAGAAAAACGTGCTCCATTGCTTTAGATGGAGTGTACATATCCGAGCCTTGTCCAGTTACTGCAG
AAGTCCTCTCGTGGAGTGTCTTTTGCACGGAGGTAAACCAAACTCTACCTEACATGTATAGGCTCGGAACAGGGTCAATGACGTC
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
I Q E E S S D D K N V P P F G L D D V Y I S P C P V C S
1390 1395 1400 1405 1410 1415
TGCATGAGGACTGCAATTCAGGAGTGTGTTTCTGTGACCTGGGATATAGTCTGCACAAAGAACTGTGTGTAAATGTCCCG
ACCGGTACCCCTGACATAAGTCCCTCACACAAAGACACTGGACCTATATGACGACGTTCTCTTGACACACAGTTACAGGGG
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
D H D D C I S G V C F L D L G Y T A A Q G T C T S N V F
1420 1425 1430 1435 1440 1445
AATCACAATGAGATGTTCCGATAGGTTTGAAGGGAAGCTCAGCCCTCTGTGTGTACAAATACAGGTGCCCAGGTTGGAAGTGGCT
TTAGTGTGTTACTGTACAAGCTATCCAAACTCCCTTCGAGTGGGAGACACCATGTTCTATTGTCCACGGGTGGAAGCTTGACGGA
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
N H N E N F D R E E L S L S F L N Y F I T G A Q V E T S
1450 1455 1460 1465 1470
GTGGAACACTTAACGATGGCAAAATCTCTCTACTTCAATGCCCCCTGGGAAAAGGAAAGCCCGACGGTCCCTCTGGACACCAGGAA
CAGCTTSTBAATTTGCTACCGTTTATAGAGAGATGAAGTTACGGGGACCTTTTCCCTTCGGGCTTGGCAGGAGACCTGTGTGTCTT
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
C G T I N D G K S L Y F N G K G V N A A N T V P L D T E N
1475 1480 1485 1490 1495 1500
TATCAGACTTGTTCAAATTTATATACAAATTTGAAGCAAAACTTCAGGCAATACCTGCAATCAAAACCAAGAACTAGAAATGAAGGG
ATAGTCTGAACAAGTTAAAAATATATGTTTAACTCTGCTTTTGAAGTCCGTAATGACGTAAGTTGCTTCTGTATCTTTACTTCCC
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
I K L Y Q T Y I Q I G S P T S S I T C I N F E T N N G
1505 1510 1515 1520 1525 1530
CTTATTGTTCAATTCAAATGACAATGGGATACTCTGGCAATTTGCTTCGAGAGTTGGACTTCAATGCTCTCTCTGGAACCAACAGA
GAATAACAAATCATAAGTTTACTGTTACCTATGAGACCCGTAACGGAAGCTCTCAACCTGAAGTACAGGAAGGACCTTGGTGTCT
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
L I V Q Y S N D N G I L W H L L P F L D F H E F L E P Q
1535 1540 1545 1550 1555 1560
TCATTTCATTGACCTGCCACAGGAGCGGAAGACACCTGCAAGGGCATTTCGATGGTGGCAACGCAACATGGGAAGCATTTCAGC
AGTAAAGGTAAGTGCACGGTGTCTCTGCTGACGTTCCCGTAAAGCTACCAACCTTGGCTTTGACCTTCTGTAAGTGG
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
I S I D L T F Q D A K T E A T E F E W N O P G N E H S
1565 1570 1575 1580 1585
CCAGTGGGCTTTGATGATGTTCTTATAGGAATGAATGACAGCTCTCAAACTGCAATTTCAAGACAAATTTGATGGCTCTATAGAT
GGTACCCGAAAGCTACTACAGGAATATECTTACTTACTGTGAGAGATTGACCTAAATTTCTGTTTAACTACCGAGATATCTA
REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
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1590 1595 1600 1605 1610 1615

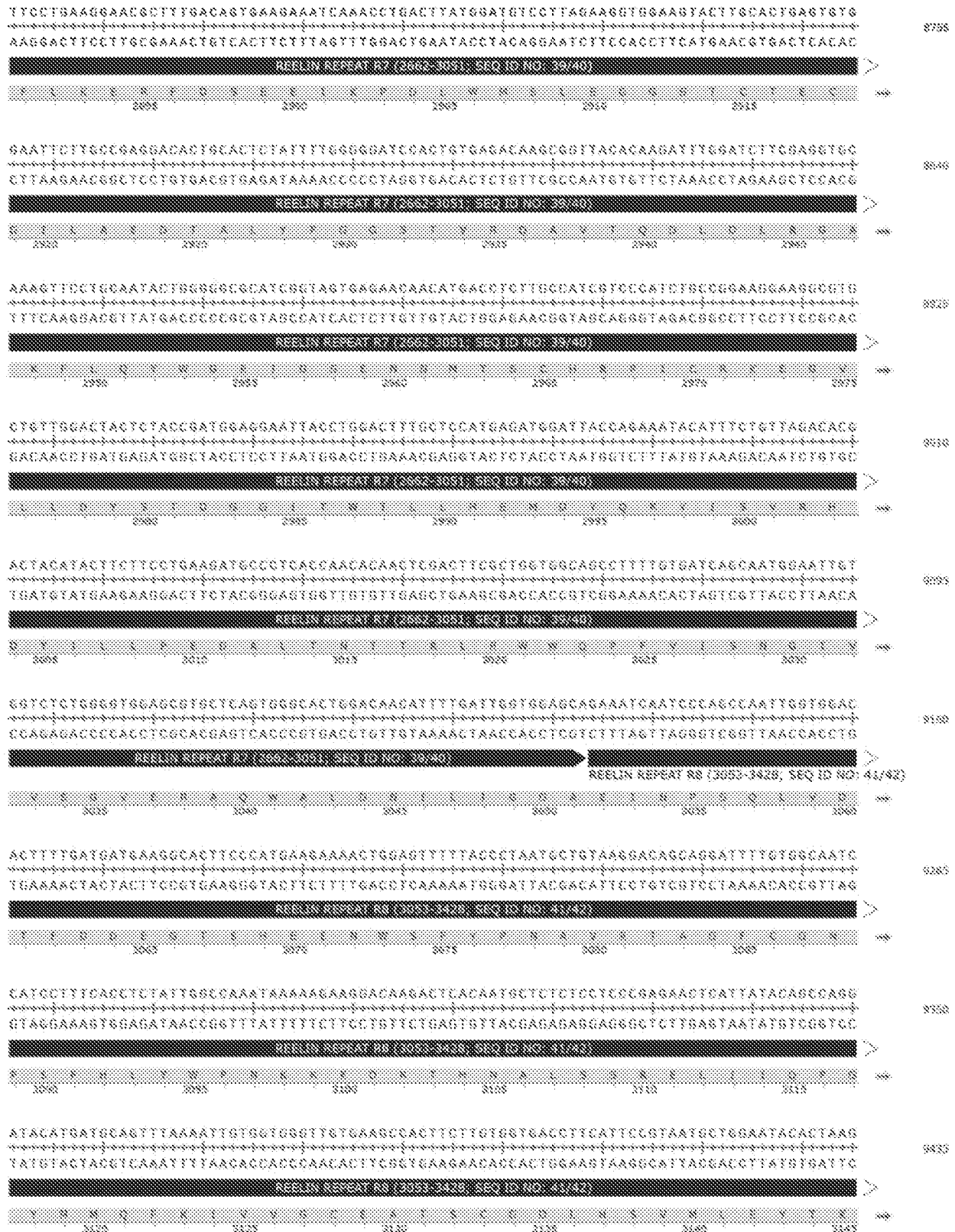
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ACATAGGAAAACTCTGTTATGCTGAGACCTGGGATTTTCATGCTGCAGCATCTACGTTTTTGCASTTTGAAAIDAGCATGGGCTG TGTATCCTTTTGGAGCAATACGACTCTGGACCTAAAGTACACAGCTCTAGATGAAAAACCTCAAACTTTACTCGTACCCGAC REPEAT P4 (1598-1647, SEQ ID NO. 25/26) S I G K F A Y A E T W D F H V S A E Y F L H E E E N G C 1649 1658 1663 1668 1673 1676	5013
TAGCAAGGCTTTCAGCACTCCACAGTGTACAGCTCCAGTATTTCTTGAACAATGGCAAGGACTGGCATCTTGTACCGAAGAG ATGCTTCGGGAAGTCTTGAGGTTGTACATGTCGAGGTCATAAGAGACTTGTACCTTCTGACGCTAGAACAGTGGCTTCTC REPEAT P4 (1598-1647, SEQ ID NO. 25/26) S E P T G N N H V Q L D Y S L N N C K D W H L Y T E E 1673 1682 1687 1692 1697 1702 1705	5100
TGTGTTCTCCAACTTGGCTGTCTGCAATTACACGGAAGTTCAATTTACACCTGGGAAGATTTCAGAAATGGAAAGCGGATCA ACACAAGGAGGTTGTAAACCGACAGACGTAAATGTGCTTCAAGTTAAATGTGGAGCTTGTAAAGTCTTAACCTTGGCTGAT REPEAT P4 (1598-1647, SEQ ID NO. 25/26) C V P F T I S C L H Y T E S S I T T E E E E F Q N N K R I 1703 1712 1717 1722 1727 1732	5183
CTGTCTACCTTCCACTCTCCACCATTTCTCTCCAGGACCCGGTTTCAGATGGATTTCAGCCAACTACACTGTGGGGGCTGATTCTCTG GACAGATGGAAGTTGAGAGGTGTAAGAGGTCCTGGGCAAGTCTAGCTAAGTCCGGTGTATGTACACCCCGACTAAGGAC REPEAT P4 (1598-1647, SEQ ID NO. 25/26) I V Y A F L S T I S F A T F R N I A N Y T V G A D S W 1733 1742 1747 1752 1757 1762	5270
GGGGATTCATAATGTTGACTAGGCTCAGGTTGGCTTGGATGTTGGTCAGGAGGAGGATTTGTGATGTTGACGCTGTGTGT CCCTAACTATTACACATGACCGGAGTCCACGGGAACCTACAGGACTCTGCTGCCCTAAACACTACGACCTGCGACACACACA REPEAT P4 (1598-1647, SEQ ID NO. 25/26) A I D N V Y I A A D C P W N C E C E N T I C D A D R C V E 1763 1772 1777 1782 1787 1792	5355
GACCGGGGCTTTGGTGGACCTATTGTGTTCTGTTGTTCTCTGCTCCCTCGATTCTTAAAGACGATTTCATGGGAATTTACATC CTGCCCCGAAACCTCTGGGATAACACAAGGACAACAAGGAGACGGGAGCTAAGAAATTTCTGCTAAAGTTACCCYTAATGTAG REPEAT P4 (1598-1647, SEQ ID NO. 25/26) D S G F G C P T C V P V V P L T S I L K D F N N L R 1793 1802 1807 1812 1817 1822	5440
CTGACCTTTGGCTGAAGTGTATGGTGCAGAGAGGGGGAATCTGAATGGTGAAGCATCAATCTGGAACATCTCTAATTTTAA GACTGGAAACCGGACTTCACATACCAGCTCTCTCCCTTAGACTTACCACCTTTGGTAGTTTAGACCTTTGAGAGATTAAAAAT REPEAT P4 (1598-1647, SEQ ID NO. 25/26) P D L W F E Y T G A E R D L R S D E T E K G G T S L I T E 1823 1832 1837 1842 1847 1852	5525
AGGGGAAGGACTAAGGATGCTTATTTCAAGAGATCTAGATTGTACAAATACAATGTATGTCCAGTTTTCACCTTAGATTATAGCA TCCCTTCTGATTCCTAGCAATAAAGTTCTCTAGATCTAACAATGTTTATGTTACATACAGGTCAAAAGTGAATCTAAATATCT REPEAT P4 (1598-1647, SEQ ID NO. 25/26) D E S L R M L I D K D L D C Y N T H Y V D F L R F I A 1843 1852 1857 1862 1867 1872	5610

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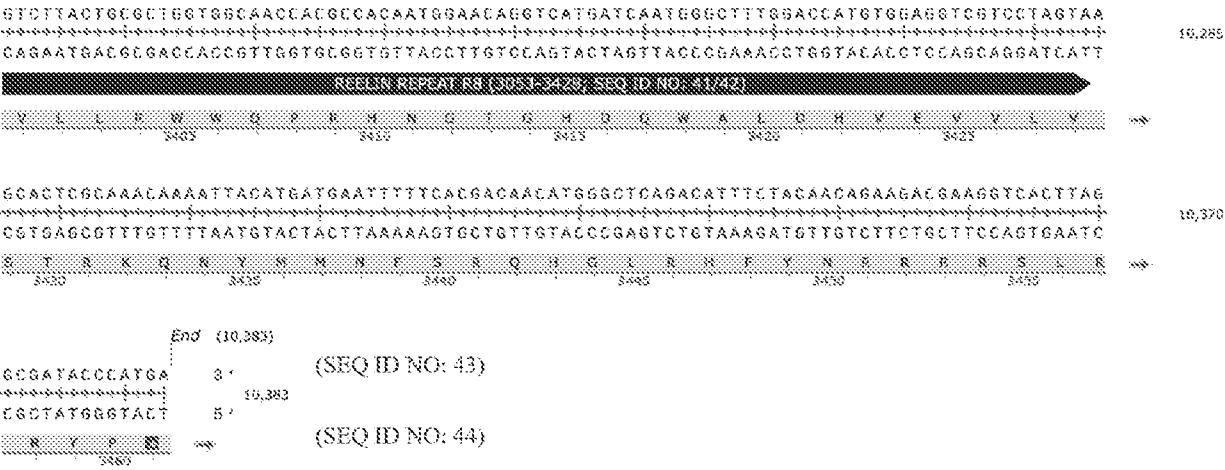




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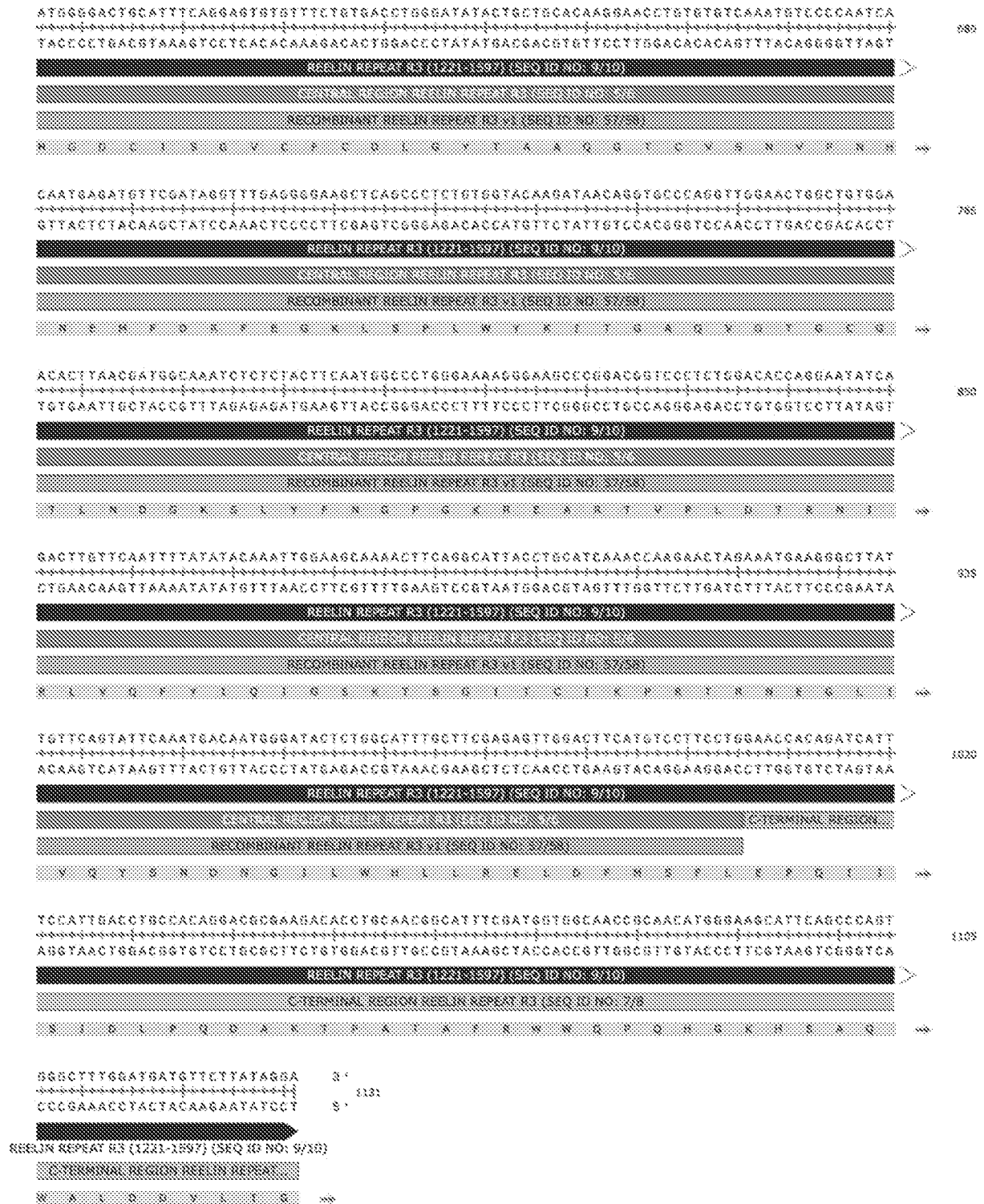
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 GTTACTCTACAAGCTATCEAACTCCCTTCGAGTCGGGAGACACCATGTTCTATTGTCCAGGGGTCCAACCTTGACGAGACACT
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)
 N E N F D R F E G K L S P L W Y R I T G A Q V G T G C Q
 ACATTTAAGGATGCAAAATCTCTACTTCAATGGCCCTGAGGAAAAGGGAAGCCCGGACGGTCCCTCTGGACACAGGAATATCA
 TGTGAATTGCTACCGTTTACAGAGAGATGAAGTTACCGGAGCCCTTTTCCTTCGGGGCTGCCAGGGAGACCTGTGGTCTTTATAGT
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)
 T L N D E K S L Y F R D S F S K R E A A T V P L D T R N I
 GACTTGTTCAAATTTATATACAAATTTGAAGCAAAAACCTTCAGGCATTACCTGCATCAAACCAAGAACTAGAAATGAAGGGCTTAT
 CTGAACAAGTTAAATATATGTTTAACTTCGTTTGAAGTCCGTAATGGACGTAGTTTGGTCTTGTATCTTTACTTCCGGAATA
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)
 R L Y Q Y F T I Q I G S K T S G I T C I K F R Y R N E G L I
 TGTTCAGTATTCAAATGACAATGGGATACTCTGGCATTGTCTTCGAGAGTTGGACTTCATGTCTCTCTGGAACACAGATCATT
 ACAAGTCATAAGTTTACTGTTACCTATGAGACCGTAAACGAAGCTCTCAACCTGAAGTACAGGAAGGACCTTGGTGTCTAGTAA
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)
 V Q Y S N D N G I L W K L L R E L D F H S F L E F Q I I
 YCCATTGACCTGCCACAGGACCGGAAGACACCTGCAACGGCATTTCGATGGTGGCAACCGCAACATGGGAAGCATTACGCCCAST
 AGGTAACCTGGACGGTGTCTCTGCGCTTCTGTGGACGTTGCCGTAAAGCTACCAACGTTGGCGTGTGTACCTTCTGTAAAGTCGGGTCA
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)
 S I D L P Q D A E T F A T A F R W W Q P Q H G E H S A Q
 GGGCTTTGGATGATGTTCTTATAGGA 3'
 CCCGAAACCTACTACAAGGAATATCCT 5'
 REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10)
 C-TERMINAL REGION REELIN REPEAT
 W A L D D Y L I S

REELIN REPEAT R3v1

TTCAAGCACTACTGCTCCAGTTCTTCTTCAGTACTCTCATGATGCTGGTATGCTCTGGTTTCTGGTGAAGAAGGCTGTTACCCGG	
AAAGTGTGATGACBAGGTCAAGAAGAAAGTCATGAGAGTACTACGAACATACAGGACCAAAGAACCACTTTCTTCBACAATGGGGC	85
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
F G S T A P V L L Q Y S H D A G M S W F L V E E C C T P	→
CTTCTGCAGGCAAGGATGCGAAGGAAACTCCAGAGAACTAAGTGAGGCCACCATGATATCACACAGGGGACTTTGAAGAAATGGAC	
GAAGACGTCCGTTTCTACGGTTCTTTGAGGTCTCTTGATTCACCTGGGTGGTACATAGTGTGTCCCTCGAAACTTCTTACCTG	170
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
K S A Q K G C E G N S K E L D E P T H Y H T G D F E E W T	→
AGAATCAACATTGTTATTCCAAGSTCTCTTGCATCCAGCAAGAACAGATTCCGATGGATCCAGGAGAGCAGCTCACAGAAAAAC	
TTCTTAGTGGTAACAATAAGGTTCCAGAGAACGTAGGTCTGTTCTGGTCTAAGGGTACCTAGGTCTCTCTGTCGAGTGTCTTTTGTG	255
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
R I T I V I F R S L A S S K T E F R W I Q E S S S Q K N	→
GTGCTCCATTGTTGTTAGATGGAGTGTACATATCCGAGCTTGTCCAGTTACTGCAAGTGGCCATGGGGACTGCAATTTCAAGSAG	
CACGGAGGTAAACCAATCTACCTCACATGTATAGGCTCGGAAACAGGGTCAATGACGTACCCGGTACCCCTGACGTAAAGTCTCTC	340
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
V P P F G L D G V Y I S E F C P S Y C S G H G D C I S G	→
TGTGTTTCTGTGACCTGGGATATACTGCTGCACAAGGAACTGTGTGTCAAAATGTCCCAATCACAAATGAGATGTTTCAATAGGTT	
ACACAAGACACTGGACCTTATATGACGACGTGTCTCTTGGACACACAGTTTACAAGGGTTAGTGTACTCTACAAGCTATCCAA	425
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
N C F C D L G Y T A A Q G T C V S N V F N H N E N F O R E	→
TGAGGGGAAGCTCAGCCCTCTGTGTGTACAAGATAACAGGTGCCAGGTTGGAACTGGCTGTGGAACTTAAAGATGGCAAAATCT	
ACTCCCTTTCAGAGTGGGAGACACCATGTTCTATTTGTCCAGGGGTCCAACCTTGACGACACCTTGTGAATTTGCTACCGTTTAGA	510
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
E G K L S P L W Y K I T G A G V G T G C G T I N O G E S	→
CTCTACTTCAATGGCCCTGGGAAAAGGGAAGCCCGACGGTCCCTCTGGACACCAGGAATATCAGACTTGTTCAAATTTTATATAC	
GAGATGAAGTTACCGGACCCCTTTTCCCTTCGGGCTGCCAGGGAGACCTGTGTCTCTTATAGTCTGAACAAGTTAAATATATG	595
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
L Y F N S P G K R E A K T V P L D I K N I R L V Q F Y I	→
AAATTGGAAGCAAACTTCAGGCATTACCTGCATCAAAACCAAGAACTAGAAATGAAGGGCTTATTGTTCAATATTCAAAATGACAA	
TTTAACCTTCTGTTTGAAGTCCGTAATGGACGTAGTTTGGTTCTTTGATCTTTACTTCCCGAATAACAAGTCATAAGTTTACTGTT	680
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
Q I G S K T S G I T C T K P R T R N E G L I Y Q Y S N D N	→
TGGGATACTCTGGCATTGCTTTCGAGAGTTGGACTTCATGTCTTCTG	3'
ACCCATGAGACGTAACGGAAGCTCTCAACCTGAAGTACAGGAAGGAC	5'
RECOMBINANT REELIN REPEAT R3v1 (SEQ ID NO. 57/58)	
G I L W H L L F E L D F H S F L	→

REELIN REPEAT R3 COMPARED WITH REELIN REPEAT R3v1

TCGAGAGAGCAGAGATCATCCAGTTATCAATCCAACTTTACCTCAGAACTTTATGAGAGCCAGCTTTTGATTACCGTA AGGCTCTTCGTCCTTCGTCTAGTAGGGTCAATAGTTAGGTTGAAATGGAGTCTTGAAATACTCTTCGGTCGAAACTAATGGGAT REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) N-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 3/4) S E K Q K Q I I P V I N P T L F Q N P Y E K P A F D Y P	85
TGAATCAGATGAGTGTGTGGTTGATGTTGGCTAATGAAGGAATGGTTAAAAATGAAACCTTCTGTCTGCCACACCATCAGCAAT ACTTAGTCTACTACACACCAACTACAAACGATTACTTCTTACCAATTTTACTTTGGAAGACACGACGGTGTGGTAGTCGTTA REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) N-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 3/4) K N Q M S V W L M L A N E G M Y K N E T F C A A Y F S A N	170
GATATTTGAAAAATCAGATGGAGATCGATTTGCAGTAACCTCGAGATTTGACCCCTGAAACCTGGATATGTGCTACAGTTCAAGCTA GTATAAACCTTTAGTCTACCTCTAGCTAAACGTCATTEAGCTCTAAACTGGGACTTTGGACCTATACACGATGTCAAGTTGAT REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) N-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 3/4) I F G K S D G D R F A V T R D L T L N P G Y V L G F K L	255
AACATAGGTTGTGCAATCAATTCAGAGTACTGCTCEAGTTCTTCTTCACTACTCTCATGATGCTGTATGTCTGTGTTCTGG TTGTATCCAACACGGTTAGTTAAGTGTCTATGACGAGGTCAAGAAGAAAGTCAATGAGAGTACTACGACCATACAGGACCAAGAGAC REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) N-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 3/4) W T D C A N Q F S S T A P V I L Q Y G H D A S M S W F L	340
TGAAAGAGAGCTGTTACCCGCTTCTGCAAGGCAAGGATGCGAAGGAAACTCCAGAGAACTAAGTGAGCCCAACCATGTATCACAC ACTTTCTTCGACAATGGGCGAAGAGCTCCGTTTCCTACGCTTCTTTGAGGCTCTCTTGATTCACTCGGTTGTTACATAGTGTG REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) SEQUENCE IDENTICAL TO REELIN REPEAT R3 (SEQ ID NO: 9/10) RECOMBINANT REELIN REPEAT R3 v1 (SEQ ID NO: 57/58) V K E G C Y P A S A G N G C E G N S R E L S E P T H Y H Y	425
AGGGGACTTTGAGGAATGGACAAGAATCACCAATTGTTATTCGAAGTCTCTTTCATCCAGCAAGACCAGATTTCGATGGATCCAG TCCCTGAAACCTTCTTACCTGTTCTTAGTGGAACAATAAGGTTCCAGAGAACTAGGCTGTTCTGTCTAAGGCTACCTAGGTC REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) SEQUENCE IDENTICAL TO REELIN REPEAT R3 (SEQ ID NO: 9/10) RECOMBINANT REELIN REPEAT R3 v1 (SEQ ID NO: 57/58) G D F E E W T R I T I V I P N S L A G S K Y R P K W I Q	510
GAGAGCAGCTCACAGAAAAAGTGCCTECATTTGCTTTAGATGGAGTGTACATATCCGAGGCTTGTCCAGTTACTGCASTGGCC CTCTGTCGASTGTCTTTTTCACGGAGGTAAACCAATCTACCTCACATGTATAGGCTCGGAACAGGGTCAATGACGTACCGG REELIN REPEAT R3 (1221-1597) (SEQ ID NO: 9/10) SEQUENCE IDENTICAL TO REELIN REPEAT R3 (SEQ ID NO: 9/10) RECOMBINANT REELIN REPEAT R3 v1 (SEQ ID NO: 57/58) E S S S Q K N Y P F F D L D G Y Y I S E F C P S Y E S S	595



REELIN REPEAT R3 SEQUENCES

• REELIN SIGNAL SEQUENCE:

ATGGAGCGCAGTGGCTGGGCCCCGGCAGACTTTCTCTCTAGCCCTGTTGCTGGGGGGCGACGCTGAGGGCGCGCGCG (SEQ ID NO: 1)
 TACCTCGCGTCACCGACCCGGGCGCTCTGAAAGGAGGATCGCGACAACGACCCCGCTGCGACTCCCGCGCGCGCG
 REELIN SIGNAL SEQUENCE (SEQ ID NO: 1/2)
 M T R S G W A K N Q T F L I A I L I L D A Y L E A R K (SEQ ID NO: 2)

• REELIN REPEAT R3 (N-TERMINAL REGION)

TCCGAGAAGCASAAGCAGATCATCCAGTTATCAATCCAACCTTACCTCAGAACTTTATGAGAAGCCAGCTTTTGAATTACCCTA 85
 AGGCTCTTCGTCTTCGTCTAGTAGGGTCAATAGTTAGGTTGAAATGGAGTCTTGAAAATACTCTTCGCTCGAAAATAAATGGGAT
 N TERMINAL R3v1 (SEQ ID NO: 3/4)
 S E K Q K Q I I P V I N P T L F Q N F Y E K P A F D Y P
 TBAATCAGATCAGTGTGTGGTGGATGTTGGCTAATGAAAGGAATGGTTAAAAATGAAACCTTCTGTGCTGCCACACCATCAGCAAT 170
 ACTTAGTCTACTCACACACCAACTACAACCGATTACTTCCTTACCAATTTTACTTTGGAAGACACGACGGTGTGGTAGTCGTTA
 N TERMINAL R3v1 (SEQ ID NO: 3/4)
 M N Q H E V W L M L A N E G M V K N E T F C A A I P S A M
 GATATTTGAAAAATCAGATGGAGATCGATTTCAGTAACCTCGABATTTGACCCCTGAAACCTGGATATGTGCTACAGTTCAAGCTA 255
 CTATAAACCTTTTAGTCTACCTCTAGCTAAACGTCATTGAGCTCTAAACTGGGACTTTGGACCTATACACGATGTCAAGTTCCGAT
 N TERMINAL R3v1 (SEQ ID NO: 3/4)
 I F C K S D C D E F A V Y T D L T L V F C Y V L Q Y K L
 AACATAGGTTGTGCCAATCAA 3'
 TTGTATCCAACACGGTTAGTT 5' 276
 N TERMINAL R3v1 (SEQ ID NO: 3/4)
 R I S C A N Q 90

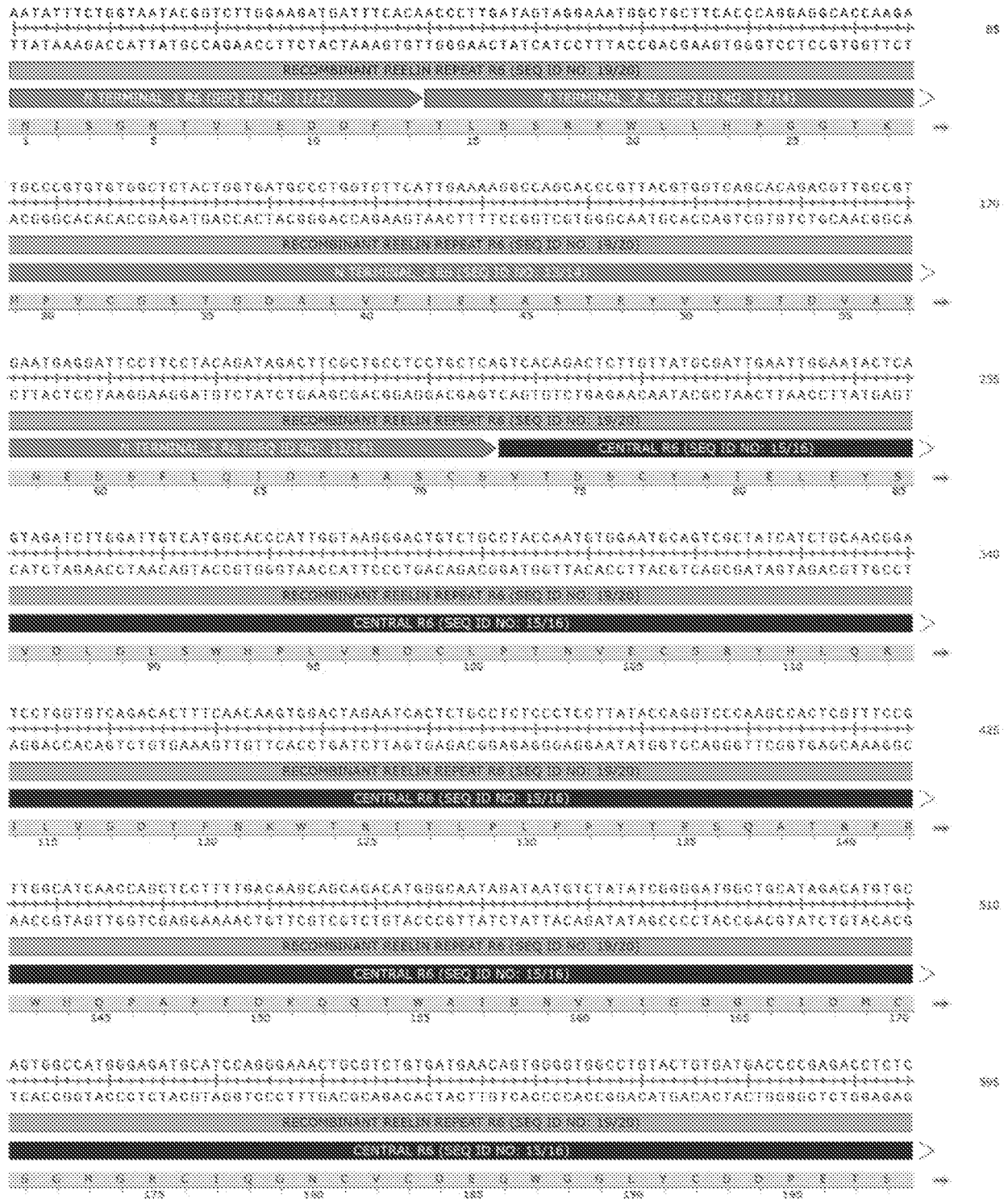
• REELIN REPEAT R3 (CENTRAL REGION):

