

NEUROD1 VECTOR

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/084,908, filed September 29, 2020, and U.S. Provisional Patent Application No. 63/246,545, filed September 21, 2021, the contents of which are incorporated herein by reference in their entireties.

INCORPORATION OF SEQUENCE LISTING

[0002] A sequence listing contained in the file named P34822WO00_SL.txt which is 21,632 bytes (measured in MS-Windows®) and created on September 27, 2021, is filed electronically herewith and incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure includes methods and compositions using an AAV vector comprising a nucleic acid sequence encoding human NeuroD1 to convert glial cells to neurons.

BACKGROUND OF THE INVENTION

[0004] Neurons are often killed or damaged and unable to regenerate in subjects with a neurological condition or following an injury to the central nervous system (CNS) or peripheral nervous system (PNS).

[0005] Glial cells become reactive following an injury to the CNS or PNS such as a brain injury or neurological condition.

[0006] Currently there are no methods available to regenerate functional new neurons in human subjects having a neurological condition using adeno-associated viruses (AAVs).

SUMMARY

[0007] In one example, this disclosure provides, and includes, an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid

sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0007a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0008] In one example, this disclosure provides, and includes, an adeno-associated virus (AAV) vector comprising a nucleic acid coding sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid sequence of SEQ ID NO: 10, where the coding sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; (e) and a SV40 polyadenylation signal sequence with a nucleic acid sequence of SEQ ID NO: 8, a hGH

polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0008a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising a nucleic acid coding sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid sequence of SEQ ID NO: 10, wherein said coding sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence with a nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0009] In an example, this disclosure provides, and