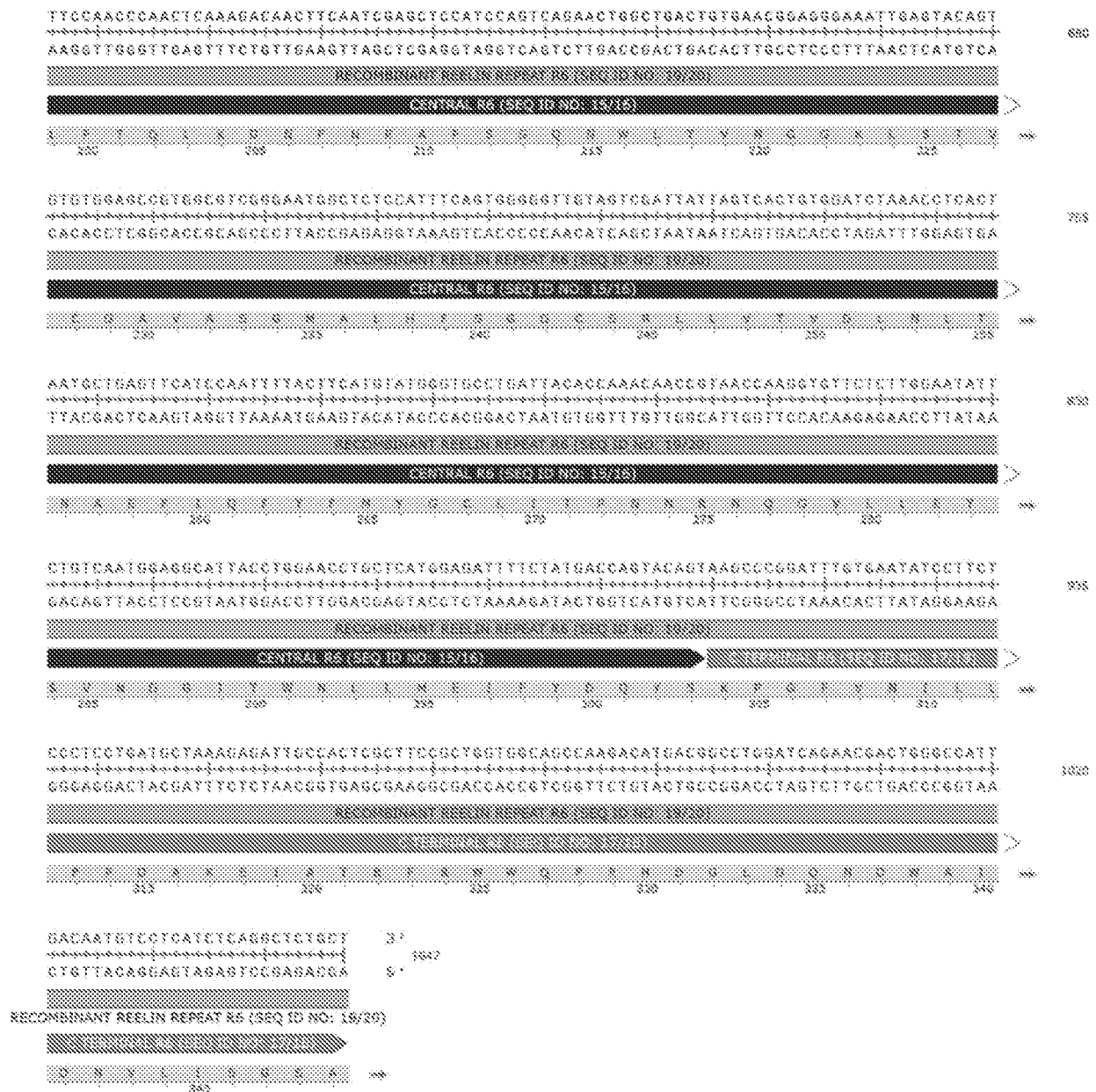
YTEACAGTAETSCYCEAGTTCTTCTTCAGTAETCTCATGATGCTGTTATGTCCTGTTCTTGGTGAAGAGAGGCTGTTACCCGG 85
 AAGTCGTATGACGAGGTCAAGAGGAAGTCAAGAGGTAAGTACTACGACCATACAGGACCAAGAGGACCACTTTCTTCGACAAATGGGCG
 CENTRAL R3 (SEQ ID NO: 5/5)
 F E S T A P V I L D Y G H D A E M E N F L V F S G C Y P
 CTTCTGCAGGCAAGGATCGGAAGGAAACTCCAGAGAACTAAGTGAGGCCCAACATGTATCACACAGGGGACTTTGAGAGATGGAC 170
 GAAGACGTCCGTTTCTACGCTTCTTTGAGGTCTCTTGATTCACTCGGGTGGTACATAGTGTGTCCCTGAAACTTCTTACCTG
 CENTRAL R3 (SEQ ID NO: 5/5)
 A T A G E G C E N S R S E L S E F I N Y T D D C E E W Y
 AAGAATCAGCATTTGTTATTCGAAGGTCTCTTBCATCCAGCAAGACDAGATTCCGATGGATCCAGGAGAGCAGCTCACAGAAAAAC 255
 YTCITAGTGGTAACAATAAGGTTCCAGAGAACGTAGGTGTCTTGGTCTAAGGCTACCTAGGTCTCTCGTGGAGTGTCTTTTG
 CENTRAL R3 (SEQ ID NO: 5/5)
 A I T I V I P K S L A G S E T S E F E W I Q E S C T Q X S

• REELIN REPEAT R3 (C-TERMINAL REGION):

34

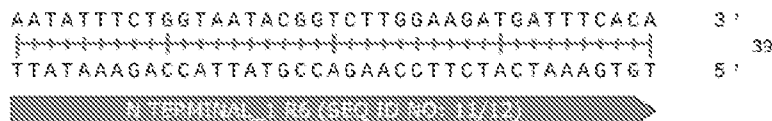
REELIN REPEAT R6





REELIN REPEAT 6 SEQUENCES:

• REELIN REPEAT R6 (N TERMINAL_1 REGION)



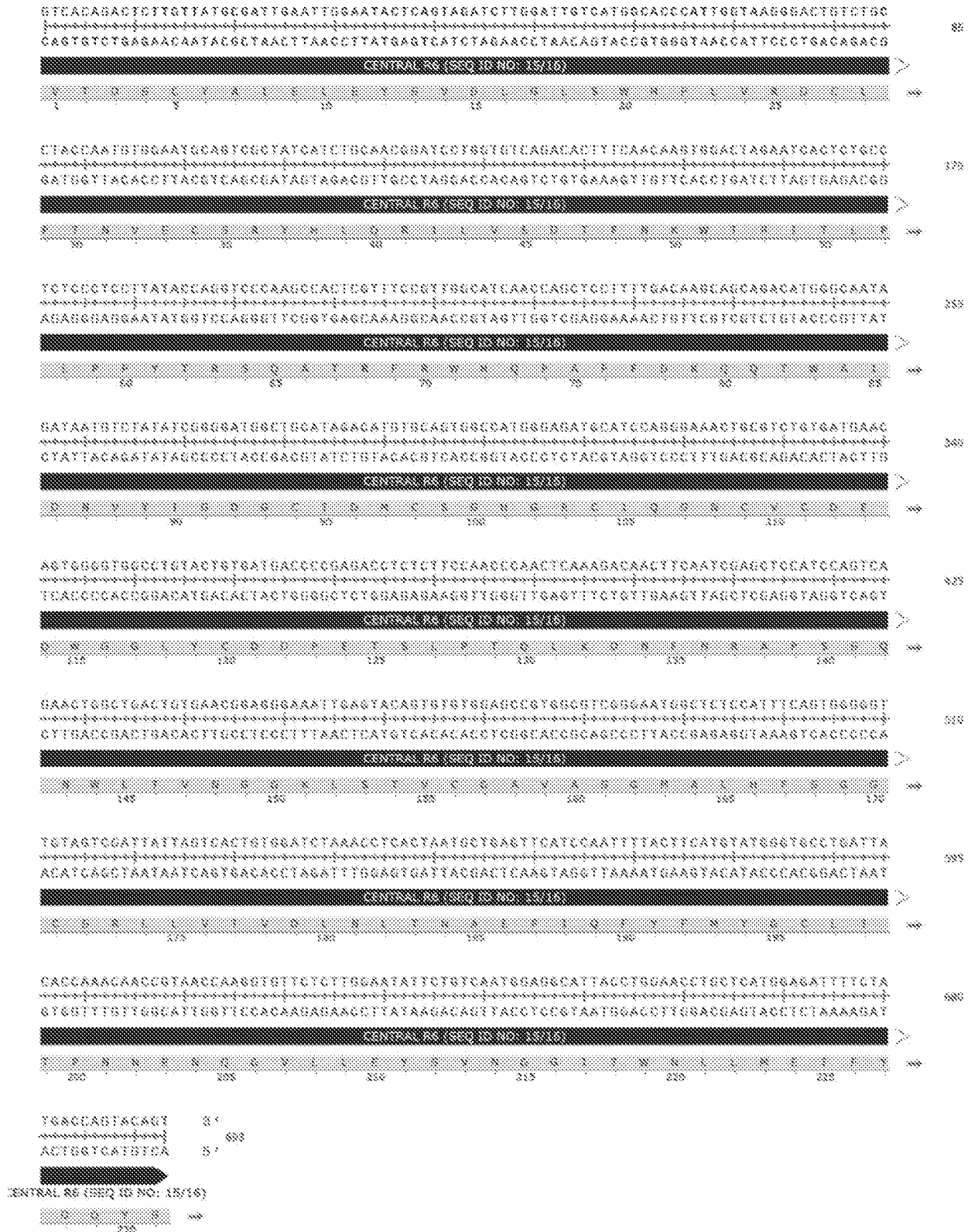
- REELIN REPEAT R6 (N TERMINAL_2 REGION)

[illegible]

● REELIN REPEAT R6: C TERMINAL REGION

[illegible]

REELIN REPEAT R6: CENTRAL REGION



RECOMBINANT REELIN REPEAT R3+R6v1 (reproduced from WO2018027037)

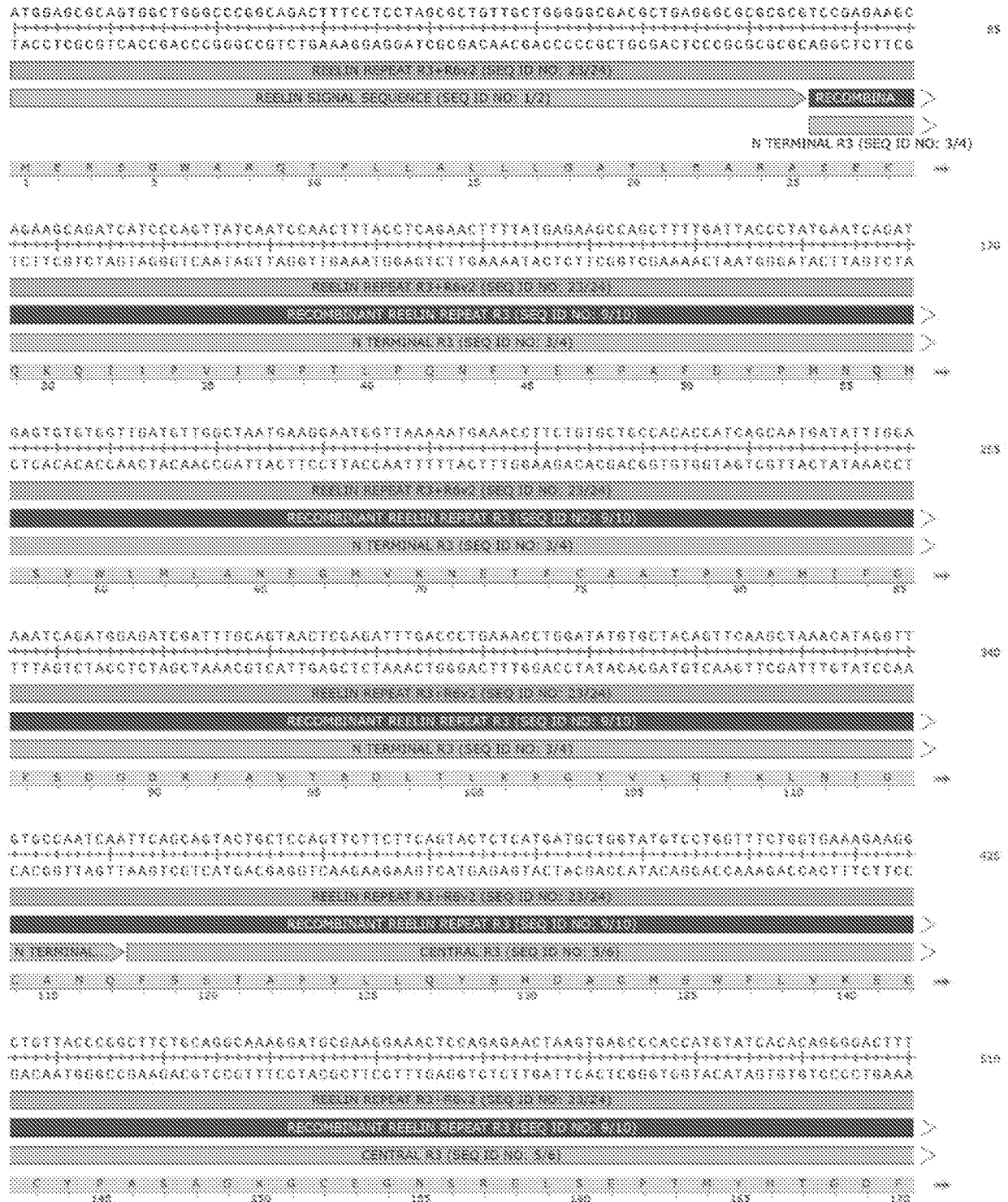
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TACCTGCGCTACCGACCCGGGCGCTCTGAAAGGAGGATCGGACACCGACCCCGCTGCGACTCCCGCGCGCGCAAGTCGTCA	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
REELIN SIGNAL SEQUENCE (175-250; SEQ ID NO: 1/2) CENTRAL RE	
M E R S G W A T Q T F L L A L L L G A T L R A R A F S S	→
CTGCTCCAGTTCTTCTTCAGTACTCTCATGATGCTGGTATGTCCTGTTTCTGGTGAAGGAAAGGCTGTTACCGGGCTTCTGCAGG	170
GACGAGGTCAAGAAAGAGTCATGAGAGTACTACGACCATACAGGACCAAGAGCACTTCTTTCGACAATGGGCGCAAGACGTCC	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
T A P V L L Q Y S H D A G H S W F L V K E G C Y P A S A G	→
CAAGGATGCGAAGGAAACTCCAGAGAACTAAGTGAGCCCAACATGATACACAGGGGACTTTGAAGAATGGACAAGAATCACC	255
GTTTCCTACGCTTCTTGGAGTCTCTTGATTAAGTCTGGGCTGATAGTGTGTCCTGAAACTTCTTACCTGTTCTTAGTGG	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
K G C E G H S R E L S E P T N Y H T G D F E E W Y R I Y	→
ATTGTTATTCCAAGGTCTCTTGCATCCAGCAAGACAGATTCCGATGGATCCAGGAGGACGCTCACAGAAAAACGTGCTCCAT	340
TAACAATAAGGTTCCAGAGAACGTAGGTCCTTCTGGTCTAAGGCTACCTAGGTCCTCTCGTGGAGTGTCTTTTTGACGGAGGTA	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
I V I F R S L A S S K Y R F R W I Q E S S S Q K N V F F	→
TTGGTTTAGATGGAGTGTACATATCCGAGCCCTTGTCCAGTTACTGCAAGTGGCCATGGGGACTSCATTTCAAGAGTGTGTTTCTG	425
AACCAATCTACCTCACATGTATAGGCTCGGAACAGGGTCAATGACGTCAACCGGTACCCCTGACGTAAAGTCTTCACACAAAGAC	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
R G L D G V Y I S E F C F S Y C S G H S D C I S G V C F C	→
TGACCTGGGATATACTGCTGCACAAAGAACCTGTGTGTCAAATGTCCCAATCACAAATGAGATGTTGATAGGTTTGAAGGGAAG	510
ACTGGACCCATATATGACGACGTGTCTCTTGGACACACAGTTACAGGGGTTAGTGTACTCTACAAGCTATCCAAACTCECCTTC	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
D L G Y T A A Q G T C V S N V F R H N E M F D R F E G K	→
CTCAGCCCTCTGTGTGTAAGATAACAGGTGCCAGGTTGGAAGTGGCTGTGGAACACTTAACGATGGCAATCTCTCTACTTCA	595
GAGTGGGAGACACCATGTTCTATTGTCCACGGTCCAACTTACCCGACACCTTGTGAATTGCTACCGTTAGAGAGATGAAGT	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
L S P I W Y K I T G A Q V G T G C C Y L N D G K S L Y F	→
ATGGCCCTGGGAAAAAGGGAAGCCGACGGTCCCTCTGGACACAGGAATATCAGACTTGTTCATTTTATATACAAATTTGGAAG	680
TACCGGACCTTTTCCCTTCGGGCTGCCAGGAGACCTGTGGTCCYATAGTCTGAACAAGTTAAAAATATGTTTAACCTTC	
REELIN REPEAT R3+R6v1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
N G P G K R E A R T Y P L D T R N J R L V Q F Y I Q I S S	→

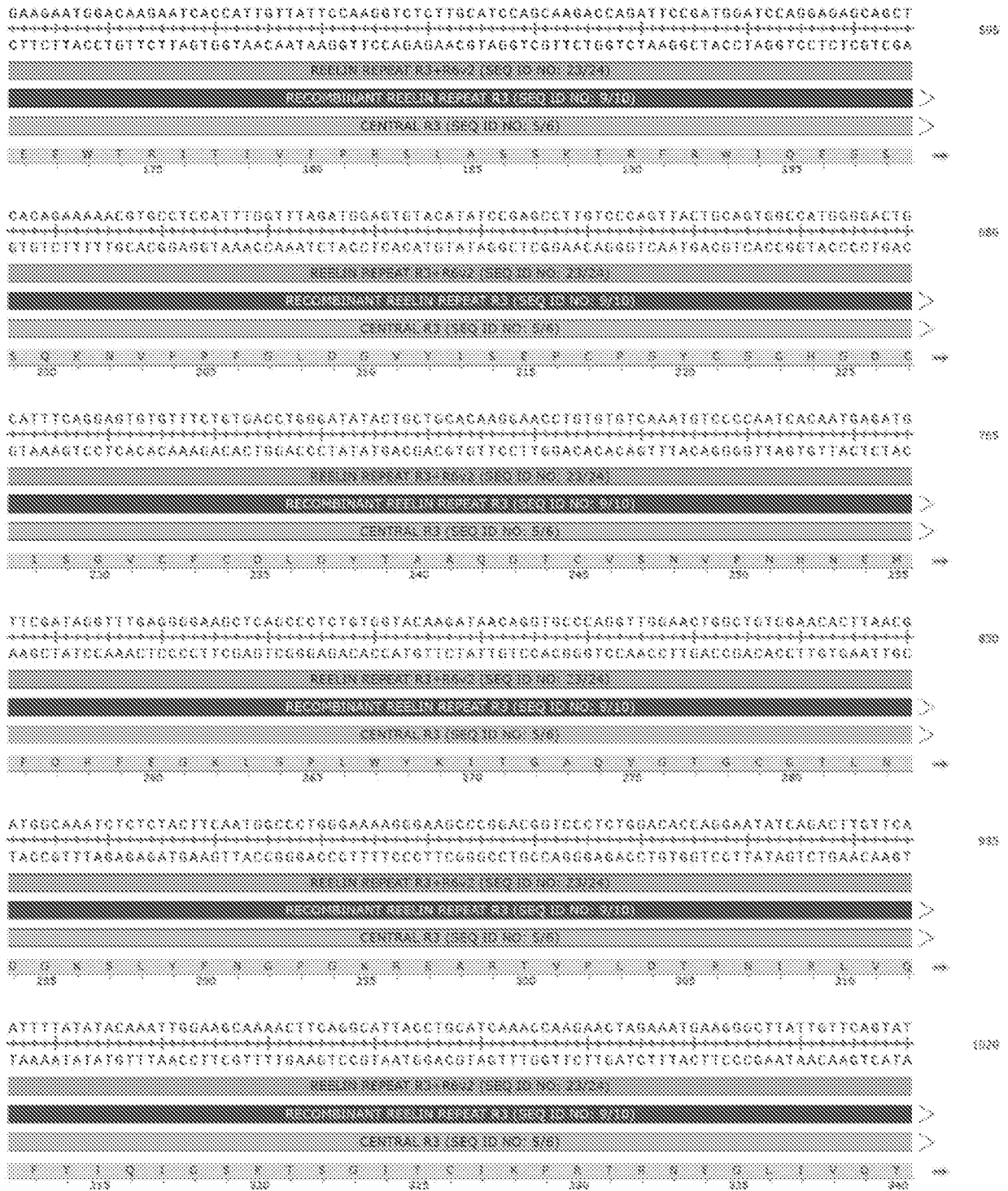
CAAAACCTTCAGGCAATTACCTGCATCAAAACCAAGAACTAGAAATGAAGGGCTTATTGTTCAATGACAAATGGGATACTC	
GTGTTGAAGTCCGTAATGGACGTAGTTTGGTTCTTGATCTTTACTTCCCGAATAACAAGTCATAAGTTTACTGTTACCCCTATGAG	788
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
K T S G I T C I K F A T R N E G L I V Q Y S N D N G I L	→
TSGCATTTGCTTCGAGAGTTGGACTTCATGTCTCTCTGGAACCAAGATCATTTCCATTGACCTGCCACAGGACGCGAAGACAC	
ACCGTAAACGAAGCTCTCAACCTGAAGTACAGGAAGGACCTTGGTGTCTAGTAAAGGTAACTGGACGGTGTCTCTGCGCTTCTGTG	890
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R3 (SEQ ID NO: 5/6)	
W H L I R E L D F M S F L E P Q I T S I D L P Q D A K T	→
CTGCAACGECATTTGATGGTGGCAACCGCAACATGGGAAGCATTCAGCCCAAGTGGGCTTTGGATGATGTTCTTATAGGAATGAA	
GACGTTGCCGTAAAGCTACCAACGCTTGGCTTGTACCTTCTGTAAGTGGGTCACCCGAAACCTACTACAAGAATATCCTTACTT	938
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)	
P A T A F R W W Q P Q H G K H S A Q W A L D S V L I C M N	→
TSACAGCTCTCAAACTGGATTCAAGACAAATTTGATGGCTCTATAACCCCTTGATAGTAGGAAATGGCTGCTTCAACCCAGGAGGG	
ACTGTGAGAGTTTGGCTAAAGTTCTGTTTAACTACCGAGATATTGGGAAGTATCATCCTTTACCGACGAAGTGGTCTCTCGG	1020
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
LINKER R3/R6 (SEQ ID NO: 53/54)	
D S S Q T G F Q D K F D D S I T L D S R Y W I L N P G S	→
ACCAAGATGCCCGTGTGTGGCTCTACTGGTGGTGGCTCTCATTTGAAAAGGCCAGCACCCTTACGTGGTCAAGCACAGAGC	
TGGTTCTAGGGGACACACACCGAGATGACCACTACGGGACCGAGAGTAACTTTTCCGCTGCTGGGCAATGACCACTCTGTCTG	1105
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)	
T K M F V C G S T G D A L V F I E K A S T R Y V V S T D	→
TTGCGGTGAATGAGGATTCCTTCTACAGATAGACTTCGCTGCTCTCTGCTCAGTCACAGACTCTTGTATGCGATTGAATTGGA	
AACGGCACTTACTCTTAAGGAAGGATGTCTATCTGAAGCGACGGAGGACGAGTCAGTGTCTGAGAACAATACGCTAACTTAACTT	1190
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
C-TERMINAL REGION REELIN REPEAT R3 (SEQ ID NO: 7/8)	
V A V N E D S F L Q I D F A A S C S V T D S C Y A I E L E	→
ATACTCAGTAGATCTTGGATTGTCTATGGCAACCCATTGGTAAGGGACTGTCTGCTACCAATGTGGAATGCAAGTCTATCTCTG	
TATGAGTCATCTAAGACCTAACAGTACCGTGGGTAACCATTCCTGACAGACGGATGGTTACACCTTACGTCAAGGATAGTAGAC	1275
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R6 (SEQ ID NO: 15/16)	
Y S V D L D I S W H F L V R D C L P T K V E C S E Y H L	→
CAACGGATCCTGGTGTGAGACACTTTCAACAGTGGACTAGAATCACTCTGCTCTCTCTCTTATACCAGGTCCCAAGCCACTC	
GTGCTTAGGACACAGTCTGTGAAGTGTCTTCACTGATCTTAGTGAAGACGGAGAGGAGGAATATGGTCCAGGGTTCGGGTGAG	1360
REELIN REPEAT R3+R6+1 (SEQ ID NO: 55/56)	
CENTRAL REGION REELIN REPEAT R6 (SEQ ID NO: 15/16)	
Q R I L V S D T F N K W T R I T L P L P Y T R S Q A T	→

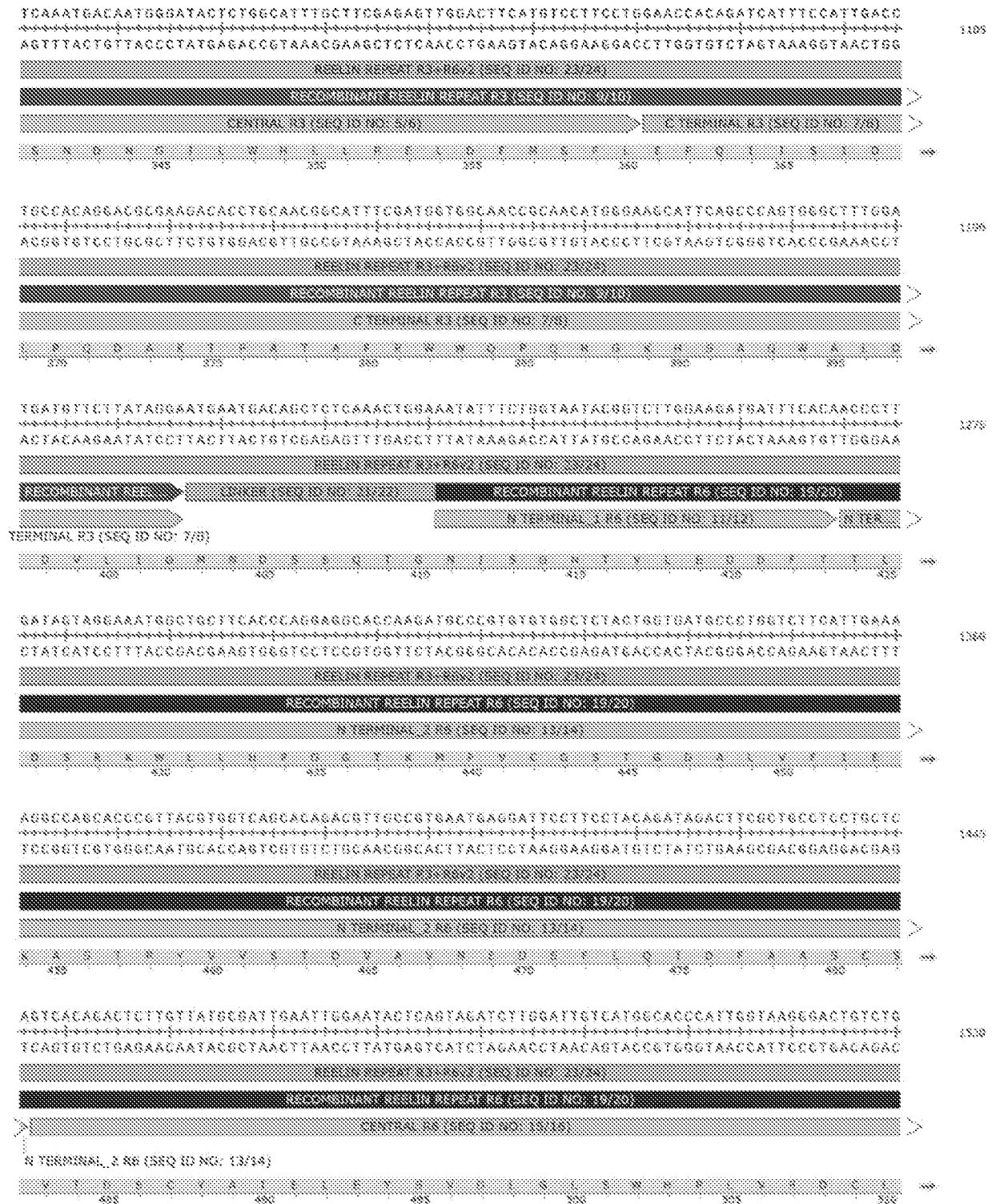
GTTTCCTTGGCATCAACCAGCTCCCTTTGACAAAGCAGCAGACATGGGCAATAGATAATGCTCTATATCGGGGATGGCTGCATAGA
 CAAAGGCAACCGTAGTTGGTCGAGGAAACTGTTCTGTCGCTGTACCGGTTATCTATTACAGATATAGCCCTACCAGCTATCT
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 E F R W H O P A F F D F Q O T N A I D N V Y I G D S C I D
 CATGTGCACTGGCCATGGGAGATGCATCCAGGGAACTGCGTCTGTGATGAACAGTGGGGTGGCTGTACTGTGATGACCCCGAG
 GTACACGTACCCGTTACCTCTACGTAGGTCCCTTTGACGCAGACACTACTTGTACGCCACCGGACATGACACTACTGGGCTC
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 N C S C H G S C I D G H C V C D E D W G G I Y C D D P E
 ACCTCTCTTCCAACCAACTCAAAGACAACCTTCAATCGAGCTCCATCCAGTCAGAACTGGCTGACTGTGAACGGAGGGAAATTGA
 TGGAGAGAAGGTTGGGTTGAGTTTCTGTTGAAETTAGCTCGAGGTAGGTCACTCTTGACCGACTGACACTTGCCCTCCCTTTAACT
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 Y S L P T Q L K D N F N H A P D S Q N W L T V N G G K L
 GTACAGTGTGTGGAGCCGTGGCGTGGGAATGECTCTCCATTTTCAGTGGGGGTTGTAGTCDATTATTAGTCACTGTGGATCTAAA
 CATGTACACACCTCGGCACCGCAGCCCTTACCGAGAGGTAAAGTCACCCCAACATCAGCTAATAATCAGTGACACCTAGATT
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 S T V C G A V A S G M A L N F S G G C S R L L V T V D L N
 CCTCACTAATGCTGAGTTCATCCAATTTTACTTTCATGTATGGGTGCTGATTACACCAACAACCGTAACCAAGGTGTTCTCTTG
 GGAGTGATTACGACTCAAGTATGTTAAATGAAGTACATACCCACGACTAATGTGGTGTGTTGGCATTGGTTCCACAAGAGAAC
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 L T N A E F I Q F Y F N Y G C L I T P N N R N Q G V L L
 GAATATTCTGTCAATGGAGGCATTACCTGGAACCTGCTCATGGAGATTTTCTATGACCACTACAGTGATTACAAGGATGACGACG
 CTTATAAGACAGTTACCTCCGTAAATGGACCTTGGACGAGTACCTCTAAAAGATACTGGTCAATGTCACTAATGTTCTACTGCTGC
 REELIN REPEAT R3+R6+1 (SEQ ID NO: 33/56)
 CENTRAL REELIN REPEAT R6 (SEQ ID NO: 15/16)
 E Y S V N G G I T W N L L H E I F Y D Q Y S D Y K D D D

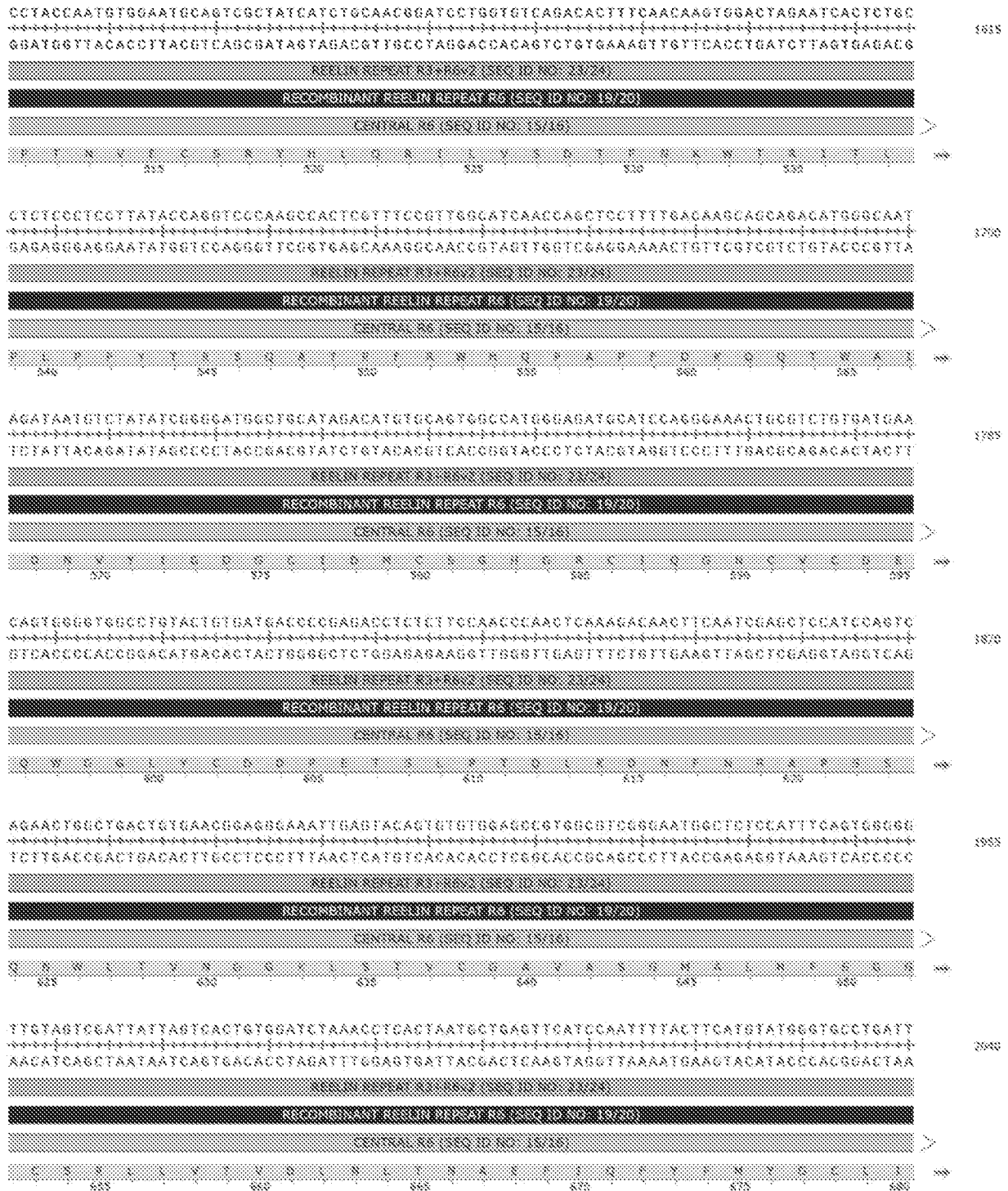
ATAAGTCACTCSAG 3'
 TATTCACTGAGCTC 5'
 REE
 D F

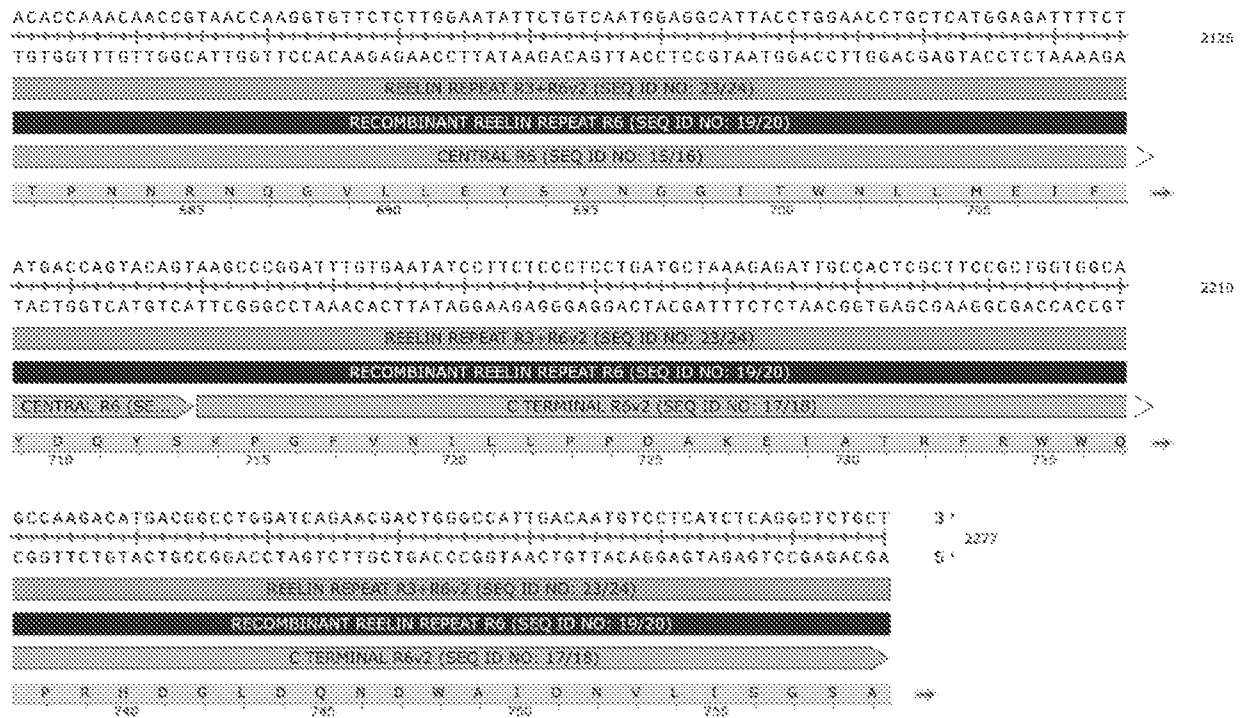
RECOMBINANT REELIN REPEAT R3+R6v2











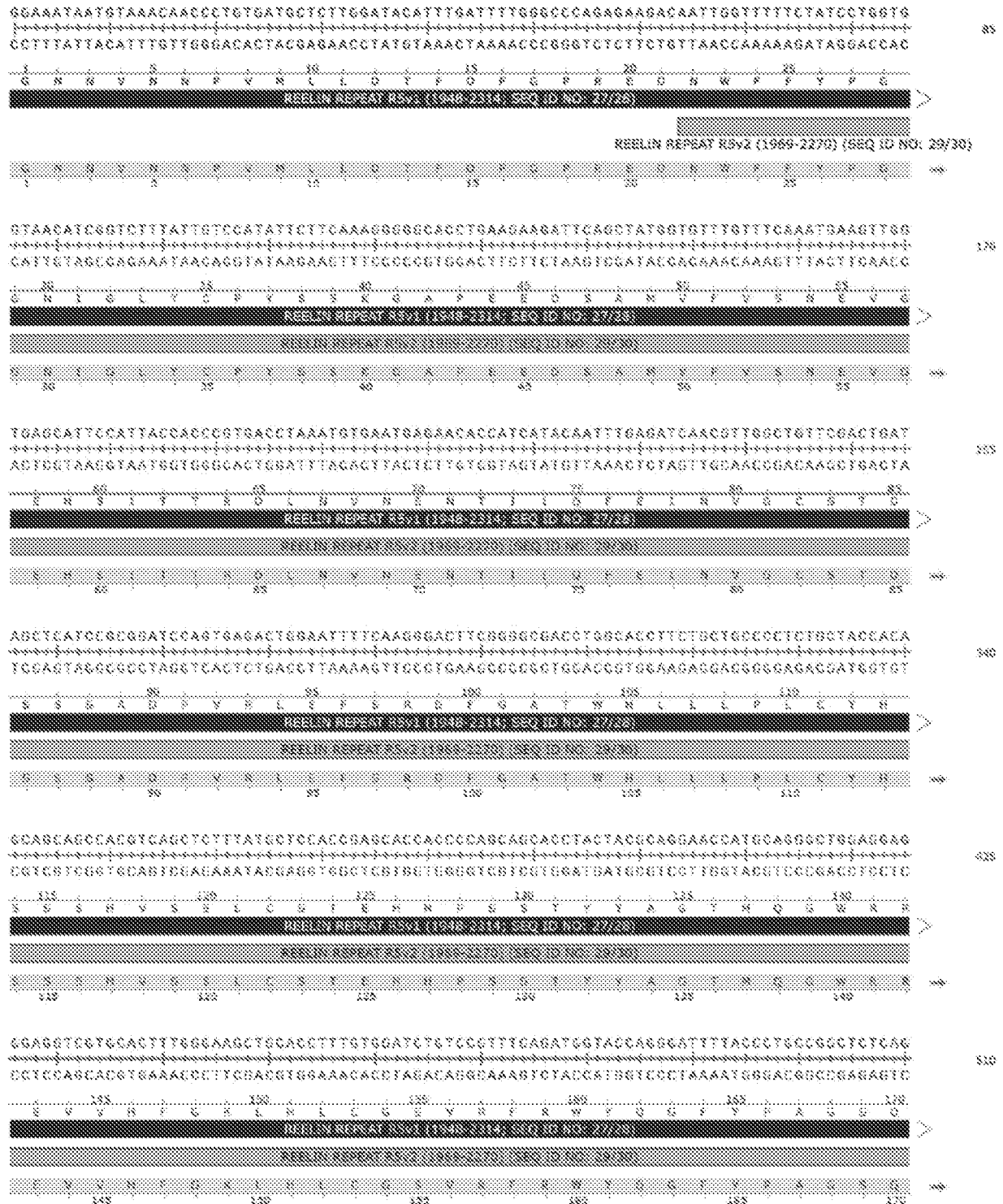
[0073] R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

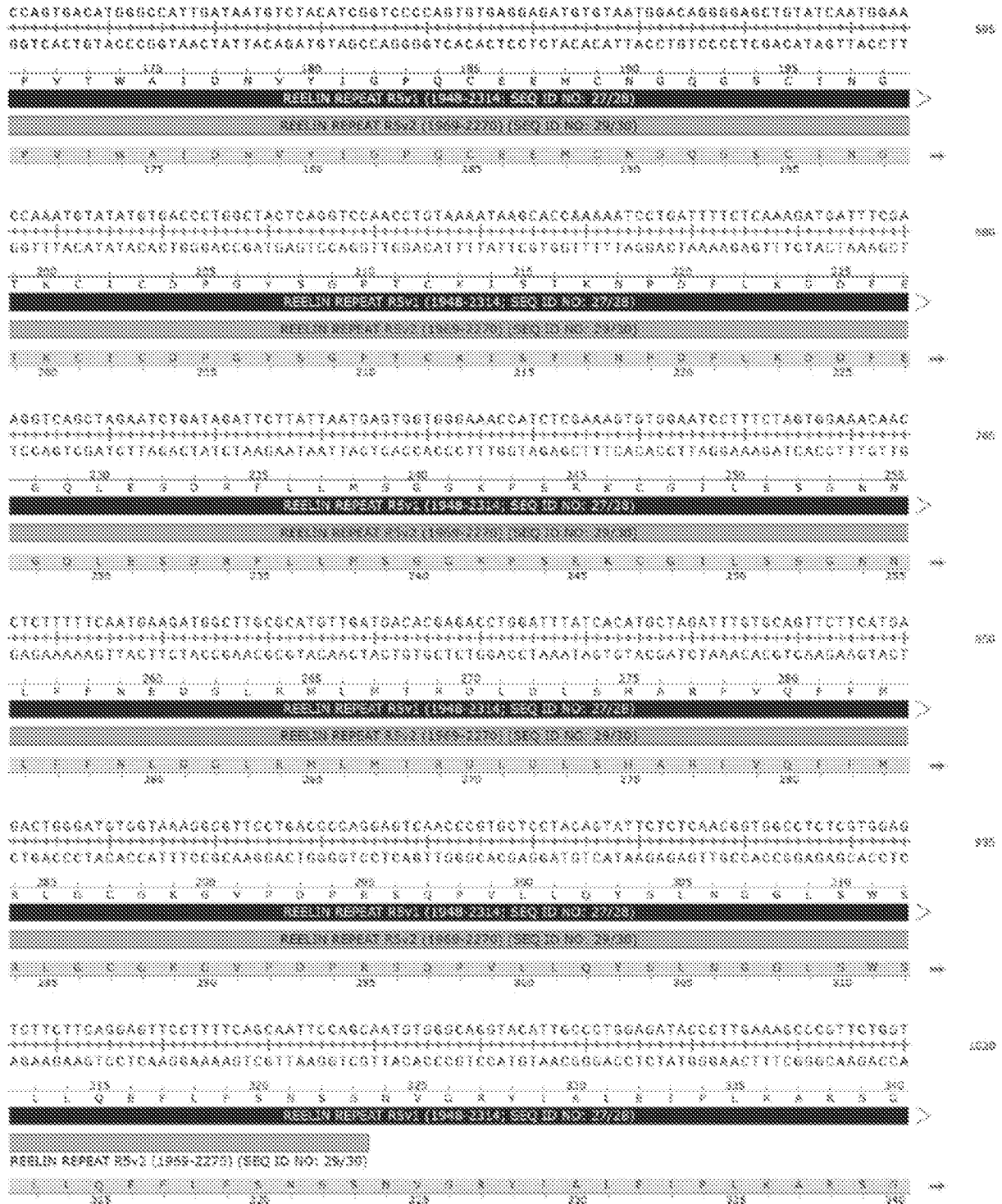
RECOMBINANT REELIN REPEAT R4

ATBAATGACAGCTCTCAAACTGGATTTTCAAGACAAATTTGATGGCTCYATAGATTTTCAABCCAACTGGTATCGAATCCAAGGAG	85
TACTTACTGTCTGASAGETTTGACCTAAAGTTCTGTGTTAAACTACCEAGATATCTAAACGTTTGGTTGACCATAGCTTAGGTTCTCTC	
N H C S S Q T G F Q D K F D G S I D L Q A N W Y H I Q E	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
N H C S S Q T G F Q D K F D G S I D L Q A N W Y H I Q E	
GTCAAGTTGATATTGACGTCTCTCTATGGATCTGCTCTGATATTTCACTGAAAAACATAGAAAAACCTTCGTTATGCTGAGACCTG	175
CAGTTCAACTATAACTGACAGAGAGATACCTATGACGAGACTATAGTGACTTTTGATCCTTTTGGAGCAATACGACTCTGGAC	
G Q V D I D C L S H D T A L I F T E N I G K F R Y A E T W	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
G Q V D I D C L S H D T A L I F T E N I G K F R Y A E T W	
GGATTTTCATGTGTCAECATCTACCTTTTTGCASTTTGAAATGAGCATGGGCTGTAGCAAGGCCCTTCAGCAACTCCACAGTGTAT	275
CCTAAAGTACACAGTCTGTAGATGGAAAAACGTCAAACTTTACTCGTACCCGACATCGTTTGGGAAAGCTGTTGAGGGTGTACAT	
D F H V S A S T F L Q F E M S M G C S K F F S H S H S V	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
D F H V S A S T F L Q F E M S M G C S K F F S H S H S V	
CAGCTCCAGTATTCTCTGAACAATGGCAAGGACTGGCATCTTTGTCACGGAAGAGTGTGTTCTTCAACCATTTGGCTGTCTGCATT	345
GTGGAGGTGATAAGAGACTTTGTTACCGTTCTGACCGTAGAACAGTGGCTTCTCACACAGGAGGTTGTAACCGACAGACGTAA	
O L Q Y S L N N E K D W H L V T E E C V P P T I G C L H	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
O L Q Y S L N N E K D W H L V T E E C V P P T I G C L H	
ACACGGAAAGTTCAATTTACACCTCGGAAGATTCAGAAATTTGGAAGGGATCACTGTCTACCTTCACCTCTCCACCATTTCTCTC	425
TGTGCTTTTCAAGTTAAATGTGGAGCCTTTCTAAGGTCTTAACTTCGCTAGTGACAGATGGAAAGGTGAGAGGTGGTAAAGAGG	
Y T E S S I Y T S E R F Q N W K R I T V Y L P L S T I S P	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
Y T E S S I Y T S E R F Q N W K R I T V Y L P L S T I S P	
CAGGACCGGTTTCAATGATGATTCAGGCGCACTACACTGTGGGGGCTGATTCCTGGGGGATTGATAATGTTGTACTGGCTCAGGG	515
GTCTTGGGCCAAGTCTACCTAAGTCCGTTGATGTGACACCCCGGACTAAGGACCCGCTAACTATTACACATGACCGGAGTCCC	
R T R F R W I G A N Y T V G A D S W A I D N V V L A S G	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
R T R F R W I G A N Y T V G A D S W A I D N V V L A S G	
TGCCCCGATGATGCTCAGGACGAGGGATTTGTGATGCTGGACGCTGTGTGTGTGACCGGGGCTTTGGTGGACCTATTGTGTTT	595
ACGGGAACCTACACGAGTCTGCTCCCTAAACACTACGACCTGCGACACACACACTGGCCCCGAAACCCCTGGGATAACACAA	
C P W H C S G R G I C D A G R C V C D R S F G G P Y C V	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
C P W H C S G R G I C D A G R C V C D R S F G G P Y C V	
CTGTTGTTCTCTGCCCCCTGATTTCTTAAAGACGATTTCAATGGGAATTTACATCCTGACCTTTGGCTGAAAGTGTATGGTGCAGA	685
GACAACAGGAGACGGGAGCTAAGAAATTTCTGCTAAGATTTACCTTAAATGTAGGACTGSAACCGGACTTCACATACCACGTCT	
P V V P L P S I L K D D F N G N L H P D L W P E V Y G A E	
REELIN REPEAT R4 (1598-1947; SEQ ID NO: 25/26)	
P V V P L P S I L K D D F N G N L H P D L W P E V Y G A E	

GAGGGGGAATCTGAATGCTGAACCATCAATCTGGAACATCTCTAATTTTTAAAGGGGAAGGACTAAGGATGCTTATTTCAAGA
 CTCCCCCTTAACTTACCACCTTTGGTAGTTTASACCTTGTASASATTAATAATTTCCCTTCTGATTGCTACGAATAAAGTTCT
 795
 R G N L N G E Y I K S G T S L I F K D E G L R H L I S R
 REELIN REPEAT R4 (1598-1947, SEQ ID NO: 25/26)
 R G N L N G E Y I K S G T S L I F K D E G L R H L I S R
 GATCTAGATTGTACAAATACAATGTATGTCCAGTTTTCACCTTAGATTATAGCAAAAAGTACCCAGASASATCTCACTCTATTC
 CTAGATCTAACAATGTTTATGTTACATACAGGTCAAAAGTGAATCTAAATATCGTTTTTCATGGGGTCTCTCTAGAGTGAGATAG
 850
 D L S C T N T A V V D F S L A P I A K S T P E K S A S I
 REELIN REPEAT R4 (1598-1947, SEQ ID NO: 25/26)
 D L S C T N T A V V D F S L A P I A V S T P E K S H O I
 TGTACAAATTCTCCATCAGTGGAGGAATCACTTTGECACCTGATGGATGAATTTTACTTTTCTCAAAACAACGAATATACTTTTCAT
 ACAATGTTAAGAGGTAGTCACTCTCTTAGTGAACCGTGGACTACCTACTTAAATGAAAAGGATTTGTTGCTTATATGAAAAGTA
 935
 L L Q F S I S G G I T W H L M D E F Y F F Q T T N I L F I
 REELIN REPEAT R4 (1598-1947, SEQ ID NO: 25/26)
 L L Q F S I S G G I T W H L M D E F Y F F Q T T N I L F I
 CAATGTTCCCTTGCCATACACTGCTCAAAACCAATGCTACAGATTCAGACTCTGGCAACCTTATAATAACGGTAAGAAAGGAAGAA
 GTTACAAGGGAACGGTATGTGACGGGTTTGGTTACGATGTTCTAAGCTCTGACACCGTTGGAATATTATTGCCATTCTTTCTTCTT
 1020
 H V F L P Y T A Q T N A T K F F L W Q P Y R N G K K E E
 REELIN REPEAT R4 (1598-1947, SEQ ID NO: 25/26)
 H V F L P Y T A Q T N A T K F F L W Q P Y R N G K K E E
 ATCTGGATTGTTGATGACTTCATTATCBAT 3'
 TAGACCTAACAACACTACTGAAGTAATAGCTA 5'
 1690
 I W I V D D F I I D
 REELIN REPEAT R4 (1598-1947, SEQ ID NO: 25/26)
 I W I V D D F I I D

RECOMBINANT REELIN REPEAT R5v1 and R5v2





RECOMBINANT REELIN REPEAT R3+R5v2

RRR-IN RSPCAT 18 (SNV TO NO. 513)

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REF IN REPEAT P3 (SEQ ID NO. 9/19)

K E D G D R T A T T G C I T L K E S Y L I O T E L N I S

RECEIVED 13 (582 ID NO. 5/13)

REF ID: A63483

WABLIN RSPCH #13 (SBO ID NO. 9/13)

REF ID: A6743 (SRQ ID NO. 9/19)

MEELIN SPECA 81 (SEQ ID NO. 8/12)



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1208



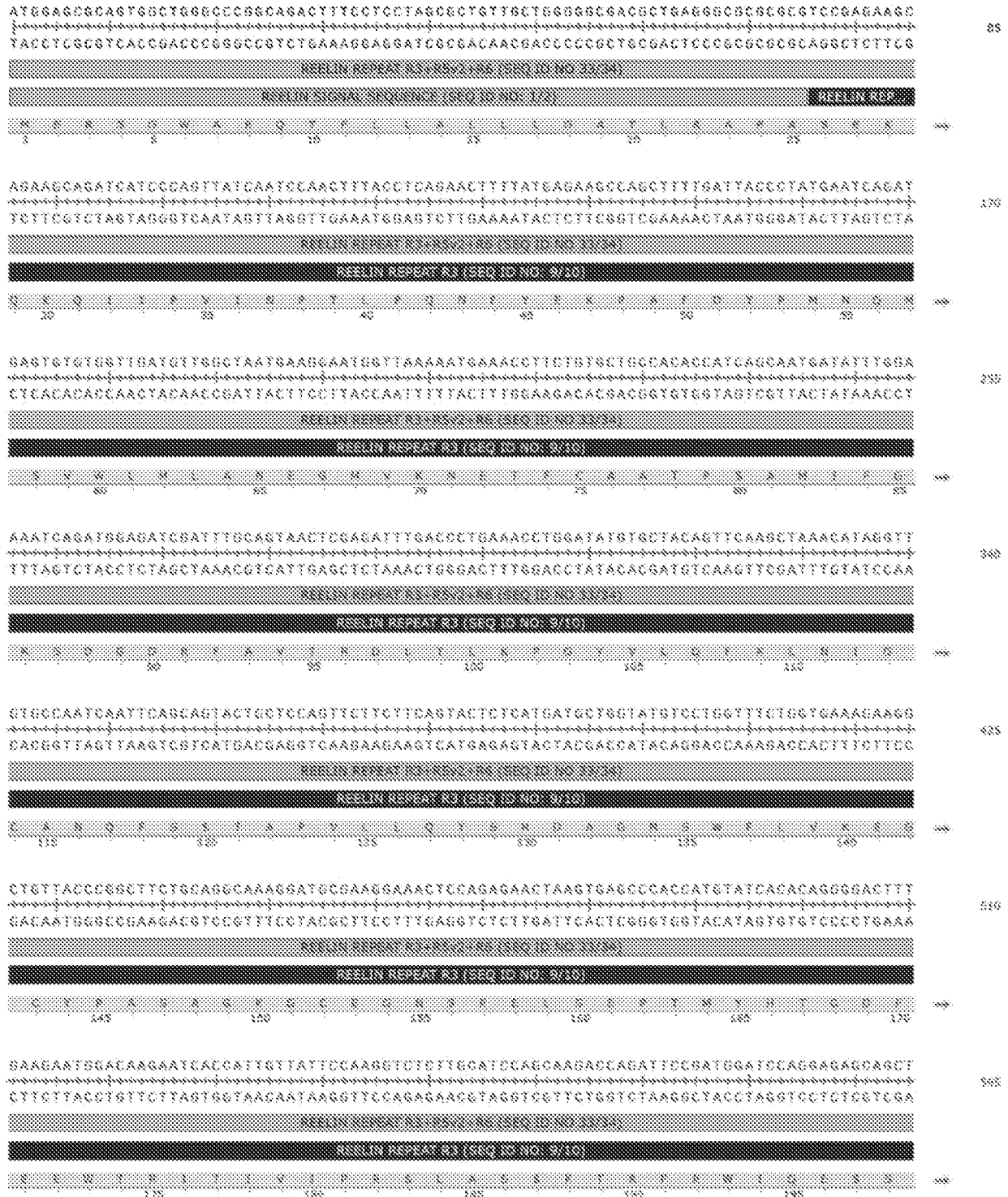
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37 (SEQ. ID NO: 32)
87

(SEQ ID NO: 33)

RECOMBINANT REELIN REPEAT R3+R5v2+ R6



CACASAAAAAGCTGCTCCATTTGGTTTAGATGGAGTGTACATATCCBAGCCTTGTCCCACTTACTGCACTGGCCATGGGGACTG
GTGTCTTTTTGCACGGAGGTAACCAAACTACCTCACATGTATAGGCTCGGAACAGGSTCAATGACGTCAACGGTACCCCTGAC

580

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

S Q P N V P F F L D G V Y I S P P S Y C S G H G C

CATTTCAAGAGTGTGTCTGTGTGACCTGGGATATACTGCTGCACAAAGAACTGTGTGTCAAAATGTCCCAATCACAATGASATG
GTAAAGTCTTCACACAAAGACACTGGACCTATATGACGACGTGTCTCTTGGACACACATTTACAGGGCTTAGTGTACTCTAC

760

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

I S G N E P C D L O Y T A A Q D T C V S N V P N H R R

TTGATAGGTTTGAAGGGGAAGCTCAGCCCTCTGTGTGTACAAGATAACAGGTGCGCAGGTTGGAACCTGGCTGTGGAACACTTAAAG
AAGCTATCCAAACTCCCTTCGAGTCCGGAGACACCATGTTCTATTGTCCACGGGTCCAAAGCTTGACCGACACCTTGTGAATTGC

850

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

F D R F E G K L S F L W Y K I T G A Q V G T G C G I L N

ATGGCAAAATCTCTTACTTCAATGSCCCTGGGAAAAAGGAAAGCCGGACGGTCCCTGTGACACCGAGGAATATCAGACTTGTTC
YACCGTTTAGAGAGATGAAGTTACCGGGACCTTTTCCCTTCGGGCTGCCAGGGAGACCTGTGGTCCCTTATAGTCTGAACAAGT

950

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

D G K S L Y T N G P D K K E A K I V F L D T R N I N L Y Q

ATTTTATATACAAATGGAGCAAAACTTCAGGCATTACCTGCATCAAAACCAAGAACTAGAAATGAAGGGCTTATTGTTCAATAT
YAAAATATATGTTAAGCTTCGTTTGAAGTDCATTAATGACATAGTTTGGTGTCTTACTTCCGAATGAAGATCATA

1030

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

F Y I Q I G S Y T N G I T C I K P T R N I S L I V G Y

TCAAATGACAAATGGGATACTCTGSCATTTCCTTCGAGAGTTGCACTTCATGTCCTTCCTGGAACCAAGATCATTTGCAATGACC
AGTTTACTGTTACCTTATGAGACCGTAAACGAAGCTCTCAACCTGAAGTACAGGAAGGACCTTGCTGTCTAGTAAAGGTAACCTGG

1100

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

S N D N D I L M H L L K E L D T M S F L E P Q I T S I D

YGCCACAGSACGCAAGACACCTGCAACGGCATTTCGATGGTGGCAACCGCAACATGGGAAGCATTCAAGCCAGTGGCTTTGGA
ACGCTGTCCTGCGCTTCCTGAGGCTTGCCTAAAGCTACACCGTTGCGCTTGTACCTTCCTAAGTGGGCTCAGCGGAAGCTT

1180

REELIN REPEAT R3 (SEQ ID NO: 33/34)

REELIN REPEAT R3 (SEQ ID NO: 9/10)

P Q D K V T P A T A P E W W Q P Q H G P N S A Q W K I D

56

ATCAATGGAACCAATGTATATGTGACCTGGCTACTCAGGTCCAACCTGTAAATAAGCACCAAAATCCTGATTTTCTCAAAG	1870
YAGTTACCTTGGTTACATATACACTGGGACCGATGAGTCCAGCTTGGACATTTTATTGCTGGTTTATGAGCTAAAAGAGTTTC	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R5+Z (SEQ ID NO: 29/30)	
I N G T F C I C D P Q Y S E P T C K I S T K N P D F L K	
590 605 610 615 620	
ATGATTTGGAAGGTCAGCTABAATCTGATAGATTCTTATTAATGAGTGTGGGAAACCATCTGAAATGTGAAATCCTTTCTAG	1955
YACTAAAGCTTCCAGTCCATCTTAGACTATCTAAGAATAATTACTCACCACCCTTTGGTAGAGCTTTCACAGCTTAGGAAAGATC	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R5+Z (SEQ ID NO: 29/30)	
D D F E G S L E S D R F L N S G G F S R F C G I L S S	
625 630 635 640 645 650	
TGGAAACAACCTCTTTTCAATGAAGATGECTTGCAGATGTTGATGACAGGAGACCTGGATTTATCAGATGCTAGATTTGTGCAG	2040
ACCTTTGTTGGAGAAAAAGTTACTTCTACCGAAGCGGTACAACCTACTGTGCTCTGACCTAAATAGTGTACGATCTAAACACGTC	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R5+Z (SEQ ID NO: 29/30)	
D N N I F F N F D G L N M L M T R D L D L S H A R F Y Q	
655 660 665 670 675 680	
YTCCTCATGAGACTGGATGTGGTAAAGGCTTCTCTGACCCGAGGAGTCAACCCGCTGCTCTACAGTATTTCTCAACGGTGGCC	2125
AAGAAGTACTCTGACCTACACCATTTCCCAAGGACTGGGGTCTCTAGTTGGGCACGAGGATGTCATAAGAGAGTTGECACCGG	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R5+Z (SEQ ID NO: 29/30)	
F F N S L G C C K S V F D P S S Q F V L L G T S L N G S	
685 690 695 700 705	
TCTCGTGAAGTCTTCTTCAGGAGTTCTCTTTTCAGCAATTCACGCAATGTGGGACAGGTACATTGCCCTGGAGATACCCCTTGAAGG	2210
AGAGCAGCTCAGAGAAAGTCTCAAGGAAAGTCTGTAAAGTCTGTACACCGTCCATGTAADGGGACCTCTATGGGAAGCTTTGG	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R5+Z (SEQ ID NO: 29/30)	
L L W S L L Q E F L C G N S S N H Y G E Y T A L E I P L K S	
710 715 720 725 730 735	
CCGTTCTGGTTCTACTGCGCTTGGCTGGTGGCAACCTCTGAGAAATGGGCACCTTCTACAGCCCTTGGTTATGCAATCAGATTCTT	2295
GGCAAGACCAAGATGAGCGGAAGGACCCAGCTTGGCAGACTCTTACCGGTGAAGATGTCGGGACCCCAATAGCTAGTCTAAGAA	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
R S D S T F L K W W Q F S E K S H I Y S F W V I D Q I A	
740 745 750 755 760 765	
ATTGGAGGAAATATTTCTGNTAATACGGTCTTGAAGATGATTTGACAAGCCCTTATAGTAGGAAATGGCTGCTTCAACCCAGGAG	2380
TAACCTCCTTTATAAAGACCAATTATGCCAGAACCTTCTACTAAAGTCTTGGGAAGTATCATCTTTACCGAGCAAGTGGGTCCTC	
REELIN REPEAT R3+R5+R6 (SEQ ID NO 33/34)	
REELIN REPEAT R6 (SEQ ID NO: 19/20)	
I S G N T S G N T V L E C D P T T L D S K N W L L N F G	
770 775 780 785 790	

SCACCAAGATGCCCGTGTGTGGCTCTACTGGTGTATGCCCTGGTCTTCATTGAAAAGGCCAGCACCCGTTACGTGGTCAGCACAGA CCTGGTTCTACGGGCACACACCGAGATGACCACTACGGGACCAAGTAACCTTTCCGGTCTGGGCAATGCACAGTCGTGTCT	2665
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
D T K R E V C G S T D D A L V F I E K A S T R T V V S T D	795 800 805 810 815 820
CCTTGGCGTGAATGAGGATTCCTTCCACAGATAGACTTCGGTGGCTGGTCTCAGTCTTGGAAATGATTTCACAACCCCTTGAT GCAACGGCACYTACTCTAAGGAAGGATGTCTATCTGAAGGCAAGGAGCAGAGTCAGAACCTTCTACTAAAGTGTGGGAACCTA	2670
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
V A V N E D S F L Q I D F A A S C S V L E D D F T I L D	825 830 835 840 845 850
ASTAGCAAATGGCTGCTTCACCCAGGAGGCACCAAGATGCCCGTGTGTGGCTCTACTGGTGTATGCCCTGGTCTTCATTGAAAAGG TCATCCCTTACCGACGAAGTGGGTCCTCCGTGGTTCTACGGGCACACAGCAGATGACCACTACGGGACCAAGTAACCTTTCC	2675
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
D N K W L L H T C G T K M P C C G S T D D A L V F I E S	855 860 865 870 875
CDAGCACCGGTTACGTGGTCAGGCADAGAGCTTGCCGTGAATGAGGATTCCTTCCACAGATAGACTTCGGTGGCTCTCTGGTCAAT GGTGGTGGCAATGCACCACTCTGTGTCTCAACGGCACTTACTCTAAGGAAGGATGTCTATCTGAAGGCAAGGAGGAGGAGTCA	2730
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
A S T R V V S T D V A V N E D S F L Q I D F A A S C S V	880 885 890 895 900 905
CACAGACTCTTGTATTATCGATTGAATTGGAATACTCAGTAGATCTTGGATTGTCTATGGCAGCCATTGGTAAGGAGTGTCTGGCT GTGTCTGABAACAATACGCTAACYTAACCTTATGATCATCTAGAACCCTAACAGTACCTGGGTAAACCAATCCCTGACAGAGGGA	2805
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
T D S C V A I E L E T S V D L S L D W H F L V R D C L F	910 915 920 925 930 935
ACCAATGTGGAATGCAGTGGCTATCATCTGCAACGGATCCTGGTGTGAGACACTTTCACCAAGTGGACTAGAATCACTCTGGCTC TGGTTACACCTTACGTCAGCAGATAGTATAGCTTGGCTAGGACCAAGTGTGTGAAAGTTGTTACCTGATCTTAGTGAGAGGGAG	2860
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
T H V E C S A Y H L G N I L V S D T F N K W T H T I L F	940 945 950 955 960
TCCCTCTTTATACCAAGTCCCAAGDCACTCGTTTCCTTGGCATCAADCAAGCTCTTTTGACAAGCAGCAGACATGGGCAATAGA AGGGAAGGAATATGCTCCAGGGTTCCGTGAGCAAAAGGCAACCGTACTTGGTCCAGGAAAACGTGTTCTCTGTCTGTACCCGTTATCT	2975
REFL IN REPEAT R3+R5+2+R6 (SEQ ID NO. 17/34)	
REFL IN REPEAT R6 (SEQ ID NO. 19/20)	
S P E Y T A S S A T V T R W H Q F A P S D K Q Q T N A I D	965 970 975 980 985 990

TAATGCTATATCGGGATGGCTGCATAGACATGTGCAGTGGCCATGGGAGATGCATCCAGGGAAACTGCCTCTGTGATGAACAG
 ATTACASATATAGCCCTACCGACATCTGTACAGTCAACGCTACCTCTACGTAGGTCCCTTTGACGCACACACTACTTGTG 3080

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

N V Y I S S C C I D N C E N E N C V C D E Q 3085

TGGGGTGGCTGTACTGTGATGACCCGAGACCTCTCTTCCAAACCAACTCAAAAGACAACCTCAATCGAGCTCCATCCAGTCAGA
 ACCCCACCGACATGACACTACTGGGCTCTGGAGAGAGAGGTGGGTTGAGTTTCTGTTGAAATTAGCTCGAGGTAGGTGAGTCT 3145

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

W G G L Y C D D P E T S L E T G L K D N F N K A T S S Q 3150

ACTGGCTGAGTGTGACGAGAGGAAATTTGAGTACAGTGTGTGGAGCCCTGGGCTCGGGAATGGCTCTCCATTTCAATGAGGTTG
 TGACCGAGTGAACCTTGGCTCCCTTTAACTCATGTGTACAGACACCTCGGACCCGACGCCCTTACCGAGAGGTAAAGTCAACCCCAAC 3210

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

N W L T Y N G G K L E T Y C G A V A S G M A L N T E G S S C 3215

YAGTCGATTATTAGTCACTGTGGATCTAAACCTCACTAATGCTGAGTTTCATCCAAATTTTACTTCATGTATGGTGGCTGATTACA
 ATCAGCTAATAATCACTGACACCTASATTTGGAGTGATTACGAGTCAAGTAGGTTAAATGAAATACATACCCACGGACTAATGT 3275

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

D R L L Y T V D L N L T N A E F I Q T Y F N Y D C L E Y 3280

CCAAACAACCGTAACCAAGGTGTTCTCTTGGAAATATCTGTCAATGGAGGCATTACCTGCAACCTGCTCATGGAGATTTTCTATG
 GGTGTTGTTGGCATTTGGTTCCACAAGAGAACCTTATAAGACAGTTACCTCGTAATGGAGCTTGAGCAGATACCTCTAAAGATAC 3345

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

P N N R N Q S V L L E Y S V N G S I T W N L I M S L F Y 3350

ACCAGTACAGTAAGCCCGATTGTTGTAATATCCTTCTCCCTCCTGATGCTAAAGAGATTGCCACTCGCTTCGGCTGGTGGCAGCC
 TGGTCATGTCATTCGGGCTTAAACACTTATAGCAAGAGGGAGGACTACGATTTCTCTAACGGTGAGCGAAGGCGACCACCGTCTCG 3415

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

D Q Y S E E S T Y N L L L F D A K E L A T D F A W D Q F 3420

AAGACATGACGGCTGGATCAGAACGACTGGGCCATTGACAATGTCTCATCTCAGGCTCTGCT 3'
 TTCTGTACTGGCGACCTAGTCTTTGCTGACCGGTAACGTGTTACAGGAGTAAAGTCCGAGACGA 5' 3540 (SEQ ID NO: 33)

REELIN REPEAT R5 (SEQ ID NO: 33/34)

REELIN REPEAT R6 (SEQ ID NO: 19/20)

A W D S I D Q N W A I D N V L I S G S R 3450 (SEQ ID NO: 34)

TABLE OF SEQ ID NOs

SEQ ID NOs:		RESIDUES (Jossin et al. 2003)	RESIDUES (Nogi et al, 2006)	REELIN SEQUENCE
NUCLEIC ACID SEQUENCE	AMINO ACID SEQUENCE			
1	2	1-75		REELIN SIGNAL SEQUENCE
3	4			REELIN REPEAT R3_v1 (N TERMINAL REGION)
5	6			REELIN REPEAT R3_v1 (CENTRAL REGION)
7	8			REELIN REPEAT R3_v1 (C TERMINAL REGION)
9	10	1221-1596	1222-1597	REELIN REPEAT R3
11	12			REELIN REPEAT R6 (N-TERMINAL_1 REGION)
13	14			REELIN REPEAT R6 (N-TERMINAL_2 REGION)
15	16			REELIN REPEAT R6 (CENTRAL REGION)
17	18			REELIN REPEAT R6 (C-TERMINAL REGION)
19	20	2315-2661	2315-2661	REELIN REPEAT R6
21	22			LINKER
23	24			REELIN REPEAT R3+R6v2
25	26	1597-1947	1598-1947	REELIN REPEAT R4
27	28	1948-2314	1948-2314	REPEAT R5v1
29	30			REPEAT R5v2
31	32			REELIN REPEAT R3+R5v2
33	34			REELIN REPEAT R3+R5v2+R62
35	36	501-860		REELIN REPEAT R1
37	38	861-1220		REELIN REPEAT R2
39	40	2662-3051		REELIN REPEAT R7
41	42	3052-3428		REELIN REPEAT R8
43	44	1-3460		REELIN FULL LENGTH (LY691723)
45, 46				WPRE
47	48			HUMAN GDNF SIGNAL SEQUENCE
49	50			HUMAN INSULIN SIGNAL SEQUENCE
51	52			IgK SIGNAL SEQUENCE
53	54			REELIN REPEAT R3/R6 LINKER REGION
55	56			REELIN REPEAT R3+R6v1
57	58			REELIN REPEAT R3_v1
	59			HUMAN SERUM ALBUMIN
	60			HUMAN AZURUCIDIN
	61			MOUSE IGK LIGHT CHAIN

III. REELIN EXPRESSION VECTORS

[0074] Nucleic acid sequences encoding the disclosed Reelin fusion proteins can be inserted into delivery vectors and expressed from transcription units within the vectors (e.g., AAV vectors). The recombinant vectors can be DNA plasmids or viral vectors. Generation of the vector construct can be accomplished using any suitable genetic engineering techniques well known in the art, including, without limitation, the standard techniques of PCR, oligonucleotide synthesis, restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook et al. *Molecular Cloning: A Laboratory Manual*. (1989)), Coffin et al. (*Retroviruses*. (1997)) and “RNA Viruses: A Practical Approach” (Alan J.

Cann, Ed., Oxford University Press, (2000)). As will be apparent to one of ordinary skill in the art, a variety of suitable vectors are available for transferring nucleic acids of the invention into cells. The selection of an appropriate vector to deliver nucleic acids and optimization of the conditions for insertion of the selected expression vector into the cell, are within the scope of one of ordinary skill in the art without the need for undue experimentation. Viral vectors comprise a nucleotide sequence having sequences for the production of recombinant virus in a packaging cell. Viral vectors expressing nucleic acids of the invention can be constructed based on viral backbones including, but not limited to, a retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV), pox virus or alphavirus. The recombinant vectors capable of expressing the nucleic acids of the disclosure can be delivered as described herein, and persist in target cells (e.g., stable transformants).

[0075] Nucleic acid sequences used to practice this invention can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Pat. No. 4,458,066.

Transgene

[0076] As used herein, a transgene refers to a nucleotide sequence encoding a Reelin fusion proteins as disclosed herein. A variant transgene is a polynucleotide sequence that encodes a variant polypeptide that is at least 55%, 65%, 70%, 75%, 80%, 85%, 90% and more preferably at least 95%, 97% or 99% homologous to a relevant region of the naturally occurring sequence over at least 20, preferably at least 30, for instance at least 40, 60, 100, 200, 300, 400 or more contiguous amino acids, or even over the entire sequence of the variant.

[0077] Homology can be measured using known methods. For example, the UWGCG Package provides the BESTFIT™ program, which can be used to calculate homology (for example used on its default settings) (Devereux et al. (1984) Nucleic Acids Research 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F.

(1993) J Mol Evol 36:290-300; Altschul, S, F et al. (1990) J Mol Biol 215:403-10. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

Homology variants sequence identity

[0078] Substitutions preferably introduce one or more conservative changes, which replace amino acids with other amino acids of similar chemical structure, similar chemical properties, or similar side-chain volume. The amino acids introduced may have similar polarity, hydrophilicity, hydrophobicity, basicity, acidity, neutrality or charge to the amino acids they replace. Alternatively, the conservative change may introduce another amino acid that is aromatic or aliphatic in the place of a pre-existing aromatic or aliphatic amino acid. Conservative amino acid changes are well known in the art and may be selected in accordance with the properties of the 20 main amino acids.

[0079] Percent (%) amino acid sequence identity with respect to the Reelin fusion protein sequence identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the Reelin fusion protein sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The % identity values used herein are generated by WU-BLAST-2 which was obtained from [Altschul et al., Methods in Enzymology, 266: 460-480 (1996); blast.wustl.edu/blast/README]. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=11. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. A % amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the “longer” sequence in the aligned region. The “longer” sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

[0080] In a similar manner, “percent (%) nucleic acid sequence identity” with respect to the coding sequence of the Reelin fusion polypeptides identified herein is defined as the percentage of

nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the Reelin fusion protein coding sequence. The identity values used herein were generated by the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

Exemplary Regulatory Sequences

Kozak sequence

[0081] In one aspect, a transgene comprises sequences required for translation initiation, e.g., a Kozak sequence. During translation initiation, the small (40S) ribosomal subunit binds to the mRNA at the 5'-untranslated region (5'-UTR) and scans in the 5' to 3' direction to reach the coding sequence (CDS) start codon, most usually an AUG. The scanning model predicts that translation initiates at the AUG codon closest to the 5' end of the mRNA, which is termed the 'first-AUG rule.' Kozak analyzed 699 vertebrate mRNAs, and from this study an expanded consensus for the translation initiation site (TIS) context emerged, namely (GCC)GCC***CCAUGG*** in which "R" at position -3, in italic font, is A or G (97% of mRNAs have a purine, most often an A (61%)) and the AUG initiation codon is bold and underlined). Mutational analysis indicated positions -3R (most often A) and +4G are both the most conserved nucleotides and exert the most critical influence on translational efficiency (reviewed by Hernández et al. (2019) Trends in Biochemical Sciences 44 (12): 1009–21).

Secretion signal sequences

[0082] In one aspect, the AAV vector comprises a secretion signal sequence operably linked to the Reelin fusion protein-encoding nucleotide sequence. Signal sequences are generally short peptides at the N-terminus of a protein that target the protein to the secretory pathways of a cell. As used herein, a "secretion signal sequence" is a peptide that promotes the secretion of a protein (e.g. Reelin) from a cell, or the nucleotide sequence encoding that peptide, as appropriate. The peptide of the secretion signal sequence may be cleaved from the protein upon its secretion from the cell, resulting in a mature form of that protein.

[0083] In one embodiment, the secretion signal sequence is a Reelin secretion signal sequence having the amino acid sequence of SEQ ID NO: 2. The secretion signal sequence of the disclosure may, for example, comprise a nucleotide sequence having at least 70%, 80%, 90%, 95%, 96%,

97%, 98% 99% or 100% identity to SEQ ID NO: 1, wherein the peptide encoded by the nucleotide sequence causes the recombinant Reelin fusion polypeptide to which it is operably linked to be secreted from the cell in which it is expressed.

[0084] Exemplary signal sequences include, but are not limited to:

HUMAN GDNF SIGNAL SEQUENCE (SEQ ID NO: 47/48)

1	M	K	L	W	D	V	V	A	V	C	L	V	L	L	H	T	A	S	A		19
1	A	T	G	A	A	S	T	T	A	T	G	G	A	T	G	T	G	T	G	T	57

HUMAN INSULIN SIGNAL SEQUENCE (SEQ ID NO: 49/50)

1 M A L W M R L L F L L A L L A L W G P D 20
1 ATGGCCCTCTGGAATGCGCCCTGCTGCCCCCTGCTGGCGCTGCTGGCCCTCTGGGSACTGAC 60

IgK SIGNAL SEQUENCE (SEQ ID NO: 51/52)

1 M E T D T L L L W V L L L W V P G S T G D 20
1 A T G G A G A C A G A C A C A C T C C T G C T A T G S S T A C T G C T C T C T G G G T T C C A S G T T C C A C T G S G T G A C 61

[0085] As used herein, a recombinant Reelin repeat fusion protein, e.g., R3+R6v1 or R3+R6v2, is deemed to be “secreted” from a cell if the amount of recombinant protein secreted is greater than about 1% of the total amount of the recombinant Reelin repeat fusion protein synthesized by the cell.

[0086] In one aspect, a recombinant Reelin repeat fusion protein, e.g., R3+R6v1 or R3+R6v2, is deemed to be “secreted” from a cell if the amount of recombinant protein secreted is at least 5, 6, 7, 8, 9, or 10% or more of the total amount of the recombinant Reelin repeat fusion protein synthesized by the cell.

[0087] In another aspect, a recombinant Reelin repeat fusion protein, e.g., R3+R6v1 or R3+R6v2, is deemed to be “not secreted” from a cell if the amount of recombinant protein secreted is equal to or less than about 1% of the total amount of the recombinant Reelin repeat fusion protein synthesized by the cell.

[0088] In one aspect, the cell is a HEK293 cell.

Promoters

[0089] The term “promoter” or “promoter sequence” as used herein is a DNA regulatory sequence capable of facilitating transcription (e.g., capable of causing detectable levels of transcription and/or increasing the detectable level of transcription over the level provided in the absence of the promoter) of an operatively linked to a downstream (3' direction) coding or non-coding sequence. In some aspects, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements needed to initiate transcription at levels detectable above background. In some aspects, a promoter sequence may comprise a transcription initiation site, as well as binding sites for transcription factors. In addition to sequences sufficient to initiate transcription, a promoter may also include sequences of other cis-acting regulatory elements that are involved in modulating transcription (e.g., enhancers, silencers and/or insulators, such as locus control regions (LCRs) or matrix attachment regions (MARs)).

[0090] Examples of promoters known in the art that may be used in some aspects, e.g., in viral vectors disclosed herein, include constitutive promoters, e.g., unregulated promoters that allow for the continual transcription of its associated transgene in any cell type and/or under any conditions. Examples of constitutive promoters include, but are not limited to, a human β -actin promoter, a human elongation factor-1 α promoter (Kim, D.W., Uetsuki, T., Kaziro et al. (1990) *Gene* 91, 217–223), a cytomegalovirus (CMV) promoter (Thomsen et al. (1984) *Proc. Natl. Acad. Sci. U.S.A.* 81, 659–663), a modified CMV promoter that is resistant to gene silencing (see, for example, published U.S. Patent Application No. 2014/0017726 and International PCT patent application WO2012099540, the contents of which are incorporated by reference herein in their entireties), a chicken β -actin promoter combined with cytomegalovirus early enhancer (CBA) (see, for example, U.S. Patent No. 5,770,400, the content of which is incorporated by reference herein in its entirety), a truncated chimeric CMV-chicken β -actin (smCBA) promoter (see, for example, U.S. Patent No. 8,298,818, the content of which is incorporated by reference herein in its entirety), a Cbh promoter (see, for example, Gray et al. (2011) *Human Gene Therapy* 22 (9): 1143–53, the content of which is incorporated by reference herein in its entirety), a CMVd2 promoter; an shCMV promoter; a SCP3 promoter (Even et al. (2016) *PLoS ONE* 11(2): e0148918; a PGK promoter (Gilham et al. (2010) *J. Gene Med.* 12, 129–136; a UbC promoter (Gill et al., *Gene*

Ther. 8, 1539–154), an SV40 minimal promoter, an RSV promoter (Yamamoto et al. (1980) Cell 22, 787–797), or a murine stem cell virus (MSCV) promoter (Hawley et al. (1994) Gene Ther. 1, 136–138).

[0091] In addition, standard techniques are known in the art for creating functional promoters by mixing and matching known regulatory elements. Fragments of promoters, e.g., those that retain at least minimum number of bases or elements to initiate transcription at levels detectable above background, may also be used. In some aspects, a CMV enhancer may be combined with a tissue-specific promoter.

[0092] In some aspects, a promoter can be a synthetic promoter (see, for example, Jüttner et al. (2019) Nature Neuroscience 22 (8): 1345–56; Leeuw et al. (2016) Molecular Brain 9 (1): 52; Sanches-Medeiros et al. (2019) J Med Artif. Intell. 2:25; Wu et al. (2019) Nature Communications 10 (1): 2880, the contents of which are incorporated by reference herein in their entireties).

[0093] In some aspects, the promoter is tissue-specific such that, in a multi-cellular organism, the promoter drives expression only in a subset of specific cells. For example, a tissue-specific promoter is constitutively active in a connective, epithelial, muscle or nervous tissue. For example, tissue-specific promoters include, but are not limited to, neuron-specific promoters, adipocyte-specific promoters, cardiomyocyte-specific promoters, smooth muscle-specific promoters, photoreceptor-specific promoters, etc.

[0094] A neuron-specific promoter refers to a promoter that, when administered e.g., peripherally, directly into the central nervous system (CNS), or delivered to neuronal cells, including in vitro, ex vivo, or in vivo, preferentially drives or regulates expression of an operatively-linked transgene in neurons as compared to expression in non-neuronal cells.

[0095] Non-limiting example of tissue-specific expression elements for neurons include neuron-specific enolase (NSE) (see, e.g., EMBL HSEN02, X51956); an aromatic amino acid decarboxylase (AADC) promoter; a neurofilament promoter (see, e.g., GenBank HUMNFL, L04147); a synapsin promoter (see, e.g., GenBank HUMSYNIB, M55301); a thy-1 promoter (see, e.g., Chen et al., (1987) Cell, 51 :7-19; Llewellyn et al. (2010) Nat. Med., 16(10):1 161-1 166); a serotonin receptor promoter (see, e.g., GenBank S62283); a tyrosine hydroxylase promoter (TH) (see, e.g., Oh et al., (2009) Gene Ther., 16:437; Sasaoka et al., (1992) Mol. Brain Res., 16:274; Boundy et al., (1998) J. Neurosci., 18:9989; and Kaneda et al., (1991) Neuron, 6:583-594); a

methyl-CpG binding protein 2 (MeCP2) promoter, an optimized methyl-CpG binding protein 2 (MeCP2) promoter (the published International Patent Application No. WO2020180928, the content of which is incorporated by reference herein in its entirety), a Ca²⁺-calmodulin-dependent protein kinase II- α (CaMKII α) promoter (see, e.g., Mayford et al., (1996) *Proc. Natl. Acad. Sci. USA*, 93:13250; and Casanova et al., (2001) *Genesis*, 31 :37); a GnRH promoter (see, e.g., Radovick et al., (1991) *Proc. Natl. Acad. Sci. USA*, 88:3402-3406); an L7 promoter (see, e.g., Oberdick et al., (1990) *Science*, 248:223-226); a DNMT promoter (see, e.g., Badge et al., (1988) *Proc. Natl. Acad. Sci. USA*, 85:3648-3652); an enkephalin promoter (see, e.g., Comb et al., (1988) *EMBO J.*, 17:3793-3805); a myelin basic protein (MBP) promoter; a CMV enhancer/platelet-derived growth factor- β promoter (see, e.g., Liu et al., (2004) *Gene Ther.*, 11 :52-60); and the like. In some aspects, a portion of or all the minimal human synapsin 1 promoter (SYN) can be used (Kugler et al., (2003) *Gene Ther.*, 10(4): 337-47; Thiel et al., (1991) *Proc. Natl. Acad. Sci. USA*, 88(8) 3431 -5; Castle et al., (2016) *Methods Mol. Biol.*, 1382: 133-49; McLean et al., (2014) *Neurosci. Lett.*, 576: 73-78; Kugler et al., (2003) *Virology*, 311 (1): 89-95). In other aspects, the neural-specific promoter can be mGluR2, NFL, NFH, n β 2, PPE, Enk and EAAT2 promoters. A non-limiting example of a tissue-specific expression elements for astrocytes include the glial fibrillary acidic protein (GFAP) and EAAT2 promoters. A non-limiting example of a tissue-specific expression element for oligodendrocytes include the myelin basic protein (MBP) promoter. In certain aspects, a neuronal promoter can include a neuronal enhancer to direct expression to specific regions of the brain (see, for example, published U.S. Patent Application No. 2019/0247516, the content of which is incorporated by reference herein in its entirety). In one aspect, the promoter can be a fugu SST (somatostatin) promoter (Nathanson, et al. *Frontiers in Neural Circuits* 3: 19).

[0096] In another aspect, the promoter can be a BM88 88bp promoter (Papadodima, Olga, Maritina Sergaki, Catherine Hurel, Avgi Mamalaki, and Rebecca Matsas. 2005. "Characterization of the BM88 Promoter and Identification of an 88 Bp Fragment Sufficient to Drive Neurone-Specific Expression." *Journal of Neurochemistry* 95 (1): 146--59, Pignataro, Diego, Diego Sucunza, Lucía Vanrell, Esperanza Lopez-Franco, Iria G. Dopeso-Reyes, Africa Vales, Mirja Hommel, Alberto J. Rico, Jose L. Lanciego, and Gloria Gonzalez-Aseguinolaza. 2017. "Adeno-Associated Viral Vectors Serotype 8 for Cell-Specific Delivery of Therapeutic Genes in the Central

Nervous System.” *Frontiers in Neuroanatomy* 11 (February): 2. the contents of which are incorporated by reference herein in their entireties).

[0097] In other aspects, a promoter can be an inducible promoter (i.e., a promoter whose activity is controlled by an external stimulus, e.g., the presence of a particular temperature, compound, or protein). In some aspects, a promoter may be a temporally restricted promoter that drives expression depending on the temporal context in which the promoter is found. For example, a temporally restricted promoter may drive expression only during specific stages of a biological process.

[0098] Prokaryotic (Gossen et al. *TIBS* 18: 471-475, 1993) and insect regulatory systems (No et al. *Proc. Natl. Acad. Sci. USA* 93: 3346-3351, 1996) have been adapted to construct gene switches that function in mammalian cells. Since inducer molecules are not expected to have targets in mammalian cells, the possibility of interference with cellular processes is reduced. Of the prokaryotic proteins, the repressors from the lac operon (Brown, M., et al. *Cell* 49: 603-612, 1987; and Hu, M. C. -T. and N. Davidson *Cell* 48: 555-566, 1987), the tet operon (e.g., U.S. Patent No. 7,541,446, the content of which is incorporated by reference herein in its entirety) and the cumate operon (e.g., U.S. Patent No. 7,745,592, the content of which is incorporated by reference herein in its entirety) have been shown to function in mammalian cells. Many have been incorporated in eukaryotic inducible expression systems using different strategies to control activation and repression of expression. Activation of expression is mediated by a chimeric transactivator protein formed by the fusion of the bacterial repressor with an activation domain (Gossen, M. and H. Bujard, *Proc. Natl. Acad. Sci. USA* 89: 5547-5551, 1992, and Gossen, M., et al. *Science* 268: 1766-1769, 1995, U.S. Patent No. 7,745,592, the contents of which are incorporated by reference herein in their entireties). The transactivator can activate transcription when bound to its DNA recognition sequence placed upstream of the minimal promoter. The ability of the activator to bind DNA is dependent on the presence/absence of the inducer molecule (e.g., doxycycline or cumate depending on the inducible system being used). Repression of expression is mediated by the repressor bound to operator sites placed downstream of the minimal promoter in the absence of inducer and repression is relieved on the addition of the inducer (Brown, M., et al. *Cell* 49: 603-612, 1987).

[0099] In one aspect, the promoter may be a promoter which is less than 1 kb. The promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. The promoter may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800 nucleotides.

[0100] In one aspect, the promoter can be a pol III-dependent promoter, e.g., a U6 snRNA or H1-RNA promoter, for the expression of non-coding RNAs including, but not limited to, U snRNAs or miRNAs. In another aspect, the promoter can be a polymerase II U snRNA-dependent promoter, e.g., a human U1 snRNA gene and of its promoter and terminator regions (see, for example, published U.S. Patent No. 7,947,823, the content of which is incorporated by reference herein in its entirety).

Exemplary cis-acting regulatory elements

[0101] Non-limiting examples of elements to enhance the transgene target specificity and expression include endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (polyA) sequences and 5'-UTR introns.

[0102] In one aspect, an expression vector may comprise at least one element to enhance the transgene target specificity and expression (See e.g., Powell et al. Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015, the contents of which are herein incorporated by reference in its entirety).

5'-UTR introns

In one aspect, the 5'-UTR may include an intron. Non-limiting examples of 5'-UTR introns include, CBA-MVM 5'-UTR intron, MVM (67-97 bps), FIX truncated intron 1 (300 bps), (β -globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobulin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps). In one aspect, the 5'-UTR introns may be 100-500 nucleotides in length. The 5'-UTR introns may have a length of 80,

90, 100, 110, 120, 130, 140, 150, 160, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490 or 500.

Post-transcriptional regulatory elements (PREs)

[0103] In certain aspects, an expression vector may comprise a “post-transcriptional regulatory element” (“PRE”) that can form a tertiary structure enhancing expression of an mRNA transcript. Examples of post-transcriptional regulatory element that may be used with the nucleic acid molecules and vectors disclosed herein include a PRE derived from hepatitis B (HPRE), bat (BPRE), ground squirrel (GSPRE), arctic squirrel (ASPRE), duck (DPRE), chimpanzee (CPRE) and woolly monkey (WMPRE) or woodchuck (WPRE), optionally wherein said post-transcriptional regulatory element is disposed 3' to the transgene.

[0104] WPRE is a tripartite regulatory element with gamma, alpha, and beta components. The alpha component is 80bp long:

GCCACGGCGGAACATCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTG
TTGGGCACTGACAATTCCGTGGT (SEQ ID NO: 45)

[0105] When used alone without the gamma and beta WPRE components, the alpha component is only 9% as active as the full tripartite WPRE. The sequence for full tripartite WPRE is:

AAACAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCCTTAACATGTTGC
TCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCG
TATGGCTTTTCATTTTCTCCTCCTTGTATAAAICCTGGTTGCTGTCTCTTTATGAGGAGTT
GTGGCCCGTTGTGTCAGGCAACGTTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCC
CACTGGTTGGGGCAATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTTCGCTTTCCCC
CTCCCTATTGCCACGGCGGAACATCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGG
GCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTCGGGGAAGCTGACGTCCTTT
CCATGGCTGCTCGCCTGTGTTGCCACCTGGATTCCTGCGCGGGACGTCCTTCTGCTACG
TCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCG
GCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCGGCC
TCCCGCCTG (SEQ ID NO: 46)

[0106] This sequence has 100% homology with base pairs 1093 to 1684 of the Woodchuck hepatitis B virus (WHV8) genome. Usually, unspliced mRNAs are exported into the cytoplasm with low efficiency. PRE cis-acting RNA elements can increase the accumulation of cytoplasmic mRNA of an intronless gene by promoting mRNA exportation from the nucleus to the cytoplasm, enhancing 3' end processing and stability.

Polyadenylation sequences

[0107] With the notable exception of replication-dependent histone transcripts, most eukaryotic mRNAs have a poly(A) tail at their 3' ends. 3' end processing is a nuclear co-transcriptional process that promotes transport of mRNAs from the nucleus to the cytoplasm and affects the stability and the translation of mRNAs. Formation of this 3' end occurs in a two-step process requiring the presence of two sequence elements in mRNA precursors (pre-mRNAs); a highly conserved hexanucleotide AAUAAA (polyadenylation signal) and a downstream G/U-rich sequence. In a first step, pre-mRNAs are cleaved between these two elements 10-35 nucleotides downstream of the AAUAAA sequence. In a second step, tightly coupled to the first step, the newly formed 3' end is extended by addition of a poly(A) sequence consisting of 200-250 adenylates which affects subsequently all aspects of mRNA metabolism, including mRNA export, stability and translation (Dominski, Z. and W. F. Marzluff (2007), *Gene* 396(2): 373-90, the content of which is incorporated by reference herein in its entirety). Polyadenylation signals known in the art include, but are not limited, polyadenylation signals from simian virus 40 (SV40) (Schek et al., *Mol. Cell Biol.* 12(12): 5386-93 (1992)), α -globin (Thein et al., *Blood* 71(2): 313-19), β -globin (Orkin et al., *EMBO J.* 4(2): 453-6 (1985)), human collagen, polyoma virus (Batt et al., *Mol. Cell Biol.* 15(9): 4783-90 (1995)) and bovine growth hormone (bGH) (Woychik et al., *Proc. Natl. Acad. Sci. USA* 81(13): 3944-8 (1984); U.S. Pat. No. 5,122,458, the content of which is incorporated by reference herein in its entirety).

miRNAs

[0108] The AAV viral vectors as disclosed herein may comprise binding sites for one or more microRNAs (or miRNAs or miRs). miRNAs are small, non-coding, single stranded ribonucleic acid molecules (RNAs), which are usually 19-25 nucleotides in length. Mature microRNAs primarily bind to the 3' untranslated region (3'-UTR) of target messenger RNAs (mRNAs) through partially or fully pairing with the complementary sequences of target mRNAs, promoting the

degradation of target mRNAs at a post-transcriptional level, and in some cases, inhibiting the initiation of translation. miRNA genes are generally transcribed as long primary transcripts of miRNAs (i.e. pri-miRNAs). The pri-miRNA is cleaved into a precursor of a miRNA (i.e. pre-miRNA) which is further processed to generate the mature and functional miRNA (see, for example, U.S. Patent No. 10,570,395, the content of which is incorporated by reference herein in its entirety).

Recombinant protein domains

[2478] In one aspect, the viral vector composition may comprise nucleotide sequences encoding a regulatory polypeptide sequence that can modulate the activity of the protein encoded by the transgene, for example, an mRNA sequence encoding a nuclear localization signal, a signal sequence, a cell penetrating peptide (CPP), a protease cleavage site (e.g., furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, granzyme B, factor XA, enterokinase, genenase, sortase, preScission protease, thrombin, TEV protease or elastase 1), a destabilization domain (DD) (e.g. an FKBP12 protein), a nucleic acid binding domain, a protein binding domain, or any protein domain of a native protein or recombinant fusion protein having a regulatory function.

Cell penetrating peptides (CPPs) refer to a heterogeneous group of peptide molecules that when fused to a “cargo” polypeptide, e.g., a Reelin fusion protein, facilitates the uptake of the polypeptide into cells. Prominent examples of CPPs include the HIV-1 TAT translocation domain (Green; M. and Loewenstein, P. M. (1988) Cell 55, 1179-1188) and the homeodomain of the Antennapedia protein from *Drosophila* (Joliet; A. et al. (1991) Proc. Natl. Acad. Sci. USA 88, 1864-1868). Mutation studies of the Antennapedia protein revealed that a sequence of 16 amino acids called penetratin or pAntp (Derossi, D. et al. (1994) J. Biol. Chem. 269, 10444-10450) is necessary and sufficient for membrane translocation. In the following, other protein-derived CPPs were developed such as the basic sequence of the HIV-1 Tat protein (Vives, E. et al. (1997) J. Biol. Chem. 272, 16010-16017). A synthetic peptide developed is the amphipathic model peptide MAP (Oehlke, J. et al. (1998) Biochim. Biophys. Acta 1414, 127-139).

IV. DESIGN OF REELIN VIRAL EXPRESSION VECTORS

[0109] A recombinant adeno associated virus or rAAV is a replication-deficient parvovirus, the single stranded DNA genome of which is about 4.7 kb in length including 145 nucleotide inverted

terminal repeat (ITRs). The nucleotide sequence of the AAV serotype 2 (AAV2) genome is presented in Srivastava et al., *J Virol*, 45: 555-564 (1983) as corrected by Ruffing et al., *J Gen Virol*, 75: 3385-3392 (1994). Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins.

[0110] As the signals directing AAV replication, genome encapsidation and integration are contained within the ITRs of the AAV genome, some, or all the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) can be replaced with foreign DNA such as an expression cassette, as disclosed herein, with the rep and cap proteins provided in trans. The sequence located between ITRs of an AAV vector genome is referred to herein as the “payload”.

[0111] The actual capacity of any AAV particle may vary depending on the viral proteins employed. Typically, the vector genome (including ITRs) is not more than about 5 kb, e.g., not more than about 4.9 kb, 4.8 kb or 4.7 kb.

[0112] The ITRs are each 145 bases in length. Thus, the payload is typically not more than about 4.7 kb, 4.6 kb, 4.5 kb or 4.4 kb in length. Preferably it is not more than 4.4 kb in length. A recombinant AAV (rAAV) may therefore contain up to about 4.7 kb, 4.6 kb, 4.5 kb or 4.4 kb of unique payload sequence.

[0113] However, following infection of a target cell, protein expression and replication from the vector requires synthesis of a complementary DNA strand to form a double stranded genome. This second strand synthesis represents a rate limiting step in transgene expression. The requirement for second strand synthesis can be avoided using so-called “self-complementary AAV” (scAAV)

vectors in which the payload contains two copies of the same transgene payload in opposite orientations to one another, i.e., a first payload sequence followed by the reverse complement of that sequence. These scAAV genomes can adopt either a hairpin structure, in which the complementary payload sequences hybridize intramolecularly with each other, or a double stranded complex of two genome molecules hybridized to one another. Transgene expression from such scAAVs is much more efficient than from conventional rAAVs, but the effective payload capacity of the vector genome is halved because of the need for the genome to carry two complementary copies of the payload sequence.

[0114] An scAAV vector genome may contain one or more mutations in one of the ITR sequences to inhibit resolution at one terminal repeat, and consequently increase yield in an scAAV preparation. Thus, one of the ITRs in an scAAV may be deleted for the terminal resolution site or may contain an inactivating mutation in the terminal resolution site. See, for example, Wang et al., *Gene Therapy* (2003) 10, 2105-2111 and McCarty et al., *Gene Therapy* (2003) 10, 2112-2118. It will therefore be apparent that the two ITR sequences at either end of an AAV genome need not be identical. scAAVs are reviewed in McCarty, *Molecular Therapy*, 16(10), 2008, 1648-1656.

[0115] In this specification, the term “rAAV vector” is generally used to refer to vectors having only one copy of any given payload sequence (i.e., a rAAV vector is not an scAAV vector), and the term “AAV vector” is used to encompass both rAAV and scAAV vectors.

Serotypes

[0116] AAV sequences in the AAV vector genomes (e.g., ITRs) may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11 and AAV PHP.B. The nucleotide sequences of the genomes of the AAV serotypes are known in the art. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., *J. Virol.*, 45: 555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_01829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249,

respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004); AAV PHP.B is described by Deverman et al., Nature Biotech. 34(2), 204-209 and its sequence deposited under GenBank Accession No. KU056473.1. Reviews of AAV serotypes may be found in Choi et al (2005) Curr Gene Ther 5(3): 299-310 and Wu et al (2006) Molecular Therapy 14(3), 316-327.

[0117] The skilled person can select an appropriate serotype, Glade, clone or isolate of AAV for use in the present vector on the basis of their common general knowledge. It should be understood, however, that also encompassed herein is the use of an AAV genome of other serotypes that may not yet have been identified or characterized. The AAV serotype can assist in determining the tissue specificity of infection (or tropism) of an AAV virus. Accordingly, preferred AAV serotypes for use in the AAV-Reelin to be administered to patients, as described herein, are those which have natural tropism for or a high efficiency of infection of target cells within the brain and CNS. Depending on the mode of administration, it may also be advantageous for the AAV serotype to have the ability to cross the blood-brain barrier (e.g., AAV9).

Packaging virions

[0118] Virion particles comprising vector genomes are generated in packaging cells capable of replicating viral genomes, expressing viral proteins (e.g., rep and cap proteins), and assembling virion particles. Packaging cells may also require helper virus functions, e.g., from adenovirus, El-deleted adenovirus, or herpesvirus. Techniques to produce AAV vector particles in packaging cells are standard in the art. Production of pseudotyped AAV is disclosed in, for example, WO 01/83692, the content of which is incorporated by reference herein in its entirety. In various aspects, AAV capsid proteins may be modified to enhance delivery of the recombinant vector. Modifications to capsid proteins are generally known in the art (see, for example, published U.S. Patent Application Nos. US 2005/0053922 and US 2009/0202490, the contents of which are incorporated by reference herein in their entirety).

[0119] One method of generating a packaging cell is to create a cell line that stably expresses all the necessary components for AAV particle production. For example, a plasmid (or multiple plasmids) comprising an AAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the AAV genome, and a selectable marker, such as a neomycin resistance gene, are

integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing (Samulski et al., 1982, *Proc. Natl. Acad. Sci. USA*, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin et al., 1983, *Gene*, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, *J. Biol. Chem.*, 259:4661-4666). The packaging cell line is then infected with a helper virus such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of AAV. Other examples of suitable methods employ adenovirus or baculovirus rather than plasmids to introduce AAV genomes and/or rep and cap genes into packaging cells.

[0120] Alternatively, a packaging cell can be generated by simply transforming a suitable cell with one or more plasmids encoding an AAV genome, AAV proteins, and any required helper virus functions. The so-called “triple transfection” method utilizes three plasmids each carrying one of these sets of genes. See Grieger et al., *Nature Protocols* 1(3), 1412-128 (2006) and references cited therein. General principles of AAV production are reviewed in, for example, Carter, 1992, *Current Opinions in Biotechnology*, 1533-539; and Muzyczka, 1992, *Curr. Topics in Microbial, and Immunol.*, 158:97-129). Various approaches are described in Ratschin et al., *Mol. Cell. Biol.* 4:2072 (1984); Hermonat et al., *Proc. Natl. Acad. Sci. USA*, 81:6466 (1984); Tratschin et al., *Mol. Cell. Biol.* 5:3251 (1985); McLaughlin et al., *J. Virol.*, 62:1963 (1988); and Lebkowski et al., 1988 *Mol. Cell. Biol.*, 7:349 (1988). Samulski et al. (1989, *J. Virol.*, 63:3822-3828); U.S. Pat. No. 5,173,414; WO 95/13365 and corresponding U.S. Pat. No. 5,658,776; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin et al. (1995) *Vaccine* 13:1244-1250; Paul et al. (1993) *Human Gene Therapy* 4:609-615; Clark et al. (1996) *Gene Therapy* 3:1124-1132; U.S. Pat. Nos. 5,786,211; 5,871,982; and 6,258,595, the contents of each of which are incorporated by reference herein in their entireties.

[0121] Techniques for scAAV production are described, for example, in Grieger et al., *Molecular Therapy* 24(2), 287-297, 2016.

[0122] In one aspect, packaging cells may be stably transformed cell lines such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another aspect, packaging cells are cells that are not

transformed cells such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[0123] The term "recombinant virus" as used herein is intended to refer to a non-wild-type and/or an artificially produced recombinant virus (e.g., a parvovirus, adenovirus, lentivirus or adeno-associated virus etc.) that comprises a transgene or other heterologous nucleic acid. The recombinant virus may comprise a recombinant viral genome (e.g. comprising a cis-acting regulatory sequences as described herein and a transgene) packaged within a viral (e.g.: AAV) capsid.

CpG content

[0124] In one aspect, a rAAV vector, including an rAAV vector genome as described herein, comprises at least one synthetic AAV ITR, wherein one or more CpG islands (a cytosine base followed immediately by a guanine base (a CpG) in which the cytosines in such arrangement tend to be methylated) that typically occur at, or near the transcription start site in an ITR are deleted and/or substituted. In one aspect, deletion, or reduction in the number of CpG islands can reduce the immunogenicity of the rAAV vector. This results from a reduction or complete inhibition in TLR-9 binding to the rAAV vector DNA sequence, which occurs at CpG islands. It is also well known that methylation of CpG motifs results in transcriptional silencing. Removal of CpG motifs in the ITR is expected to result in decreased TLR-9 recognition and/or decreased methylation and therefore decreased transgene silencing. In some aspects, it is the minimal functional ITR in which one or more CpG islands are deleted and/or substituted. In one aspect, AAV ITR2 is known to contain 16 CpG islands of which one or more, or all 16 can be deleted.

[0125] In some aspects, at least 1 CpG motif is deleted and/or substituted, e.g., at least 4 or more or 8 or more CpG motifs, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 CpG motifs. The phrase "deleted and/or substituted" as used herein means that one or both nucleotides in the CpG motif is deleted, substituted with a different nucleotide, or any combination of deletions and substitutions.

[0126] In certain aspects, the transgene nucleic acid sequence can also be codon optimized to enhance expression *in vivo* and/or to reduce the number of CpG islands and avoid an innate immune response to the vector.

Dual AAV vectors

[0127] Various strategies have been investigated to overcome the limitation of AAV cargo capacity. Several groups have attempted to “force” large genes into one of the many AAV capsids available by developing the so-called oversize vectors. Although administration of oversize AAV vectors achieves therapeutically-relevant levels of transgene expression in rodent and canine models of human inherited diseases, including the retina of the *Abca4*^{−/−} and shaker 1 (*sh1*) mouse models of STGD andUSH1B, the mechanism underlying oversize AAV-mediated transduction remains elusive. Oversize AAV vectors do not contain a pure population of intact large size genomes but rather a heterogeneous mixture of mostly truncated genomes ≤5 kb in length. Following infection, reassembly of these truncated genomes in the target cell nucleus has been proposed as a mechanism for oversize AAV vector transduction. Independent of transduction mechanism and *in vivo* efficacy, the heterogeneity in oversize AAV genome sizes is a major limitation for their application in human gene therapy.

[0128] Alternatively, the inherent ability of AAV genomes to undergo intermolecular concatemerization is exploited to transfer large genes *in vivo* by splitting a large gene expression cassette into halves (<5 kb in size), each contained in one of two separate (dual) AAV vectors. In the dual AAV trans-splicing strategy, a splice donor (SD) signal is placed at the 3′ end of the 5′-half vector and a splice acceptor (SA) signal is placed at the 5′ end of the 3′-half vector. Upon co-infection of the same cell by the dual AAV vectors and inverted terminal repeat (ITR)-mediated head-to-tail concatemerization of the two halves, trans-splicing results in the production of a mature mRNA and full-size protein. Trans-splicing has been successfully used to express large genes in muscle and retina.

[0129] Alternatively, the two halves of a large transgene expression cassette contained in dual AAV vectors may contain homologous overlapping sequences (at the 3′ end of the 5′-half vector and at the 5′ end of the 3′-half vector, dual AAV overlapping), which will mediate reconstitution of a single large genome by homologous recombination. This strategy depends on the recombinogenic properties of the transgene overlapping sequences.

[0130] A third dual AAV strategy (hybrid) is based on adding a highly recombinogenic region from an exogenous gene [i.e. alkaline phosphatase, AP] to the trans-splicing vector. The added region is placed downstream of the SD signal in the 5'-half vector and upstream of the SA signal in the 3'-half vector in order to increase recombination between the dual AAVs. The published US patent application No 2010/003218 and US Patent No.10,494,645, both of which are incorporated by reference herein in their entireties, provide additional examples of dual vector systems.

V. PHARMACEUTICAL COMPOSITIONS AND MODES OF ADMINISTRATION

Reelin expression vector administration in vivo

[0131] As is well known in the art, a vector is a tool that allows or facilitates the transfer of an entity from one environment to another. By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell. Optionally, once within the target cell, the vector may then serve to maintain the heterologous DNA within the cell. Examples of vectors used in recombinant DNA techniques include plasmids, chromosomes, artificial chromosomes, or viruses.

[0132] Non-viral delivery systems include but are not limited to DNA transfection methods. Here, transfection includes a process using a non-viral vector to deliver a gene to a target mammalian cell. Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), and combinations thereof.

[0133] Viral delivery systems include but are not limited to adenovirus vector, an adeno-associated viral (AAV) vector, a herpes viral vector, retroviral vector, lentiviral vector, baculoviral vector. Other examples of vectors include ex vivo delivery systems, which include but are not limited to DNA transfection methods such as electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection.

[0134] For gene therapy applications, the expression vectors disclosed herein can be incorporated into a non-replicating viral vector, for example, an adeno-associated virus (AAV).

[0135] In certain aspects, the rAAV vectors and/or rAAV genome as disclosed herein can be formulated in a solvent, emulsion, or other diluent in an amount sufficient to obtain a desired titer of an rAAV vector disclosed herein. In other aspects, the rAAV vectors and/or rAAV genome as disclosed herein may be formulated in a solvent, emulsion or a diluent in an amount of, e.g., less than about 90% (v/v), less than about 80% (v/v), less than about 70% (v/v), less than about 65% (v/v), less than about 60% (v/v), less than about 55% (v/v), less than about 50% (v/v), less than about 45% (v/v), less than about 40% (v/v), less than about 35% (v/v), less than about 30% (v/v), less than about 25% (v/v), less than about 20% (v/v), less than about 15% (v/v), less than about 10% (v/v), less than about 5% (v/v), or less than about 1% (v/v). In other aspects, the rAAV vectors and/or rAAV genome as disclosed herein can disclosed herein may comprise a solvent, emulsion or other diluent in an amount in a range of, e.g., about 1% (v/v) to 90% (v/v), about 1% (v/v) to 70% (v/v), about 1% (v/v) to 60% (v/v), about 1% (v/v) to 50% (v/v), about 1% (v/v) to 40% (v/v), about 1% (v/v) to 30% (v/v), about 1% (v/v) to 20% (v/v), about 1% (v/v) to 10% (v/v), about 2% (v/v) to 50% (v/v), about 2% (v/v) to 40% (v/v), about 2% (v/v) to 30% (v/v), about 2% (v/v) to 20% (v/v), about 2% (v/v) to 10% (v/v), about 4% (v/v) to 50% (v/v), about 4% (v/v) to 40% (v/v), about 4% (v/v) to 30% (v/v), about 4% (v/v) to 20% (v/v), about 4% (v/v) to 10% (v/v), about 6% (v/v) to 50% (v/v), about 6% (v/v) to 40% (v/v), about 6% (v/v) to 30% (v/v), about 6% (v/v) to 20% (v/v), about 6% (v/v) to 10% (v/v), about 8% (v/v) to 50% (v/v), about 8% (v/v) to 40% (v/v), about 8% (v/v) to 30% (v/v), about 8% (v/v) to 20% (v/v), about 8% (v/v) to 15% (v/v), or about 8% (v/v) to 12% (v/v).

[0136] A viral composition may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected regarding the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient, or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilizing agent(s), and other carrier agents that may aid or increase the viral entry into the target site (such as for example a lipid delivery system).

[0137] In certain aspects, the pharmaceutically acceptable excipient is 1×PBS, (e.g., 0.154M NaCl, 0.056M Na₂HPO₄, and 0.0106 M KH₂PO₄) or DPBS (e.g., 0.337M NaCl, 0.27 M KCl, 0.015M Na₂HPO₄, and 0.0015M KH₂PO₄).

[0138] In certain aspects, the viral vector is present at a concentration of 2.5×10^1 vg/ml, 7.5×10^{11} vg/ml, or 2.5×10^{12} vg/ml.

[0139] In certain aspects, the pH of the composition is 6.5 to 7.5; 7.0 to 7.5; 6.8 to 7.2. In some aspects, the pH of the composition is 7.0 or 7.4.

[0140] The composition may further comprise empty capsids at a percentage of about 95% cp/cp, 90% cp/cp, 85% cp/cp, 80% cp/cp, 75% cp/cp, 70% cp/cp, 65% cp/cp, 60% cp/cp, 55% cp/cp, 50% cp/cp, 45% cp/cp, 40% cp/cp, 35% cp/cp, 30% cp/cp, 25% cp/cp, 20% cp/cp, 15% cp/cp, 10% cp/cp, 5% cp/cp or less.

[0141] In some embodiments, the intrathecal administration is via a pump. The pump may be a surgically implanted osmotic pump. In certain embodiments, the osmotic pump is implanted into the subarachnoid space of the spinal canal to facilitate intrathecal administration.

[0142] In certain embodiments, human subjects receive a one-time treatment of intrathecally delivered vector (e.g., AAV9) comprising nucleotide sequences Reelin fusion protein in an amount of about 1×10^{13} GC to about 10×10^{13} GC over a period of about 24 hours.

[0143] The slow continuous intrathecal infusion of the AAV9-Reelin fusion protein can be scaled up to humans by using an osmotically driven pump such as the DUROS® implant, ALZA Corporation (Mountain View, Calif.). See also, J. C. Wright, J. Culwell, Long-term controlled delivery of therapeutic agents by the osmotically driven DUROS® implant, in: M. J. Rathbone, J. Hadgraft, M. S. Roberts (Eds.), *Modified-Release Drug Delivery Technology*, Informa Healthcare, New York, 2008, pp. 143-149.

[0144] Osmotic delivery devices and their component parts have been described, for example, in U.S. Pat. Nos. 5,609,885; 5,728,396; 5,985,305; 5,997,527; 6,113,938; 6,132,420; 6,156,331; 6,217,906; 6,261,584; 6,270,787; 6,287,295; 6,375,978; 6,395,292; 6,508,808; 6,544,252; 6,635,268; 6,682,522; 6,923,800; 6,939,556; 6,976,981; 6,997,922; 7,014,636; 7,207,982; 7,112,335; 7,163,688; U.S. Patent Publication Nos. 2005-0175701, 2007-0281024, and 2008-0091176.

[0145] The DUROS® delivery device typically consists of a cylindrical reservoir which contains the osmotic engine, piston, and drug formulation. The reservoir is capped at one end by a controlled-rate water-permeable membrane and capped at the other end by a diffusion moderator

through which drug formulation is released from the drug reservoir. The piston separates the drug formulation from the osmotic engine and utilizes a seal to prevent the water in the osmotic engine compartment from entering the drug reservoir. The diffusion moderator is designed, in conjunction with the drug formulation, to prevent body fluid from entering the drug reservoir through the orifice.

[0146] The DUROS® device releases a therapeutic agent at a predetermined rate based on the principle of osmosis. Extracellular fluid enters the DUROS® device through a semi-permeable membrane directly into a salt engine that expands to drive the piston at a slow and even delivery rate. Movement of the piston forces the drug formulation to be released through the orifice or exit port at a predetermined sheer rate. In one embodiment of the present invention, the reservoir of the DUROS® device is load with a suspension formulation of the present invention, comprising, for example, 1×10^{11} gc AAV9-Reelin, wherein the device is capable of delivering the suspension formulation to a subject over an extended period of time at a pre-determined, therapeutically effective delivery rate.

[0147] Other implantable, drug delivery devices may be used in the practice of the present invention and may include regulator-type implantable pumps that provide constant flow, adjustable flow, or programmable flow of the compound, such as those available from Codman & Shurtleff, Inc. (Raynham, Mass.), Medtronic, Inc. (Minneapolis, Minn.), and Tricumed Medinzintechnik GmbH (Germany).

[0148] For example, a conventional syringe and needle can be used to inject a rAAV virion suspension into a subject. Parenteral administration of rAAV vectors, by injection can be performed, for example, by bolus injection or continuous infusion, intracavernosally, intravenously, intramuscularly or subcutaneously. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain agents for a pharmaceutical formulation, such as suspending, stabilizing and/or dispersing agents or enough salts or monosaccharides to make the solution isotonic with blood. Alternatively, the rAAV vectors and/or rAAV genome as disclosed herein can be in powder form (e.g., lyophilized) for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

[0149] For example, administration to skeletal muscle includes, but is not limited to, administration to skeletal muscle in the limbs (e.g., upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (e.g., tongue), thorax, abdomen, pelvis/perineum, and/or digits. Administration to cardiac muscle includes administration to the left atrium, right atrium, left ventricle, right ventricle and/or septum. The virus vector and/or capsid can be delivered to cardiac muscle by intravenous administration, intra-arterial administration such as intra-aortic administration, direct cardiac injection (e.g., into left atrium, right atrium, left ventricle, right ventricle), and/or coronary artery perfusion. Administration to a diaphragm muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration.

[0150] Alternatively, one may administer the virus vector and/or virus capsids disclosed herein in a local rather than systemic manner, for example, in a bolus or sustained-release formulation. Further, the virus vector and/or virus capsid can be delivered adhered to a surgically implantable matrix (see, for example, published U.S. Patent Application No. 2004/0013645, the content of which is incorporated by reference herein in its entirety). The virus vectors and/or virus capsids disclosed herein can be administered to the lungs of a subject by any suitable means, optionally by administering an aerosol suspension of respirable particles comprised of the virus vectors and/or virus capsids, which the subject inhales. The respirable particles can be liquid or solid. Aerosols of liquid particles comprising the virus vectors and/or virus capsids may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, e.g., U.S. Patent No. 4,501,729, the content of which is incorporated by reference herein in its entirety). Aerosols of solid particles comprising the virus vectors and/or capsids may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0151] In one aspect, a pharmaceutical formulation comprising a viral vector as disclosed herein, having an SMSM compound inducible viral expression vector and a pharmaceutically-acceptable excipient to at least one target site in the CNS of the subject in a dose of at least about 1×10^9 vg, 1×10^{10} vg, 1×10^{11} vg, or 1×10^{12} vg, or more. In some aspects, the dose is at least about 1×10^{13} vg, 5×10^{13} vg, 1.5×10^{14} vg, or 5×10^{14} vg.

[0152] In certain aspects, the target site is the cerebrospinal fluid (CSF) space (e.g., lumbar puncture); sub-arachnoid space, (e.g., cisterna magna); brain, (e.g., cerebroventricular space, cerebellum, cerebrum, hippocampus, interior cortex, dorsal root ganglion, or caudate nucleus); or the spine (e.g., the lumbar spine, thoracic spine, cervical spine). In some aspects, the active ingredient is delivered in two injections: one in the right cerebellum and one in the left cerebellum. In some aspects, these are two equal injections. In some aspects, the active ingredient is administered by injecting the cerebellum and providing it systemically.

[0153] In certain aspects, the pharmaceutical formulation may be administered intraparenchymally, intrathecally, intracerebroventricularly, intracisternally, systemically or a combination of these. In some aspects, the pharmaceutical formulation is administered by intrathecally in equal portions to the cisterna magna and the lumbar spine.

[0154] In some aspects, the dose is an amount of at least 3.7×10^{10} vg/g, 1.11×10^{11} vg/g, or 3.7×10^{11} vg/g on a brain weight basis. In some aspects, the pharmaceutical formulation comprises a vector concentration of at least 2×10^{12} vg/ml, 7×10^{12} vg/ml, or 2×10^{13} vg/ml.

[0155] In some aspects, the dose is an amount of about 1×10^{11} vg, 2×10^{11} vg, 3×10^{11} vg, 4×10^{11} vg, 5×10^{11} vg, 6×10^{11} vg, 7×10^{11} vg, 8×10^{11} vg, 9×10^{11} vg, 1×10^{12} vg, 2×10^{12} vg, 3×10^{12} vg, 4×10^{12} vg, 5×10^{12} vg, 6×10^{12} vg, 7×10^{12} vg, 8×10^{12} vg, 9×10^{12} vg, 1×10^{13} vg, 2×10^{13} vg, 3×10^{13} vg, 4×10^{13} vg, 5×10^{13} vg, 6×10^{13} vg, 7×10^{13} vg, 8×10^{13} vg, 9×10^{13} vg, 1×10^{14} vg.

[0156] In some aspects of the method, the pharmaceutical formulation can be administered as a single bolus injection of about 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1 ml, 2 ml, 3 ml, 4 ml, or 5 ml. In other aspects, the pharmaceutical formulation is delivered as an infusion at a rate of 0.001 ml/min to 1 ml/min, (e.g., 0.01 ml/min).

[0157] In certain aspects, more than one administration (e.g., two, three, four, five, six, seven, eight, nine, 10, etc., or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., hourly, daily, weekly, monthly, yearly, etc. Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For instance, treatment of a disease or disorder may comprise a one-time administration of an effective dose of a pharmaceutical composition virus vector disclosed herein. Alternatively, treatment of a disease or disorder may comprise multiple administrations of an effective dose of a virus vector carried out over a range of time periods, such as, e.g., once daily,

twice daily, trice daily, once every few days, or once weekly. In certain aspects, rAAV particles may be administered to multiple locations, for example, 1, 2, 3, 4, or 5 location simultaneously or staggered over time.

[0158] The timing of administration can vary from individual to individual, depending upon such factors as the severity of an individual's symptoms. For example, an effective dose of a virus vector disclosed herein can be administered to an individual once every six months for an indefinite period, or until the individual no longer requires therapy. A person of ordinary skill in the art will recognize that the condition of the individual can be monitored throughout the course of treatment and that the effective amount of a virus vector disclosed herein that is administered can be adjusted accordingly.

[0159] The pharmaceutical composition is typically in liquid form. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, magnesium chloride, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. In some cases, a surfactant, such as pluronic acid (PF68) 0.001% may be used.

[0160] For injection, the active ingredient will be in the form of an aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

VI. KITS

[0161] The term "kit" as used herein refers to a packaged product or article of manufacture comprising components. The kit preferably comprises a box or container that holds the components of the kit. The box or container is affixed with a label, or a Food and Drug Administration approved protocol. The box or container holds components of the disclosure which are preferably contained within plastic, polyethylene, polypropylene, ethylene, or propylene vessels. The vessels can be capped tubes or bottles. The kit can also include instructions for use of the reagents.

In another aspect, provided herein are kits comprising, in a container, AAV-hReelin virions as described herein, and instructions for use. In some aspects, the kits further comprise a negative control, such as phosphate buffered saline.

[0162] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

EXAMPLES

[0163] Examples have been set forth below for the purpose of illustration and to describe certain specific aspects of the disclosure. However, the scope of the claims is not to be in any way limited by the examples set forth herein. Various changes and modifications to the disclosed aspects will be apparent to those skilled in the art and such changes and modifications may be made without departing from the spirit of the disclosure and the scope of the appended claims.

[0266] [2591] The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics, and recombinant DNA, which are within the skill of the art. See, e.g., Bailey, J. E. and Ollis, D. F., *Biochemical Engineering Fundamentals*, McGraw-Hill Book Company, NY, 1986; *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY, N.Y. (1991-2015), including all supplements; Green and Sambrook, (*Molecular Cloning: A Laboratory Manual*, 4th, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2014); *Current Protocols In Molecular Biology* (F. M. Ausubel, et al. eds., (2017)) including all supplements; *Short Protocols in Molecular Biology*, (Ausubel et al., 1999)) including all supplements; the series *Methods In Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), *Antibodies, A Laboratory Manual*, Second Edition (Harlow and Lane, eds. (2014) and *Culture Of Animal Cells: A Manual Of Basic Technique*, 7th

Edition (R. I. Freshney, ed. (2016)), all the contents of which are incorporated by reference herein in their entireties.

EXAMPLE I: COMPARISON OF R3+R3v1 AND R3+R6v2 SECRETION

[0164] The expression and secretion of Reelin repeat R3+R6v2 was compared with Reelin repeat R3+R6v1 disclosed previously (see, for example, the U.S. Patent Publication No. 20190169246 and the International patent application number, PCT/US2021/013960, the contents of which are hereby incorporated by reference herein in their entirety). The nucleotide and amino acid sequences of these constructs are depicted under section II above.

[0165] The expression vector had the following structure:



[0166] The Reelin signal peptide (SP) was replaced with the signal sequence derived from mouse IgK light chain, human serum albumin, human azurocidin or mouse IgK. The R3+R6v1 expression vectors were then transiently transfected into HEK293, Hela or U87 cells. 48 hours after transduction, supernatant and cells were collected separately. Cells were lysed in RIPA buffer with protease and phosphatase inhibitors. Samples were diluted with 1x Laemmli loading buffer, heated at 95°C for 5 min and 15µg of protein were loaded onto an electrophoresis gel before transferring onto nitrocellulose membrane. Membranes were incubated with an anti-V5 antibody (1:1000) and an anti-actin antibody (1:1000) overnight at 4°C followed by secondary antibody the next day and imaged using the Odyssey CLx.

[0167] As shown in FIG. 2Ai, the R3+R6v1 protein was detectable in cell lysates but only trace amounts were secreted into the cell media. Based on the ECL signal, the amount of secreted recombinant R3+R6v1 was less than 1% of total protein. This shows the detection of secreted R3+R6v1 by dot blot analysis of the cell media, as reported in the international patent application number, PCT/US2021/013960, more likely than not, results from the detection of insoluble cytotoxic R3+R6v1 aggregates that are released into the cell media upon cell death.

[0168] FIG. 2Aii R3+R6v1 V5 tagged constructs were generated with different promoters, CMV promoter, synapsin promoter, MECP2 promoter and CAG promoter and transfected into U87 cells.

[0169] Here again the R3+R6v1 protein when expressed from a CMV or CAG promoter was detectable in cell lysates but only trace amounts secreted R3+R6v1 (less than 1% of total protein) could be found in the cell media.

[0170] To improve secretion, R3 and R3+R6v2 expression vectors were constructed where the native Reelin signal sequence is inserted immediately upstream of the Reelin repeat R3 fused to R6v2 (see section II above).

[0171] HEK293 cells were transfected with plasmids expressing either the novel Reelin-like peptides R3 or R3+R6v2. 48 hours after transduction, supernatant and cells were collected separately. Cells were lysed in RIPA buffer with protease and phosphatase inhibitors. Samples were diluted with 1x Laemmli loading buffer, heated at 95°C for 5 min and 15µg of protein were loaded onto an electrophoresis gel before transferring onto nitrocellulose membrane. Membranes were incubated with an anti-V5 antibody (1:1000) and an anti-actin antibody (1:1000) overnight at 4°C followed by secondary antibody the next day and imaged using the Odyssey CLx.

[0172] FIG. 2Aiii depicts a Western blot of HEK293 cell extracts and media. Both R3 and R3+R6v2 were detected in the cell lysate and in the supernatant indicating the Reelin fusion proteins were efficiently secreted by HEK293 cells.

[0173] To evaluate and compare the secretion of R3+R6v1 with R3+R6v2, these constructs were transiently transfected in parallel into HEK293 as described above. As shown in FIG. 2Aiv, expression of the R3+R6v1 with R3+R6v2 in cell lysates was comparable for both constructs, however only the R3+R6v2 protein was secreted into the cell media.

[0174] In conclusion, the new recombinant R3+R6v2 construct secreted significant amounts of recombinant protein into the media of HEK 293 cells. The amount of R3+R6v1 secreted into the cell media of HEK 293 cells was negligible.

EXAMPLE 2: R3+R6v2 PEPTIDE ENHANCES LONG TERM POTENTIATION (LTP) IN ACUTE HIPPOCAMPAL SLICES FROM WT ANIMALS

[0175] Wild type animals were decapitated, and the brain was immediately removed and covered with ice cold ACSF (in mM, 125 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 25 D-glucose, 1 MgCl₂, 2 CaCl₂). 400 µm thick horizontal slices were sectioned with a vibratome and the hippocampus was dissected out and incubated in ACSF at room temperature for at least 1 hour

before being positioned onto electrodes. Extracellular field recordings were collected using the MED64 Quad II system (Automate Scientific). Slices were positioned on the MED64 Quad II 8x2 electrode probes where they were perfused with oxygenated ACSF, heated to 32°C, and allowed to recover for 30-60 min. The single electrode of the 8x2 matrix which generated the most robust field excitatory postsynaptic potential (fEPSP) signal was used to stimulate the CA3-CA1 Schaeffer collaterals in the hippocampal slice in the CA3 region, while the other 15 electrodes recorded the fEPSP signals. Hippocampal slices were stimulated at 40-50% of the maximum field excitatory postsynaptic potential (fEPSP) every 20s until a stable baseline was established for 20 minutes. 5nM of R3+R6v2 peptide or vehicle were incubated after 10 minutes of baseline and washed out 20 minutes after induction of long term potentiation (LTP). LTP was induced by theta-burst stimulation (TBS) containing five trains of four pulses at 200 Hz separated by 200 ms, repeated a total of six times with a 10 s inter-train interval (Hethorn et al., 2015 The European Journal of Neuroscience 41 (10): 1372-80). Baseline recordings continued for 60 min after the end of the induction of LTP. The Med64 Mobius software (Automate Scientific) was used to collect and analyze the data. Slopes of EPSPs from the 15 recording electrodes with a signal in region CA1 containing an EPSP and a fiber volley were collected and averaged (about 2-10 electrodes depending on the positioning per slice) to generate a single trace for each slice every 20 seconds. 6 traces were then averaged to generate a single data point for every two minutes of recording per slice and were plotted on a graph. Traces from each condition were averaged and data from the final 20 min were analyzed using unpaired Student's t-test.

[0176] As shown in FIG. 2B, acute hippocampal slices from WT animals treated with the R3+6 peptide (5nM) for 20 minutes show enhanced LTP after theta burst stimulation (TBS).

[0177] FIG. 2C shows LTP is significantly enhanced in the final 20 minutes of recording in slices treated with R3+6 peptide. * = $p < 0.05$.

[0178] R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

EXAMPLE 3: ICV INJECTION OF AAV9_ R3+6 INTO REELER MICE RESULTS IN R3+6 PROTEIN EXPRESSION IN THE CORTEX AND HIPPOCAMPUS

AAV9_ R3-6 ICV injection into reeler mice

[0179] AAV9_R3+6 (with V5 tag) or vehicle control was injected ICV into postnatal day (pnd) 3 heterozygous reeler mice at a titer of 4.52×10^{10} vg/mouse. After 4-5 weeks days, the brains were collected from perfused animals and immediately immersed in 4% PFA for 1-2 days. They were washed with PBS and cryoprotected by incubation in solutions of increasing sucrose concentration (from 20% to 30%) for 24 hours apiece until the brain sank to the bottom. 20 μ m thick sagittal slices were generated using a cryostat. Slices were washed in 1X PBS followed by incubation in PBS containing 0.5% Triton x-100 for 30 min, followed by 30 min incubation in PBS containing 10% goat serum. Slices were incubated overnight with an anti-V5 antibody (Cell Signaling 1:1000) at 4°C, washed 3x in PBS + 0.01% Triton x-100 followed by incubation in secondary antibody. Slices were washed 3x in PBS + 0.01% Triton x-100, mounted on slides and imaged with 10x or 63x objectives using a Leica Thunder imaging system.

[0180] FIG. 3A shows high levels of R3+R6v2 expression in the cortex and hippocampus

Western blot analysis

[0181] Mouse brains were dissected and snap frozen with dry ice. Tissue was sonicated in ice cold RIPA buffer with protease and phosphatase inhibitors. Samples were diluted with 1x Laemmli loading buffer, heated at 95°C for 5 min (except when detecting with the anti-G10 Reelin antibody) and loaded onto electrophoresis gel before transferring onto nitrocellulose membrane. Membranes were incubated either with anti-G10 Reelin (1:1000) or anti-V5 antibodies (1:1000) overnight at 4°C followed by secondary antibody the next day and imaged and analyzed using the Odyssey CLx.

[0182] FIG. 3B depicts a Western blot of brain tissue taken from heterozygous reeler mice injected ICV with AAV9_R3+6 or vehicle control. The blots detected endogenous Reelin (top), R3+6 in the cortex (middle), or R3+6 in the hippocampus (bottom). High dose was 4.52×10^{10} /mouse, Low dose was 4.52×10^9 / mouse.

[0183] FIG. 3C depicts bar graphs illustrating the quantification of Reelin or R3+6 levels from the Western blots of FIG. 3B. WT: Wildtype, KO: reeler heterozygous mouse, LD: low dose, HD: high dose.

[0184] R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

EXAMPLE 4: ICV INJECTION OF AAV9_R3+6 INTO HETEROZYGOUS REELER MICE RESCUES IMPAIRMENT OF SPINE DENSITY IN THE HIPPOCAMPUS

[0185] AAV9_R3+6 (with V5 tag) or vehicle control was injected ICV into postnatal day (pnd) 3 heterozygous reeler mice at a titer of 4.52×10^{10} vg/mouse.

[0186] Golgi imaging was prepared by following the FD NeuroTechnologies Rapid GolgiStain Kit. Briefly, brains of AAV9_R3-R6 or vehicle control ICV injected Reeler mice were dissected out and immediately incubated into solution A/B for two weeks, followed by solution C for 3 – 7 days. 100 μ m thick slices were generated using a cryostat and stained following the sequential staining protocol described in the Rapid GolgiStain Kit. Golgi stained slices were imaged using the Leica Thunder Imaging System and images were collected using a 63x objective. 20 μ m length dendritic spines from pyramidal neurons in area CA1 of the hippocampus, free from surrounding dendritic interference were selected. The number of spines along 20 μ m length dendritic shafts were counted by hand and averaged and analyzed using a one-way ANOVA, followed by Tukey's Multiple Comparison post-hoc test.

[0187] FIG. 4A shows representative images of Golgi stained mouse hippocampus at low and high magnification.

[0188] FIG. 4B shows representative images of 20 μ m long dendritic branches from vehicle injected wildtype, heterozygous reeler mice (HRM), or AAV9_R3+6 injected HRM. Injections were ICV at pnd 3. High dose was 4.52×10^{10} /mouse, Low dose was 4.52×10^9 / mouse.

[0189] FIG. 4C shows an exemplary quantification of the number of spines per 20 μ m dendritic branch segments from apical hippocampal dendrites in area CA1. N = 40.

[0190] R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

EXAMPLE 5: FMR1 KO ANIMALS TREATED WITH AAV9_R3+6 RESCUES HYPERACTIVITY IN AN OPEN FIELD ASSAY

[0191] AAV9_R3-R6 or vehicle control ICV injected FMR $\pm$ knockout (KO) mice were placed in a brightly lit open field (40 x 40 cm) for 30 minutes. Distance traveled, velocity, time immobile

spent in center/perimeter were measured by beam-break recording (Columbus Instruments) and distance traveled, or velocity were plotted as a function of time.

[0192] FIG. 5A shows FMR1 KO animals exhibit hyperactivity as measured by distance traveled over 30 min compared with WT animals.

[0193] FIG. 5B demonstrates FMR1 KO animals injected directly into the hippocampus with a low dose of AAV9_R3+6 at pnd28 exhibit reduced hyperactivity compared with vehicle treated FMR1 KO animals over the course of 30 min.

[0194] FIG. 5C shows the total distance travelled by mice in each of the groups in either the center or periphery of the open field arena.

[0195] FIG. 5D shows a second study of FMR1 KO animals exhibiting hyperactivity as measured by distance traveled over 30 min compared with WT animals.

[0196] FIG. 5E demonstrates in a second study that FMR1 KO animals injected ICV with AAV9_R3+6 at pnd0 exhibit reduced hyperactivity with both high and low doses compared with vehicle treated FMR1 KO animals over the course of 30 min.

[0197] FIG. 5F shows the total distance travelled by mice in a second study for each of the groups in either the center or periphery of the open field arena. High dose was $4.52\text{E}10/\text{mouse}$, Low dose was $4.52\text{E}9/\text{mouse}$.

[0198] R3+6 and R3+R6v2 are used interchangeably herein unless indicated otherwise.

EXAMPLE 6: AAV9_R3+6 RESCUES ALTERATIONS IN MGLUR-LTD AND SPINE DENSITY IN FMR1 KO MICE

[0199] AAV9_R3+6 (with V5 tag) or vehicle control was injected ICV into postnatal day (pnd) 3 FMR KO mice at a high dose of $4.52\text{E}10/\text{mouse}$ or a low dose of $4.52\text{E}9/\text{mouse}$.

Western blot analysis

[0200] Mouse brains were dissected and snap frozen with dry ice. Tissue was sonicated in ice cold RIPA buffer with protease and phosphatase inhibitors. Samples were diluted with 1x Laemmli loading buffer, heated at 95°C for 5 min (except when detecting with the anti-G10 Reelin antibody) and loaded onto electrophoresis gel before transferring onto nitrocellulose membrane. Membranes

were incubated either with anti-G10 Reelin (1:1000) or anti-V5 antibodies (1:1000) overnight at 4°C followed by secondary antibody the next day and imaged and analyzed using the Odyssey CLx. FIG. 6A shows a representative Western blot illustrating R3+6 protein levels in FMR1 KO treated animals.

mGluR-LTD

[0201] Treated animals were decapitated and the brains were immediately removed and covered with ice cold ACSF (in mM, 125 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 25 D-glucose, 1 MgCl₂, 2 CaCl₂). 400 µm thick horizontal slices were sectioned with a vibratome and the hippocampus was dissected out and incubated in ACSF at room temperature for at least 1 hour before positioned onto the electrodes. Extracellular field recordings were collected using the MED64 Quad II system (Automate Scientific). Slices were positioned on the MED64 Quad II 8x2 electrode probes where they were perfused with oxygenated ACSF, heated to 32°C, and allowed to recover for 30-60 min. The single electrode of the 8x2 matrix which generated the most robust signal was used to stimulate the CA3-CA1 Schaeffer collaterals in the hippocampal slice, while the other 15 electrodes recorded the signal. Hippocampal slices were stimulated at 50-60% of the maximum field excitatory postsynaptic potential (fEPSP) every 20s until a stable baseline was established for 20 minutes. Long term depression (LTD) was induced by incubation with 100µM (*R,S*)-3,5-Dihydroxyphenylglycine (DHPG) for 10 min. Baseline recordings continued for 60 min after the end of the induction of LTD. The Med64 Mobius software (Automate Scientific) was used to collect and analyze the data. Slopes of EPSPs from the 15 recording electrodes with a signal in region CA1 containing an EPSP and a fiber volley were collected and averaged (about 2-10 electrodes depending on the positioning per slice) to generate a single trace for each slice every 20 seconds. 6 traces were then averaged to generate a single data point for every two minutes of recording per slice and were plotted on a graph. Traces from each condition were averaged and data from the final 20 min were analyzed using a one-way ANOVA, followed by Tukey's Multiple Comparison post-hoc test.

[0202] FIG. 6B shows the average fEPSP slope over time from acute hippocampal slices from each of the experimental groups treated with DHPG to induce mGluR-LTD.

[0203] The graph of FIG. 6C illustrates the average fEPSP slope for the final 20 minutes of recording after induction of mGluR-LTD. Aberrant mGluR-LTD was rescued in hippocampal slices from FMR1 KO animals treated with the low dose of AAV9_R3+6.

Spine density

[0204] Golgi imaging was prepared by following the FD NeuroTechnologies Rapid GolgiStain Kit. Briefly, brains were dissected out and immediately incubated into solution A/B for two weeks, followed by solution C for 3 – 7 days. Brains were then sent to Neurodigitech for the remainder of the staining procedure followed by digital dendritic reconstruction and subsequent morphological analysis. Basal and apical dendrites of pyramidal cells in the CA1 of the hippocampus were analyzed using the NeuroLucida software on a Nikon Eclipse Ni image microscope.

[0205] FIG. 6D shows representative images of Golgi stained pyramidal neurons from area CA1 in the hippocampus.

[0206] FIG. 6E shows the overall spine density of basal and apical dendrites from pyramidal neurons in area CA1 of all experimental groups.

[0207] FIG. 6F shows the overall spine density from dendritic segments of all experimental groups. Both high dose and low dose AAV9_R3+6 treatment rescues aberrant spine density observed in FMR1 KO animals. High dose was 4.52E10/mouse, Low dose was 4.52E9/ mouse. Data from FIGs. 6E – 6F was generated by Neurodigitech.

CLAIMS

1. A viral vector composition for expressing a secreted recombinant Reelin fusion protein comprising from one to three Reelin repeats consisting of
 - a Reelin repeat R3;
 - a Reelin repeat R3 and a Reelin repeat R5;
 - a Reelin repeat R3 and a Reelin repeat R6; or
 - a Reelin repeat R3, a Reelin repeat R5 and a Reelin repeat R6;wherein,
 - Reelin repeat R3 is amino terminal to Reelin repeats R5 and R6,
 - Reelin repeat R5 if present, is amino terminal to Reelin repeat R6, andwherein the Reelin repeat R3 comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 4.
2. The viral vector of claim 1, where the polypeptide sequence of Reelin repeat R3 comprises a proteolytic cleavage site at Pro 1244.
3. The viral vector of claim 1, where the Reelin repeat R3 has at least 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 10.
4. The viral vector of claim 1, where the Reelin repeat R3 is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity to the nucleotide acid sequence of SEQ ID NO: 9.
5. The viral vector composition of claim 1, wherein the Reelin repeat R5 polypeptide comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 30.
6. The viral vector composition of claim 1, wherein the Reelin repeat R5 polypeptide comprises at least 80, 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 30.
7. The viral vector composition of claim 1, wherein the Reelin repeat R5 polypeptide is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity

- to the nucleotide acid sequence of SEQ ID NO: 29.
8. The viral vector composition of claim 1, wherein the Reelin repeat R6 polypeptide comprises at least 10 amino acids of the polypeptide sequence of SEQ ID NO: 12, 14, 16 or 18.
 9. The viral vector composition of claim 1, wherein the Reelin repeat R6 polypeptide comprises at least 80, 85, 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 20.
 10. The viral vector composition of claim 1, wherein the Reelin repeat R6 polypeptide is encoded by a nucleotide sequence having at least 85, 90, 95 or 99% sequence identity to the nucleotide acid sequence of SEQ ID NO: 19.
 11. The viral vector composition of claim 1, where the nucleotide sequence encoding the recombinant Reelin fusion protein is operably linked to a tissue-specific promoter.
 12. The viral vector composition of claim 1, where the tissue-specific promoter comprises a synapsin or BM88 promoter.
 13. The viral vector composition of any one of the preceding claims, wherein the viral vector is an adeno-associated viral vector (AAV) selected from the group consisting of AAV-9, AAV-5, AAV-4, and AAV-1.
 14. A viral vector composition for expressing a recombinant Reelin fusion protein encoded by a nucleotide sequence having from 80, 85, 90, 95 or 99% sequence identity to the nucleotide sequence of SEQ ID NO: 23.
 15. The viral vector composition of claim 14, wherein the recombinant Reelin fusion protein comprises at least 90, 95 or 99% sequence identity to the amino acid sequence of SEQ ID NO: 24.
 16. The viral vector composition of claim 14 or 15, wherein the viral vector composition expresses a therapeutically effective amount of the recombinant Reelin fusion protein

effective at inducing Reelin-dependent ApoER2 receptor activation and signaling.

17. The viral vector composition of claim 14 or 15, wherein the viral vector composition expresses a therapeutically effective amount of the recombinant Reelin fusion protein effective at increasing dendritic spine density, long-term potentiation (LTP), or synaptic plasticity.
18. A method of treating a disease or disorder of the nervous system, comprising administering the viral vector compositions of claim 14 or 15 to a subject in need thereof.
19. The method of claim 18, wherein the disease or disorder of the nervous system comprises Fragile X Syndrome.
20. The method of claim 18 wherein the viral vector compositions are administered intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, or subcutaneously to a subject in need thereof.

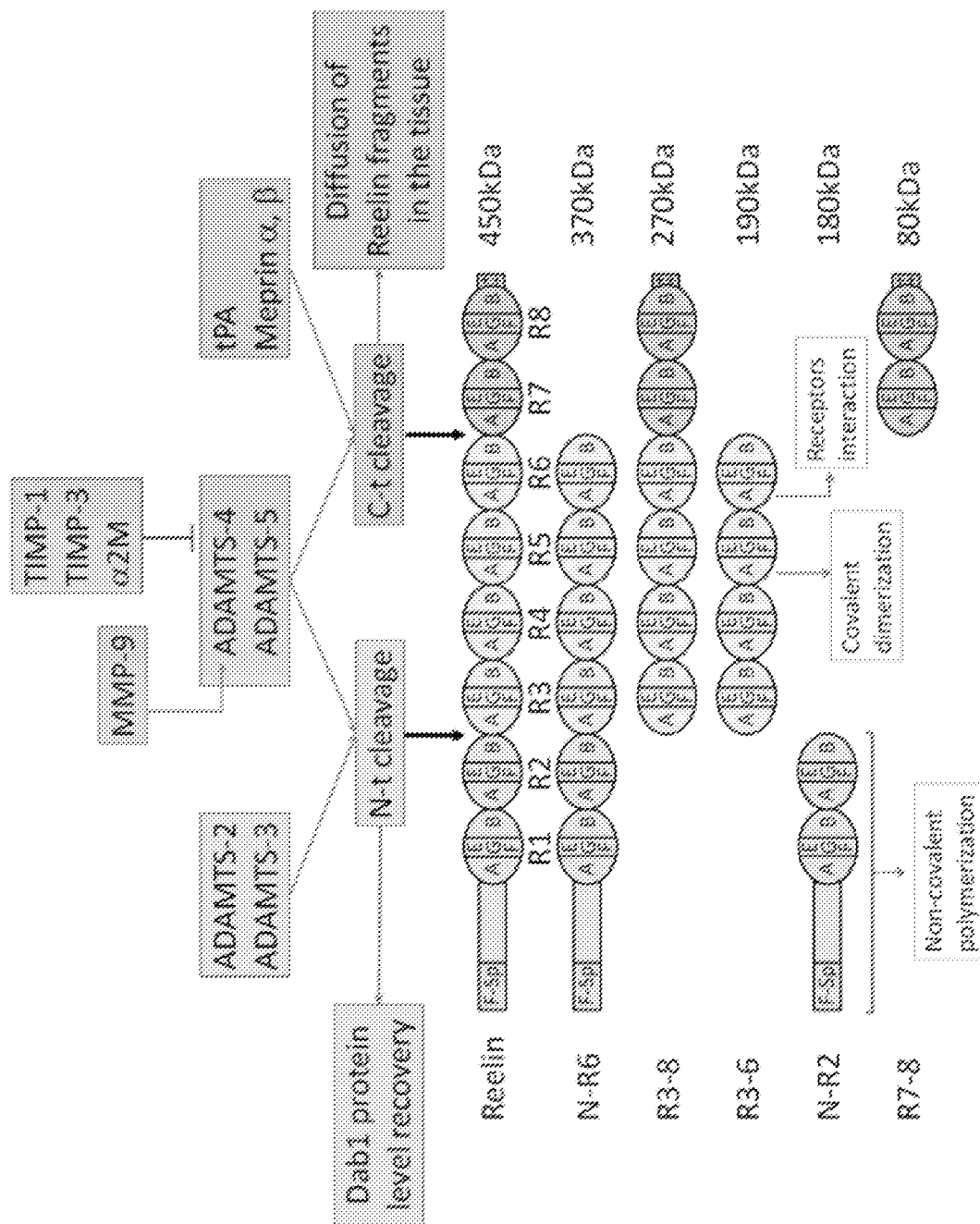


FIG. 1A

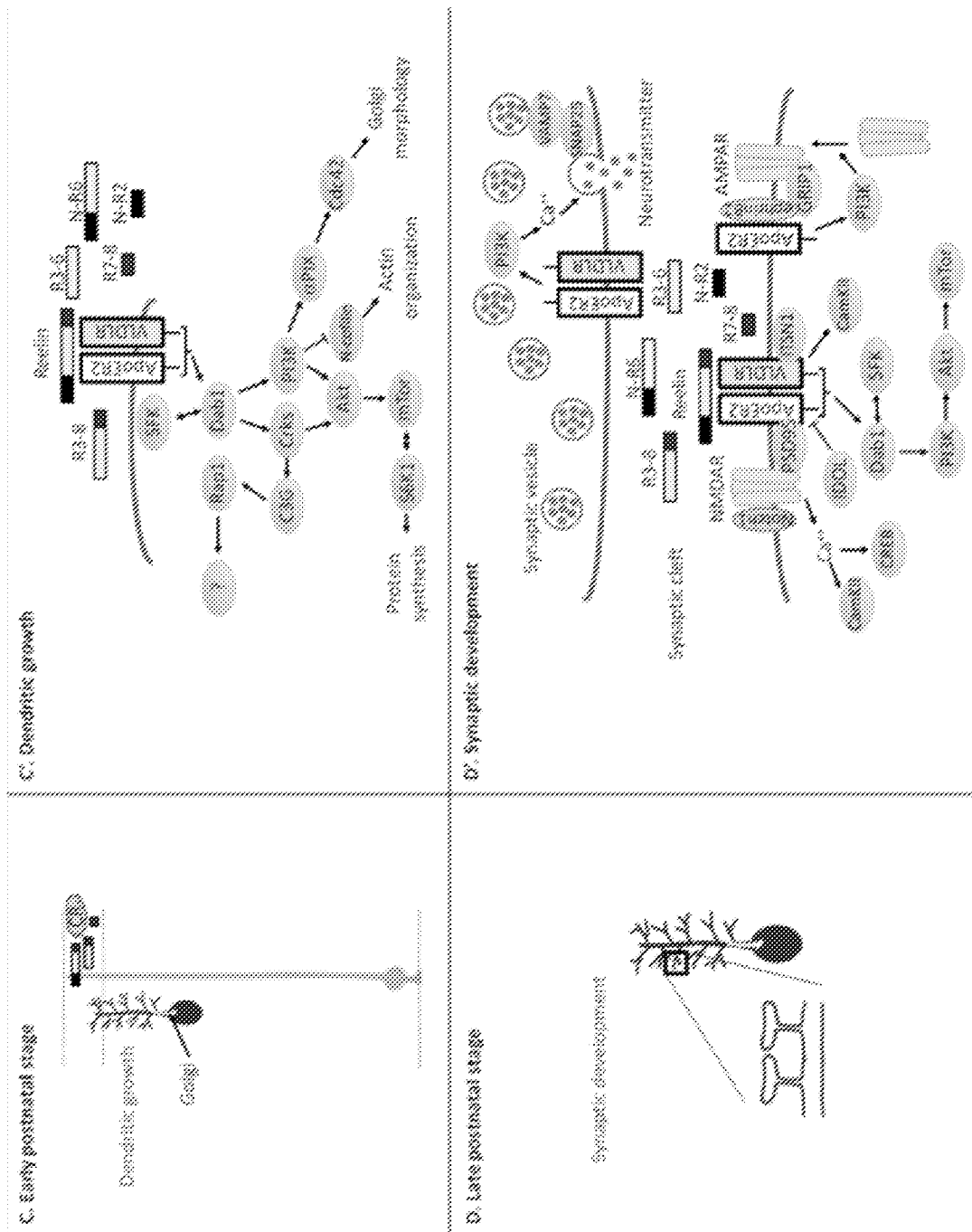
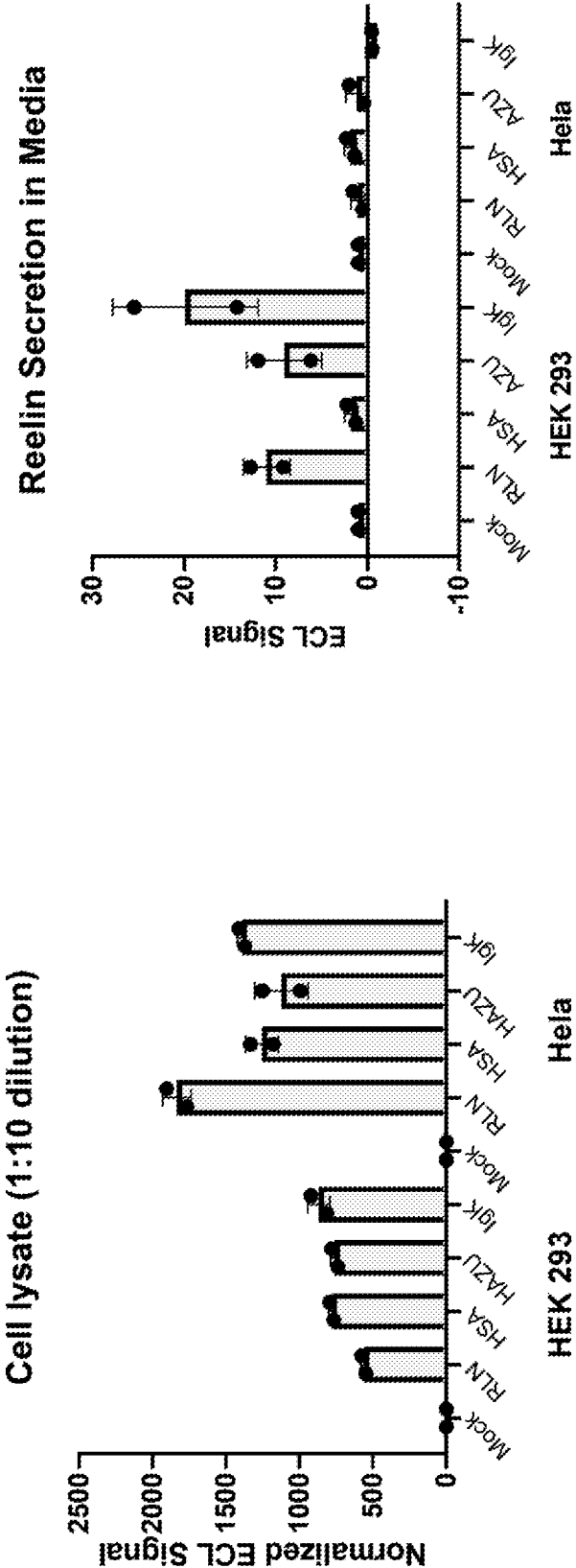


FIG. 1B

SECRETION OF REELIN REPEAT R3+R6v1

SIGNAL PEPTIDES EMPLOYED		AMINO ACID SEQUENCE	SEQ ID NO
RLN	Human Reelin	MERSGWARQTFLLALLGATLRARA	2
HSA	Human serum albumin	MKWWTFISLLFLFSSAYS	59
AZU	Human azurucidin	MTRLTVLALLAGLLASSRA	60
IgK	Mouse IgK light chain	METDTLLLVWLLWVPGSTGD	61



Secreted is < 1% total protein

FIG. 2Ai

SECRETION OF REELIN REPEAT R3+R6v1

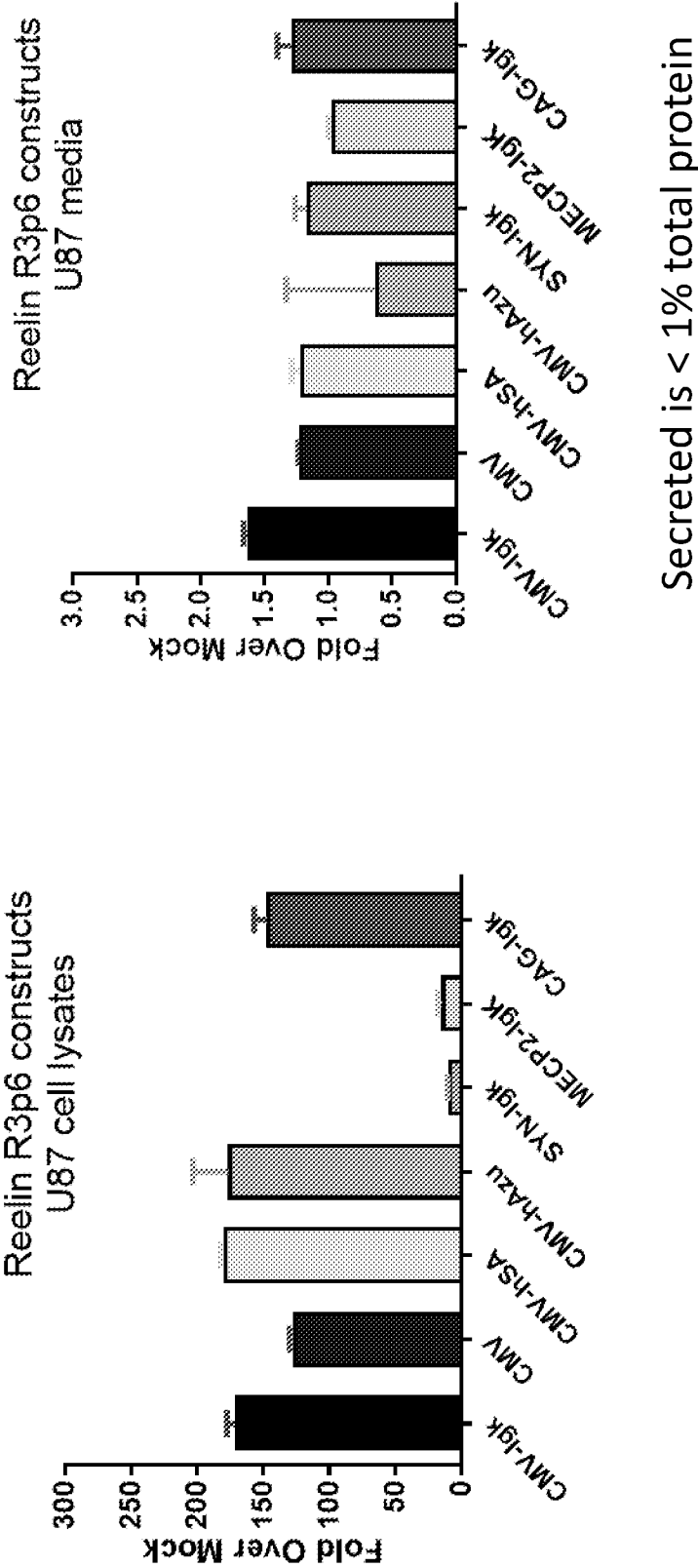


FIG. 2Aii

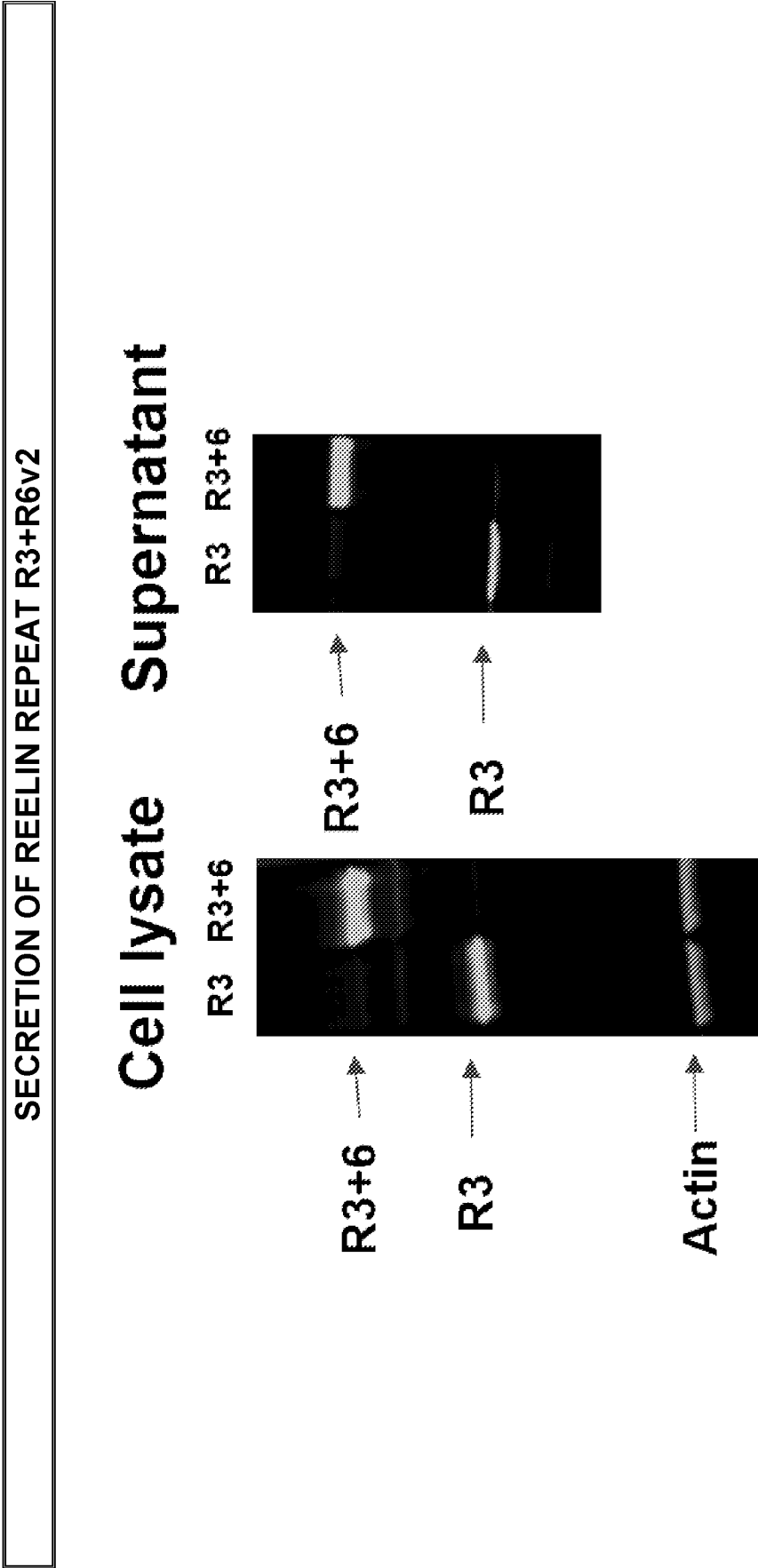


FIG. 2Aiii

SECRETION OF REELIN REPEAT R3+R6v1 vs. R3+R6v2

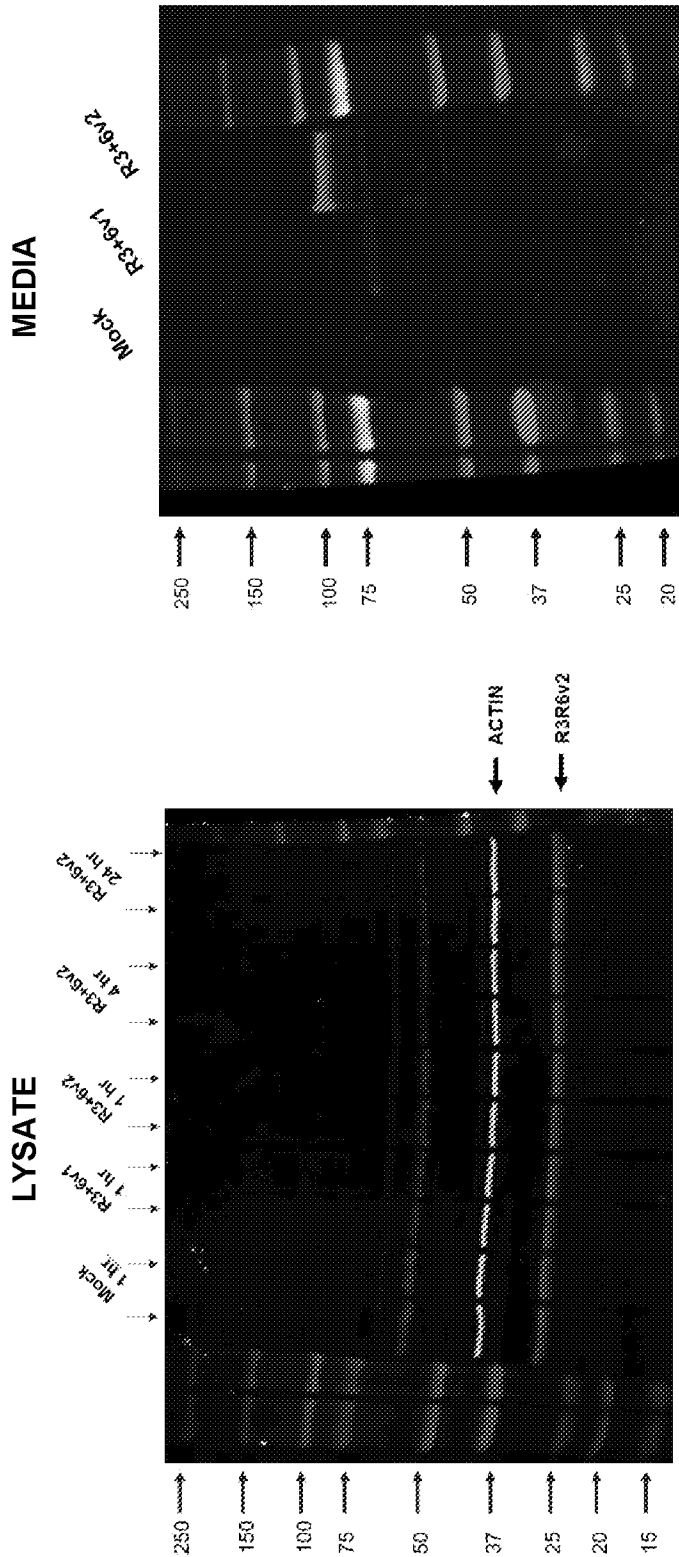


FIG. 2Aiv

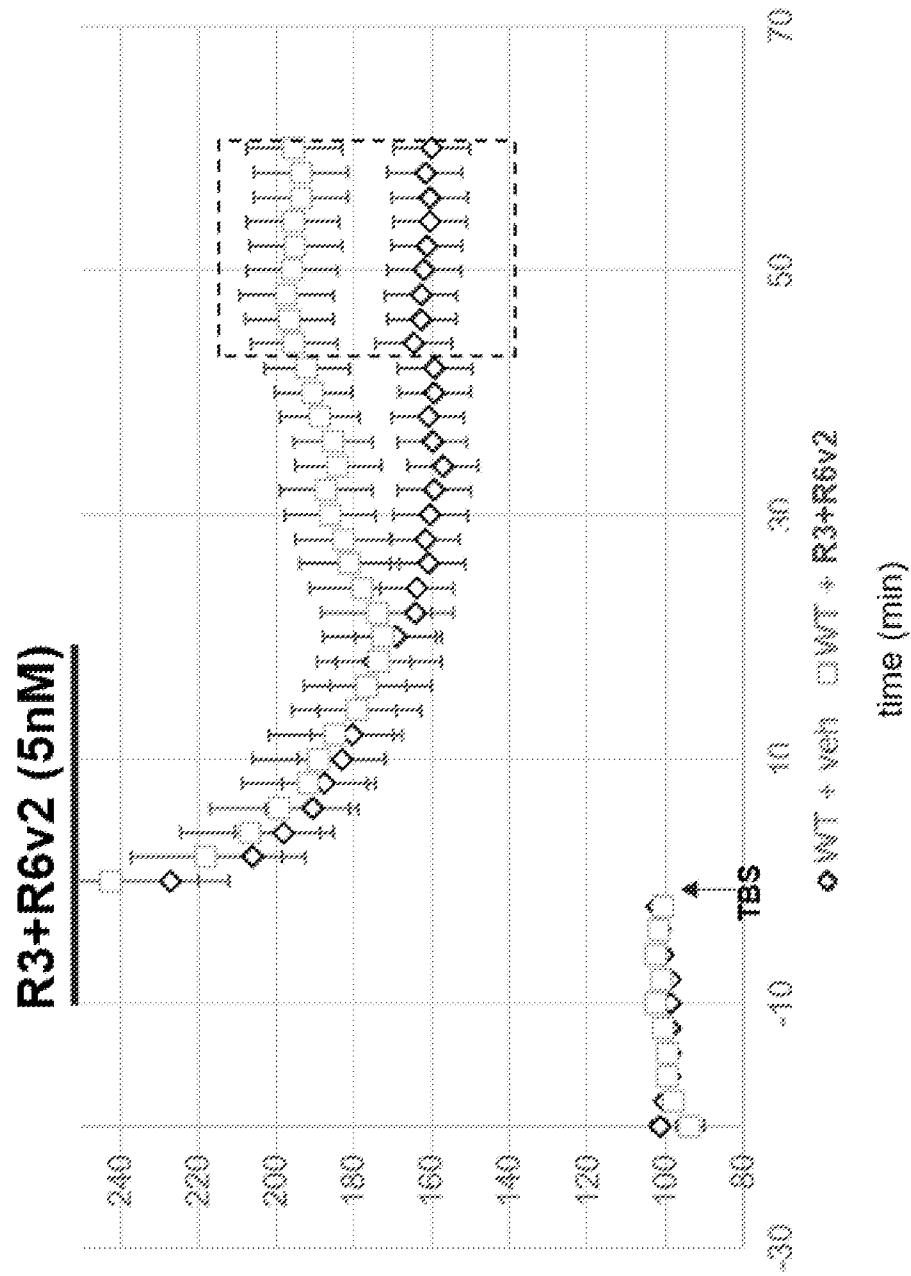


FIG. 2B

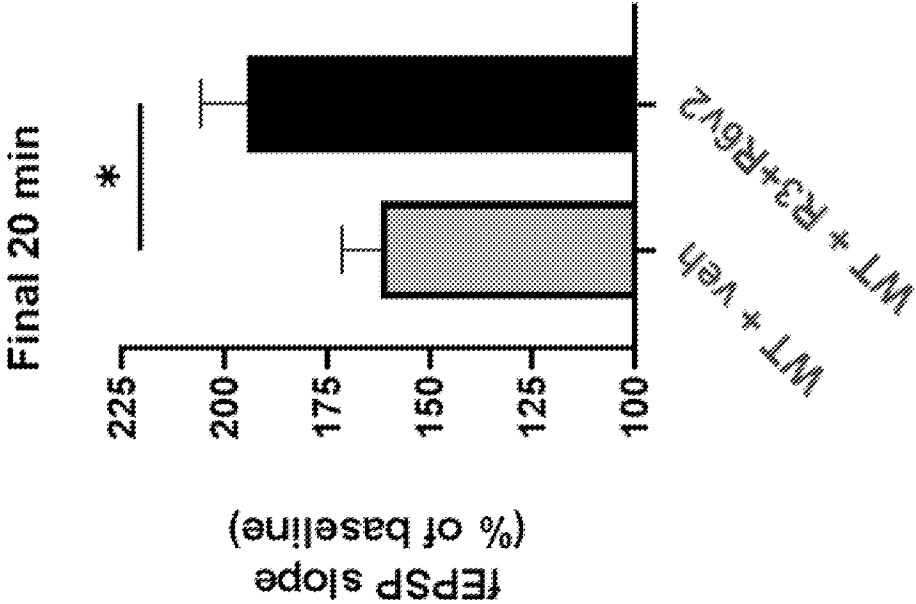


FIG. 2C

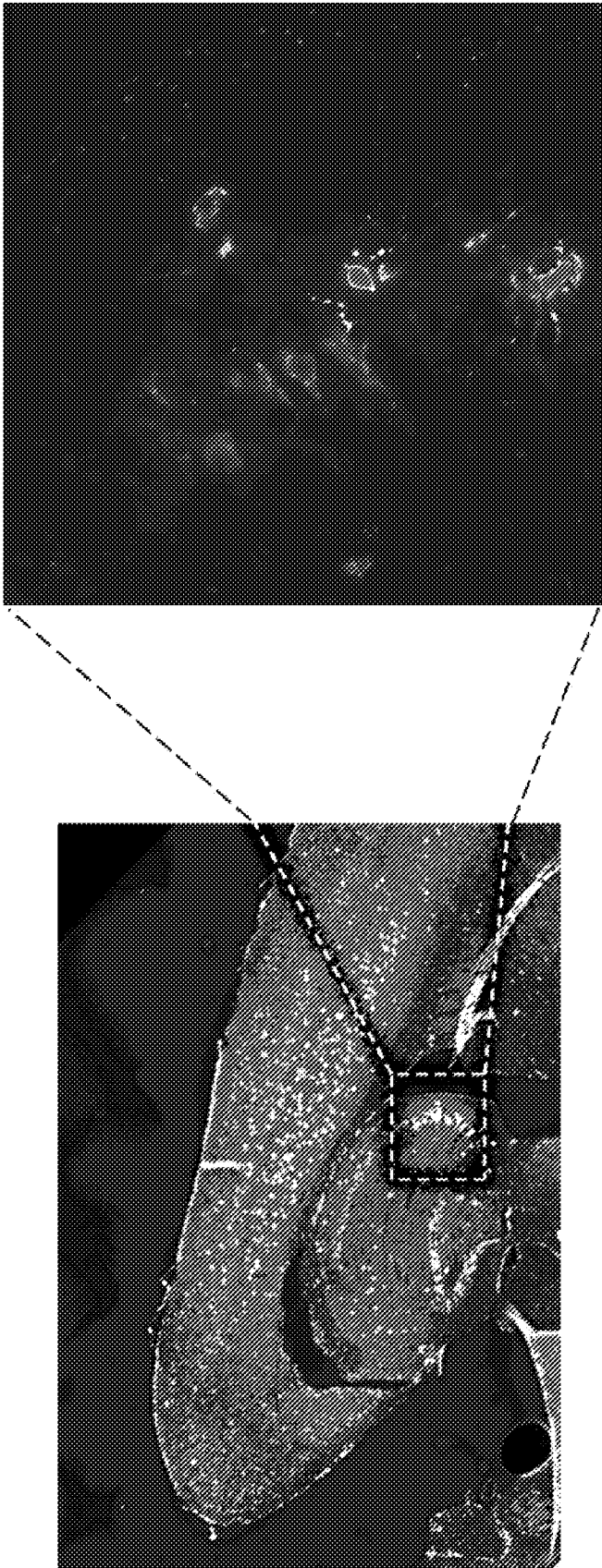


FIG. 3A

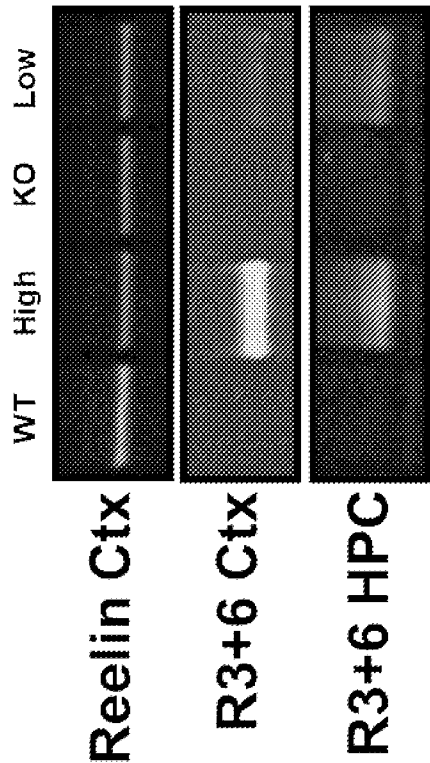


FIG. 3B

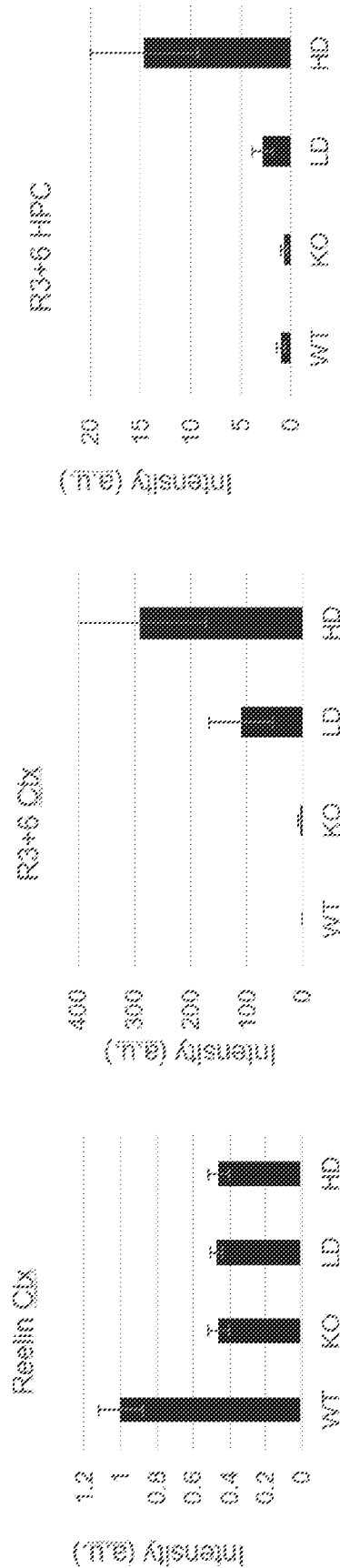


FIG. 3C



FIG. 4A

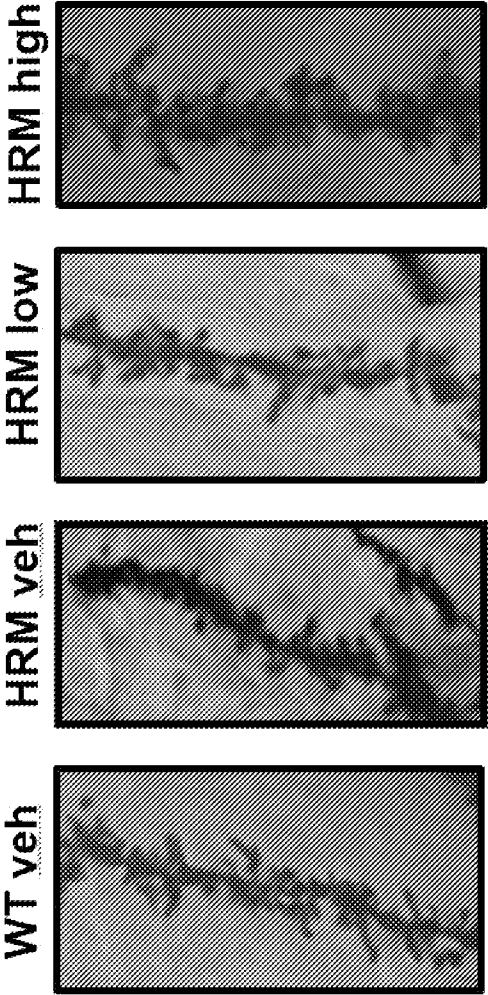


FIG. 4B

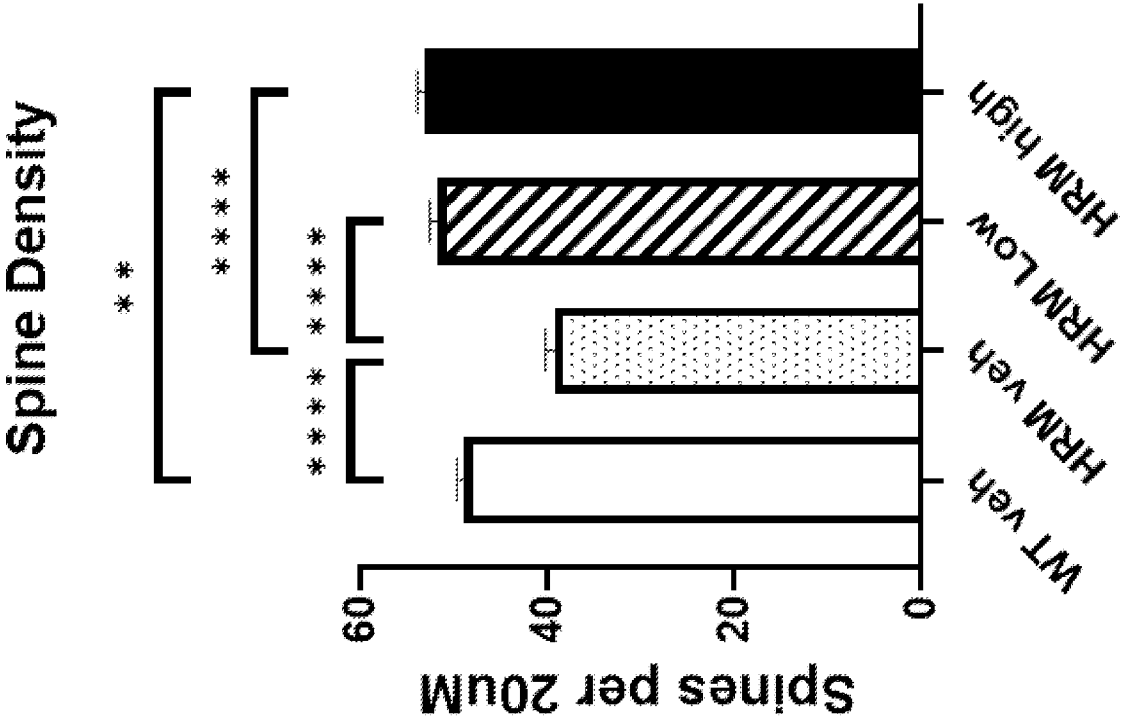


FIG. 4C

Open Field Distance Over Time

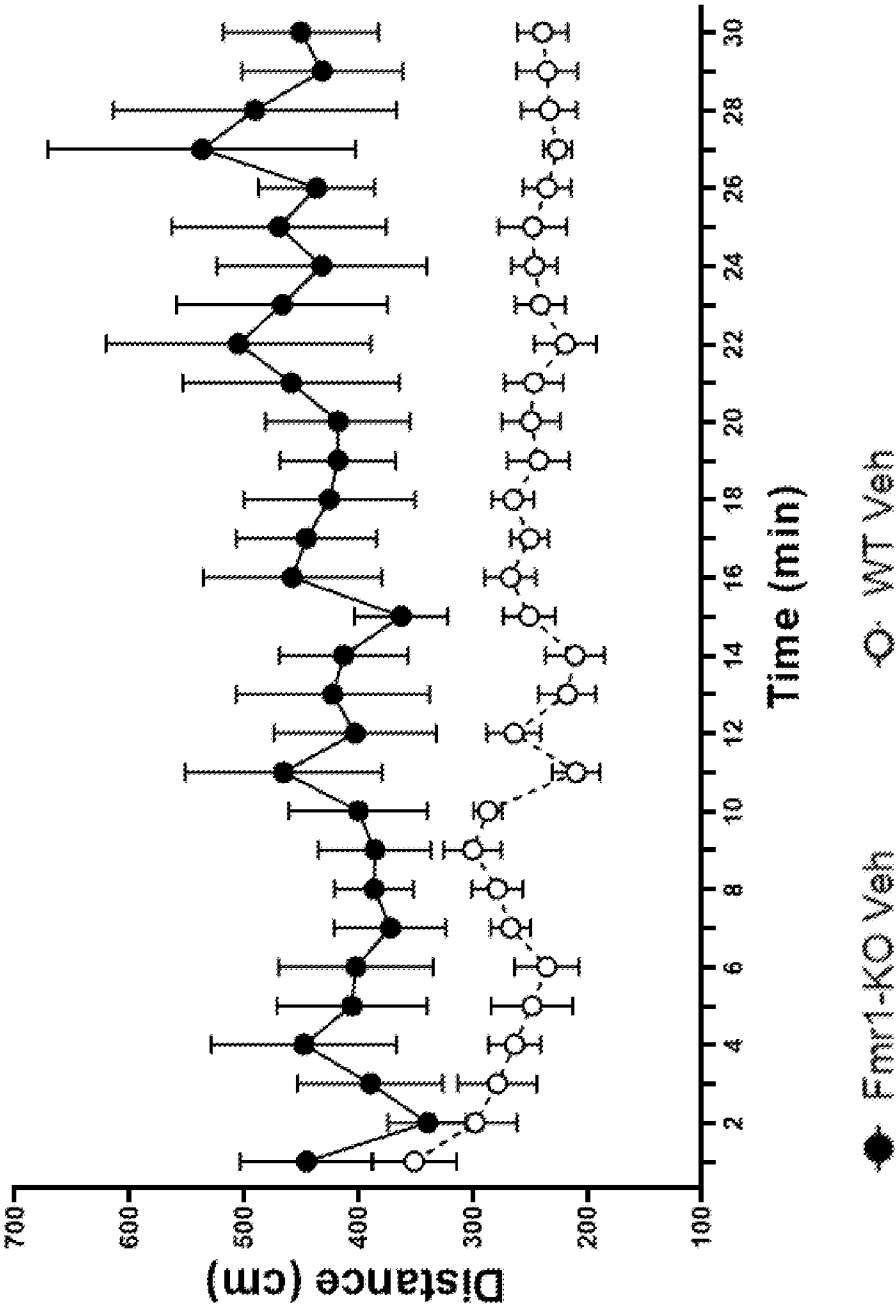


FIG. 5A

Open Field Distance Over Time

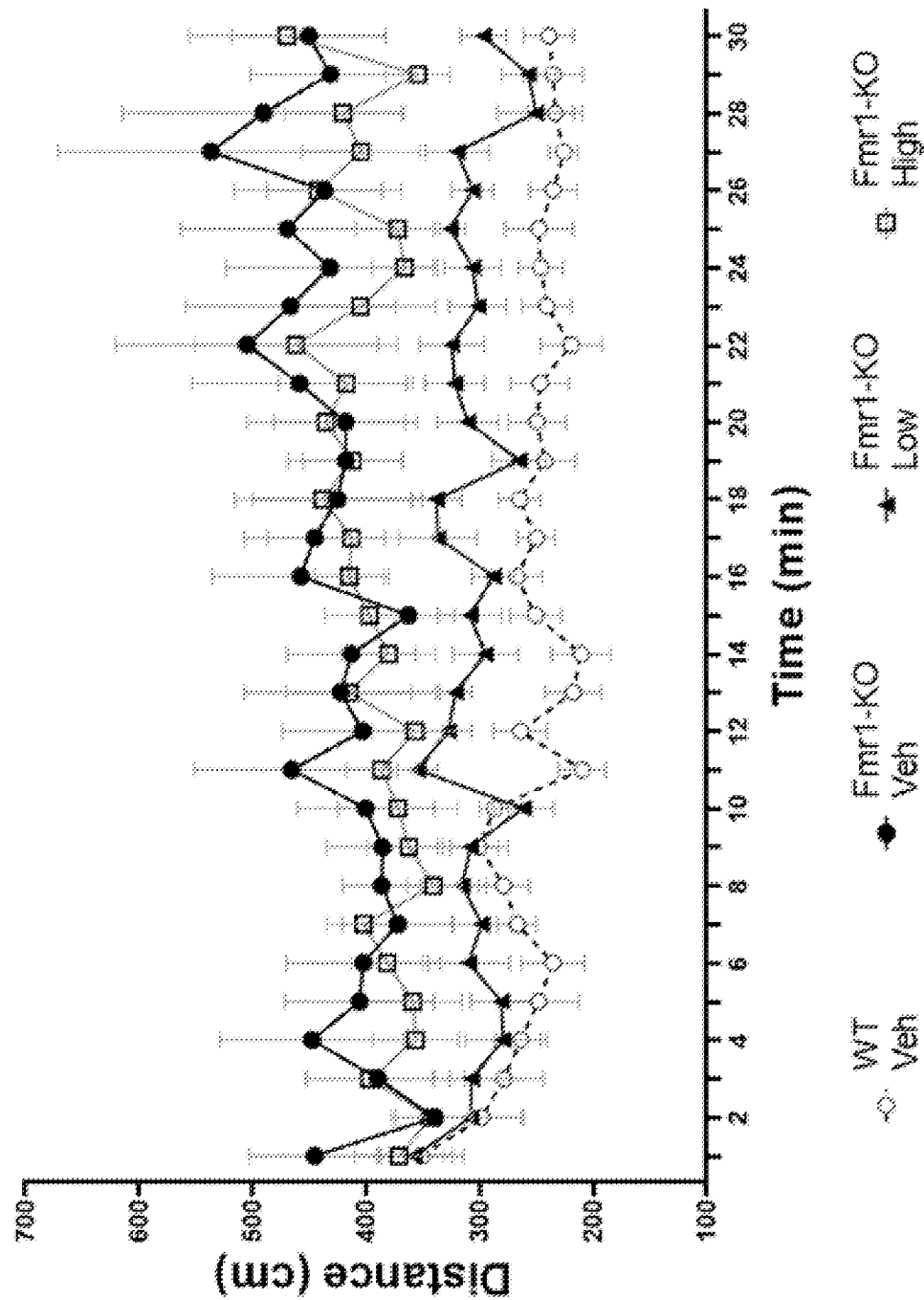


FIG. 5B

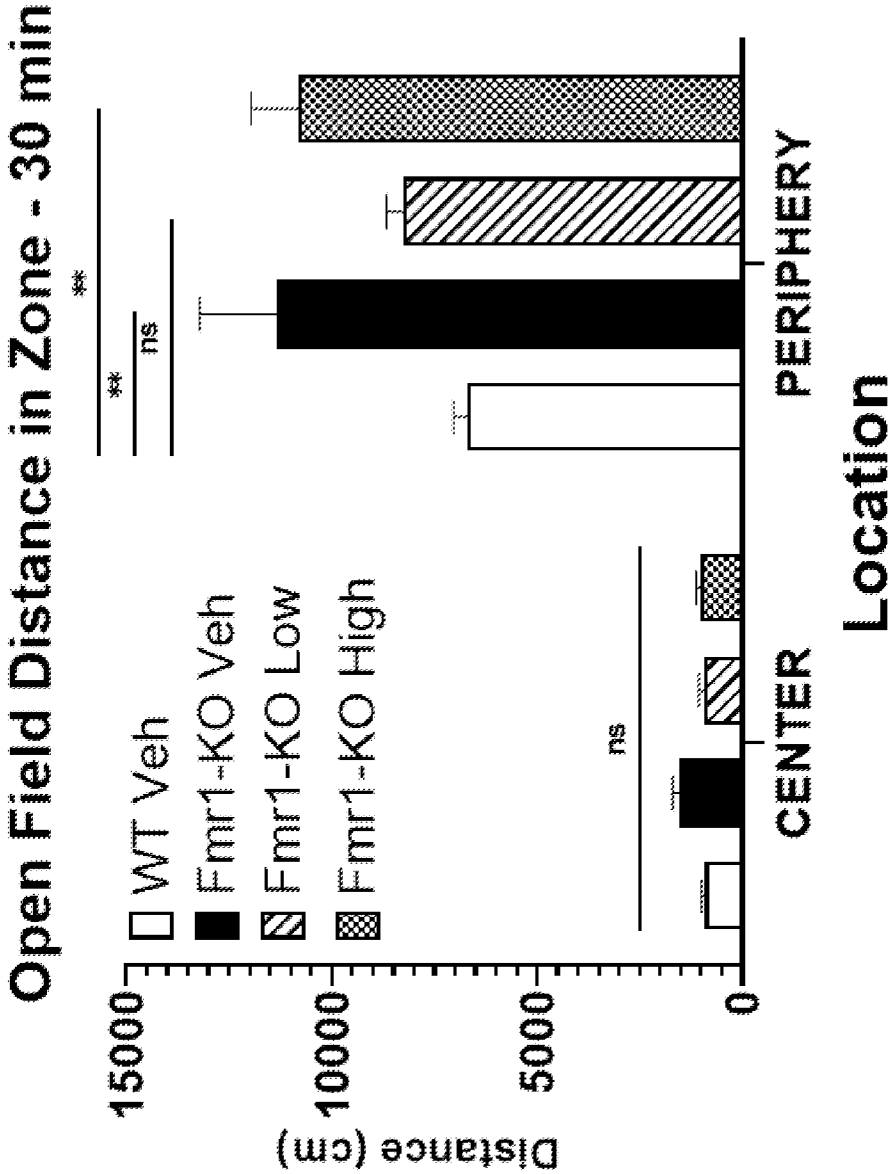


FIG. 5C

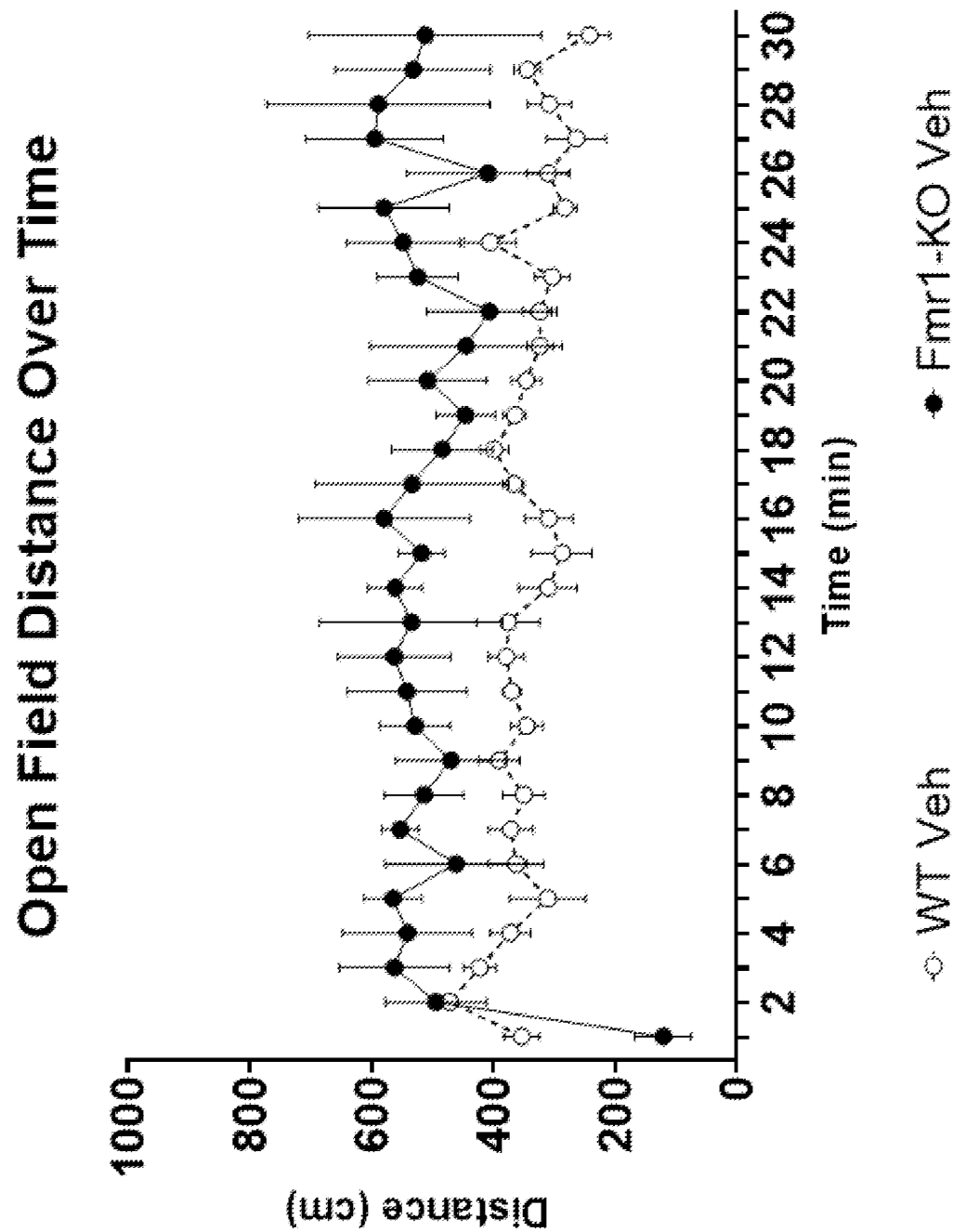


FIG. 5D

Open Field Distance Over Time

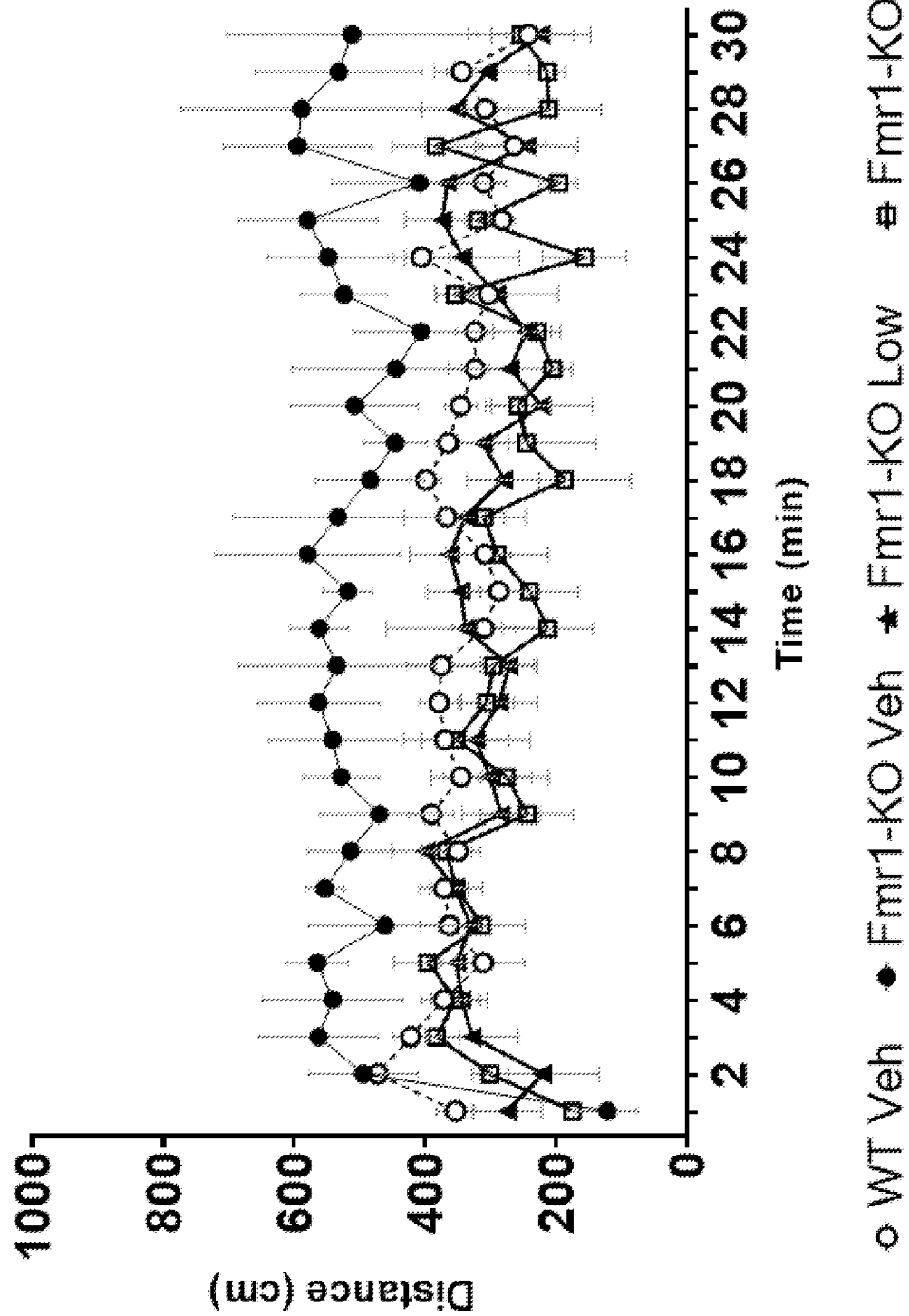


FIG. 5E

Open Field Distance in Zone

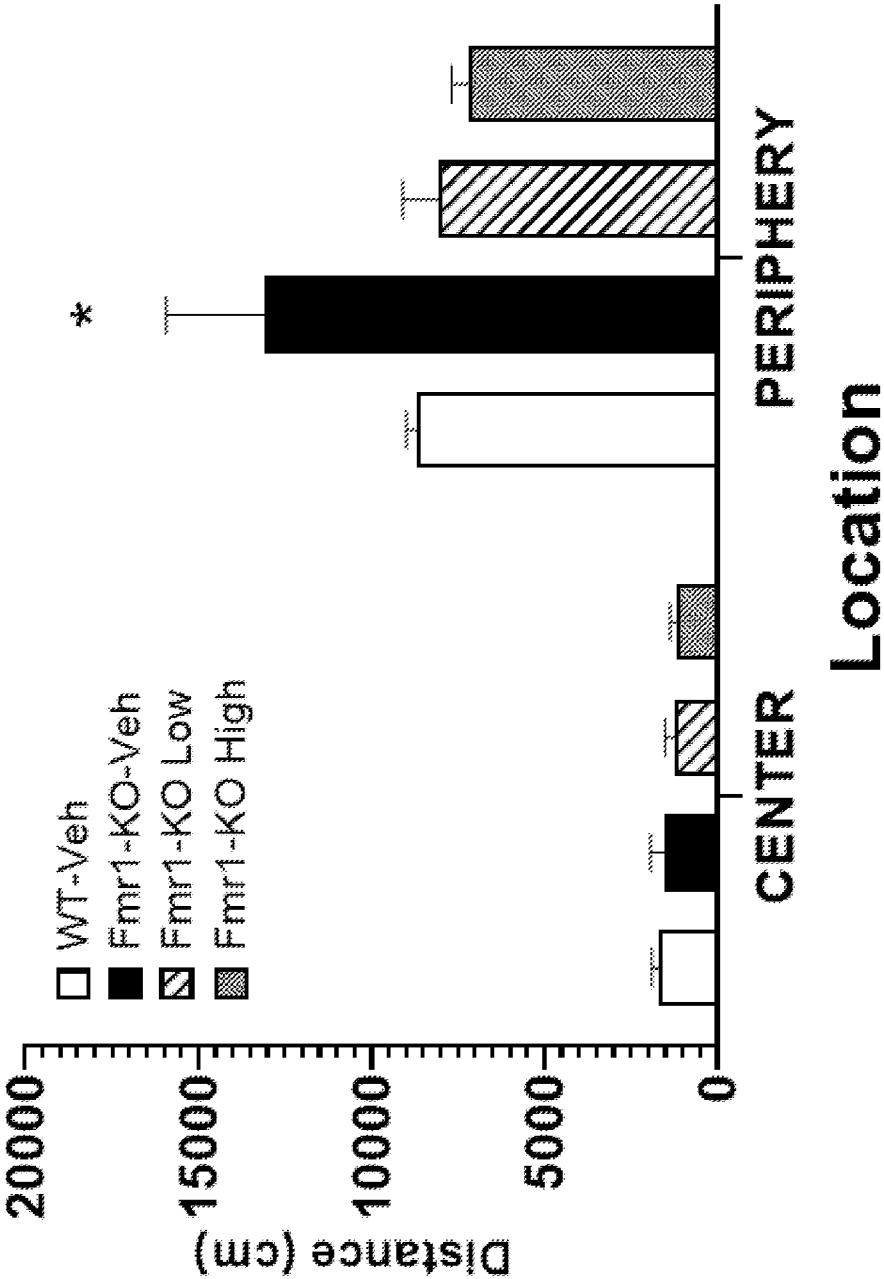


FIG. 5F

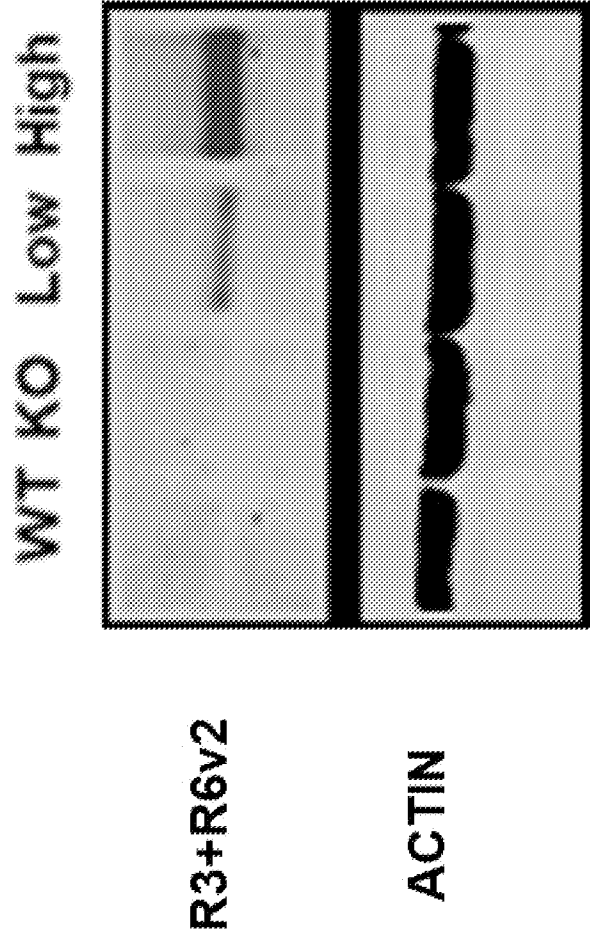


FIG. 6A

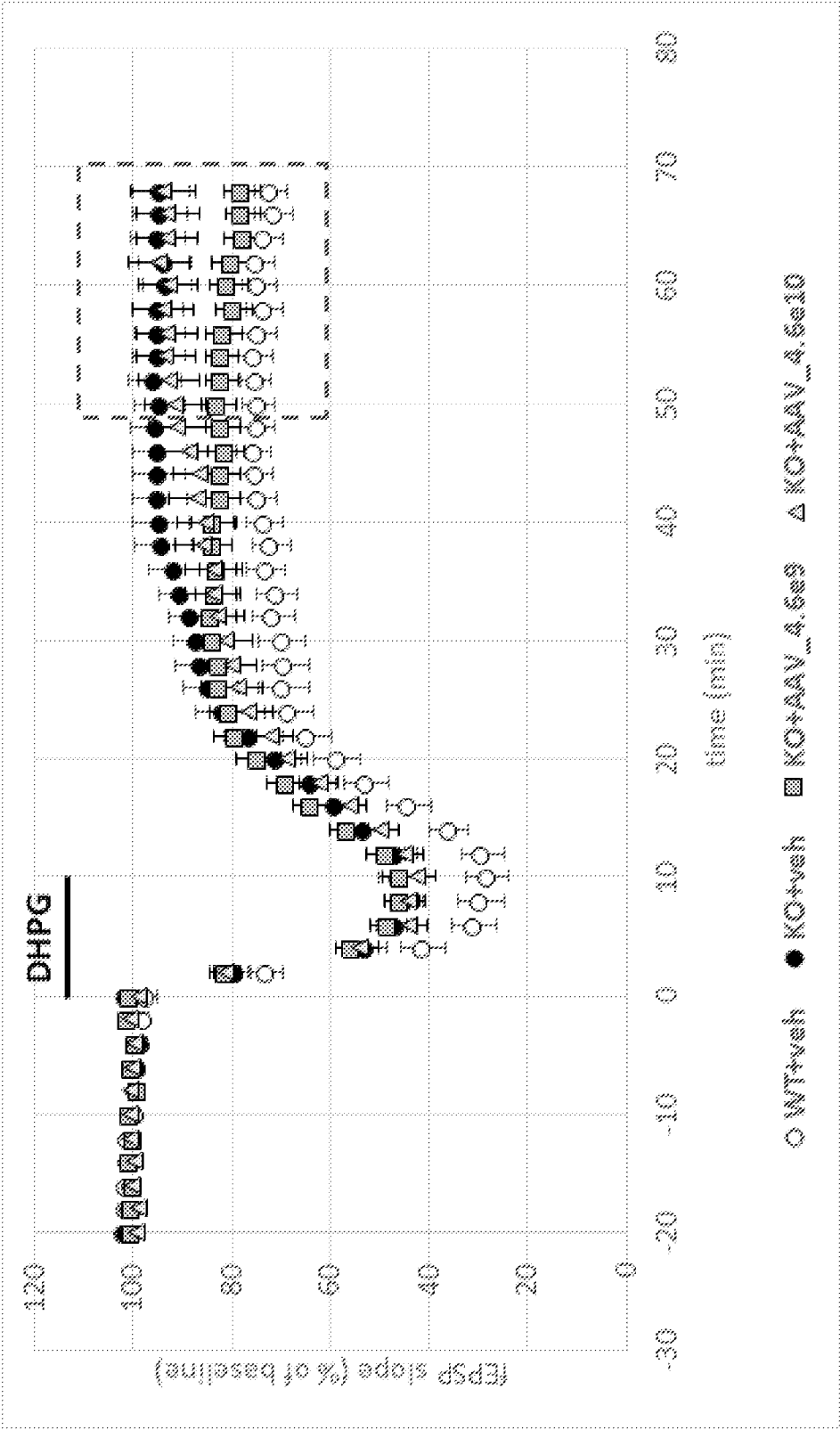


FIG. 6B

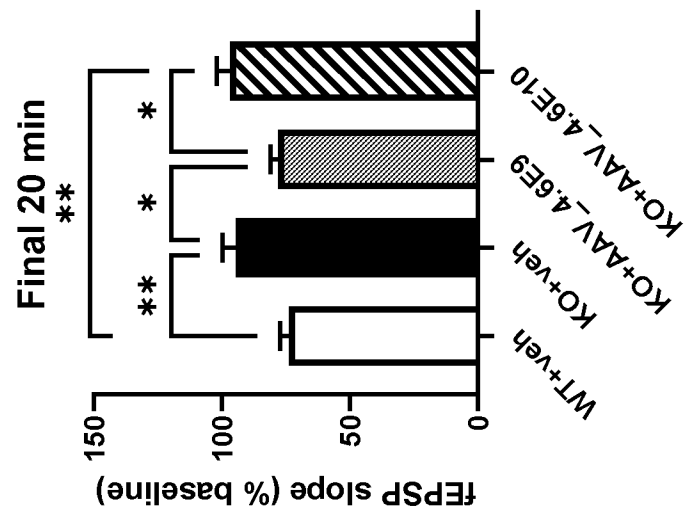


FIG. 6C

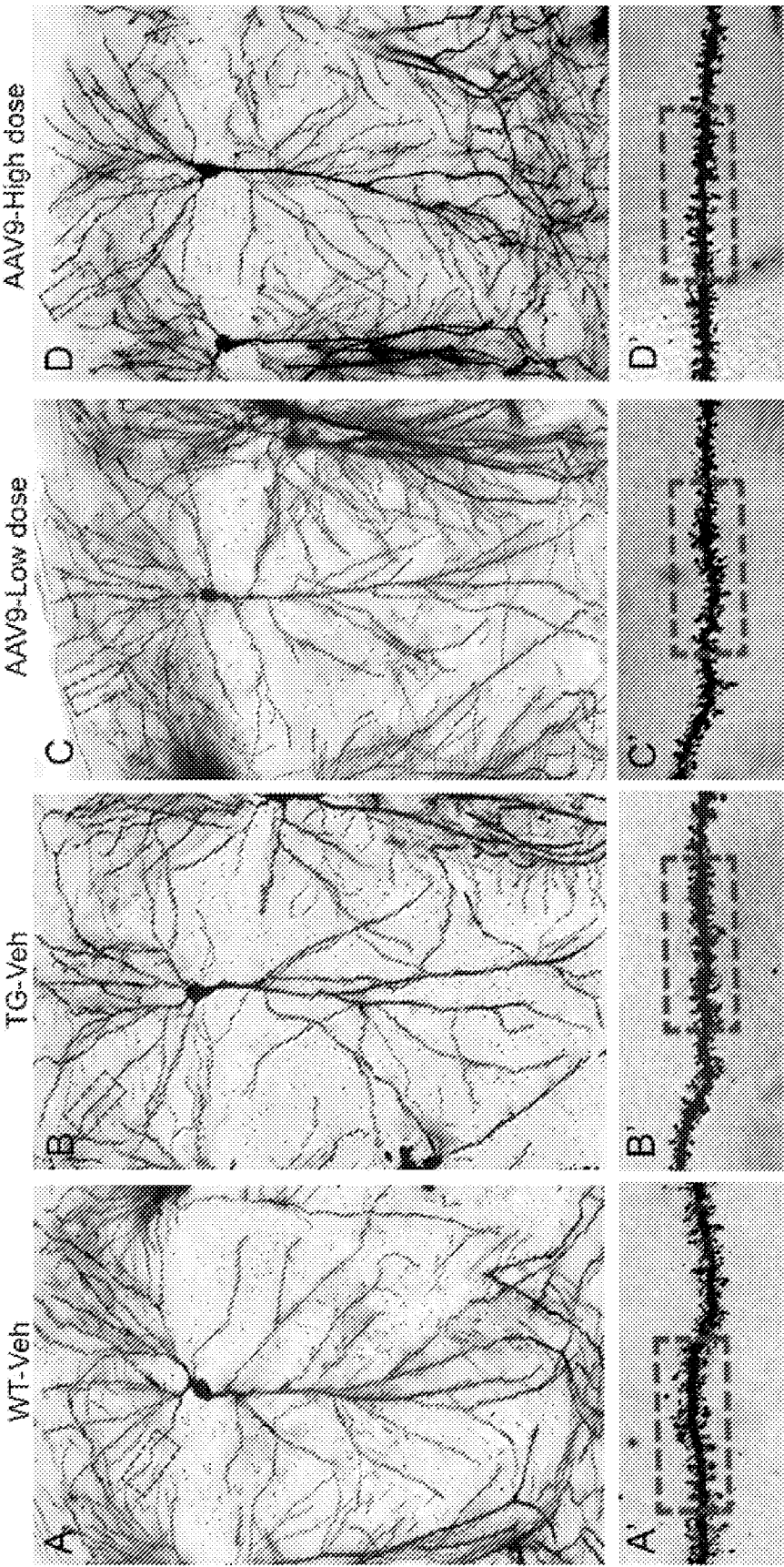


FIG. 6D

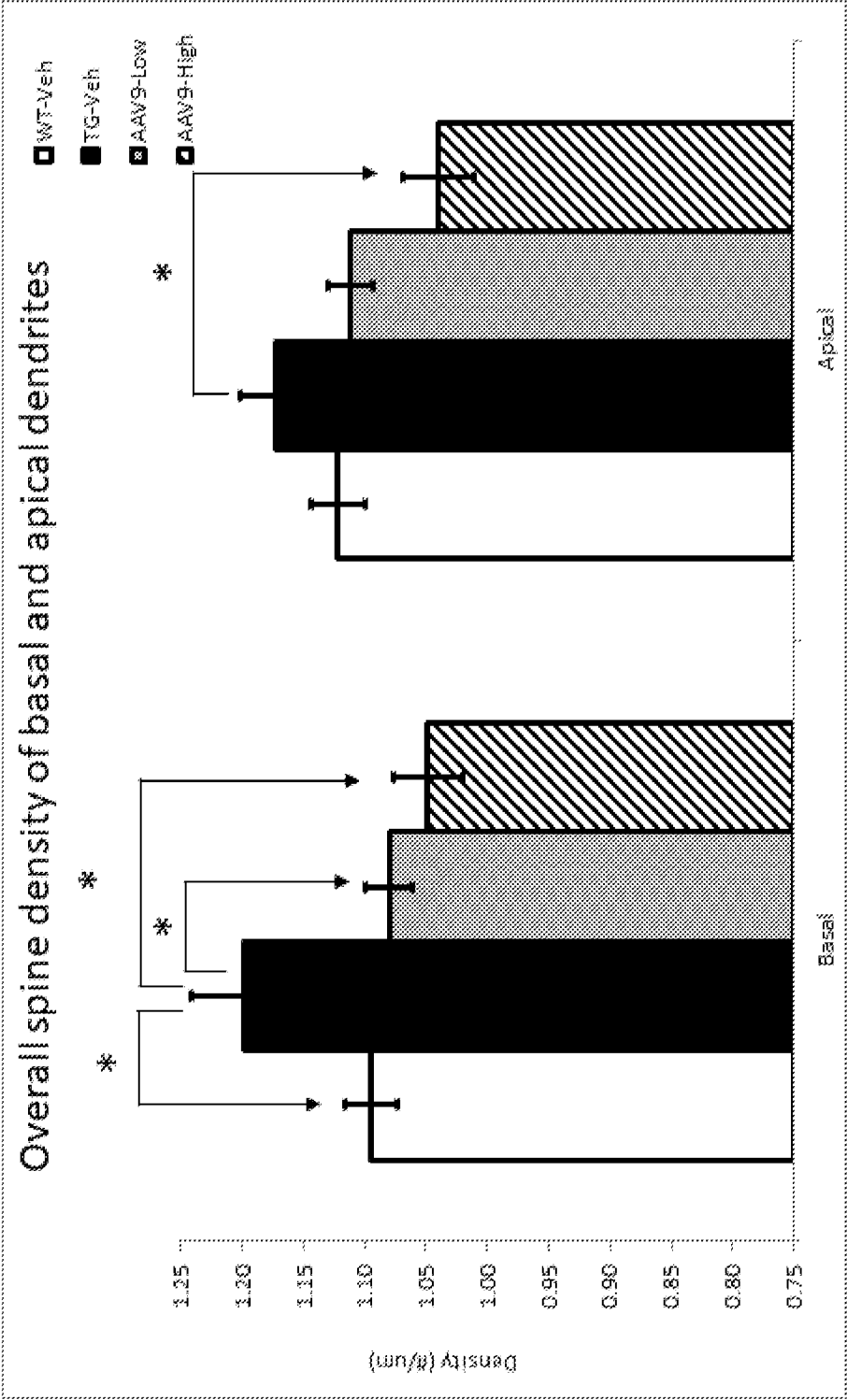


FIG. 6E

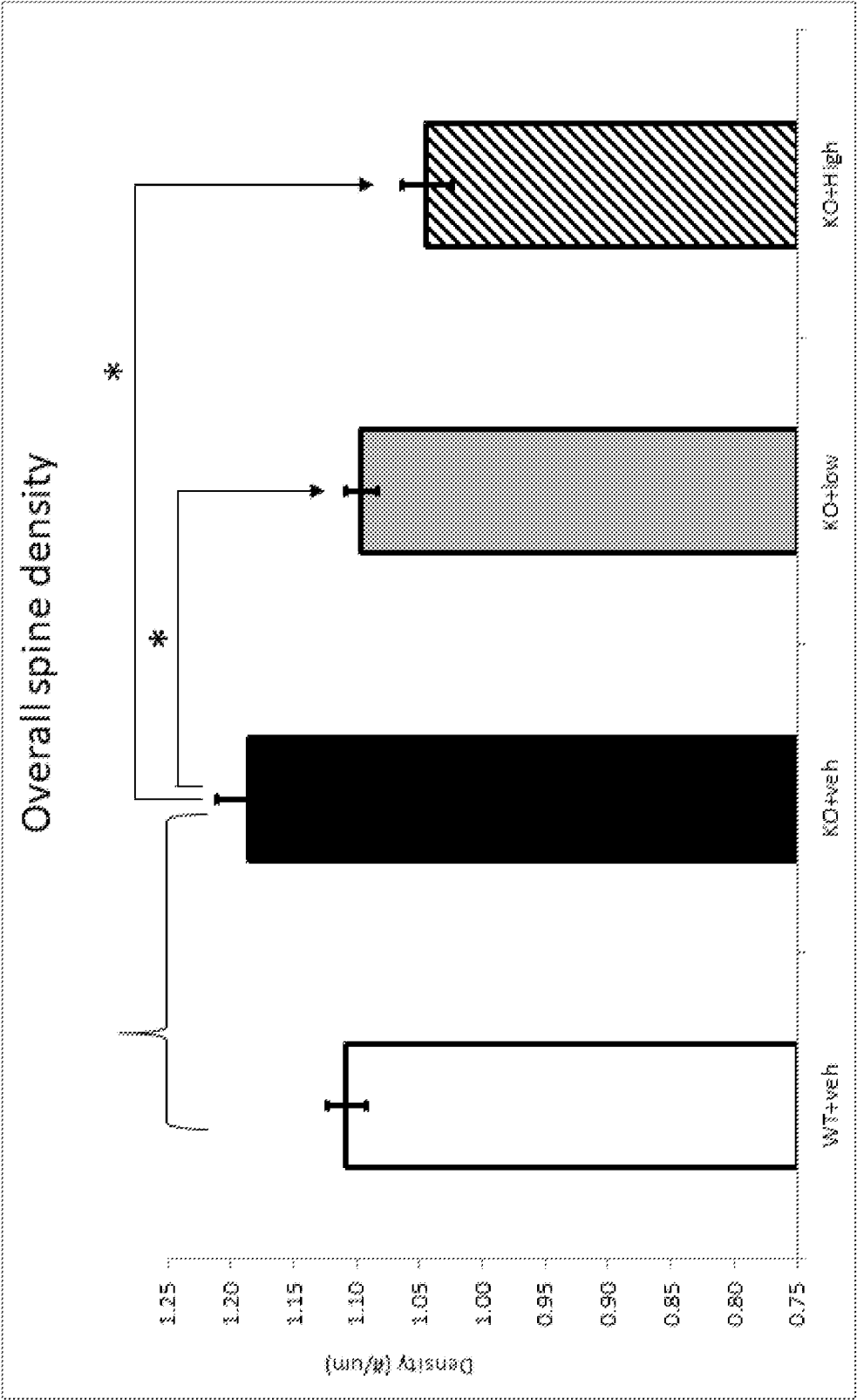


FIG. 6F

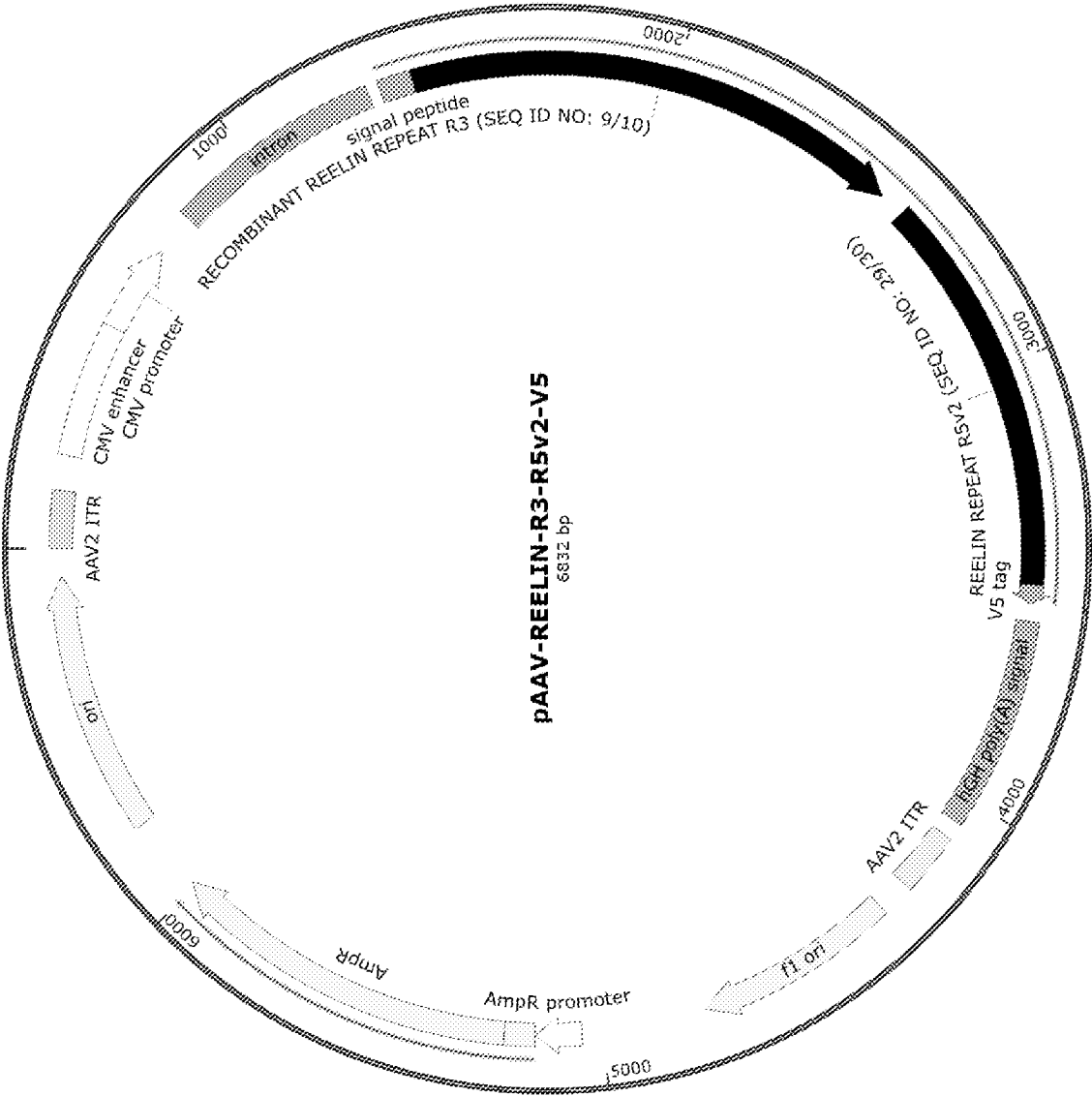


FIG. 7A

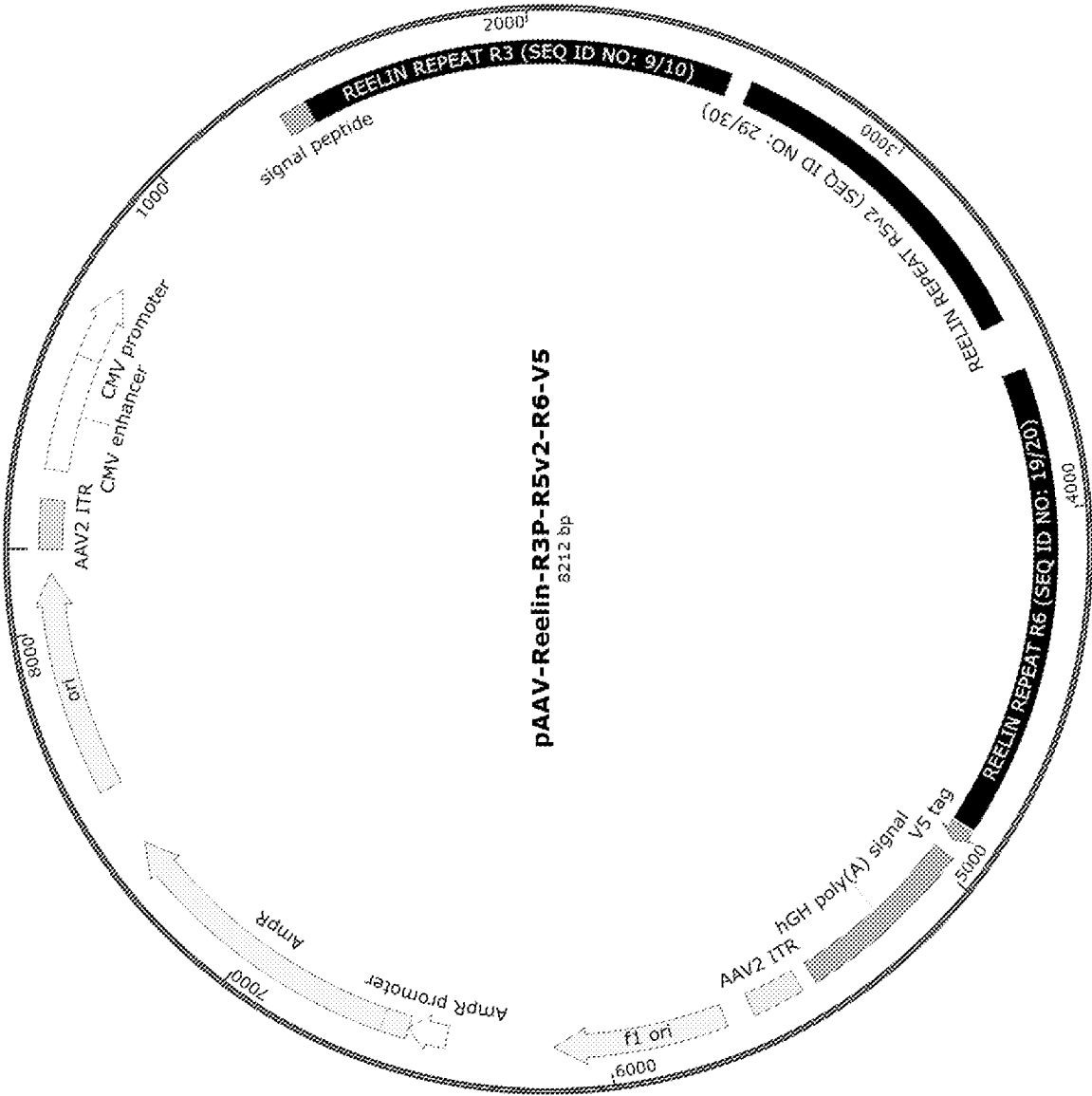


FIG. 7B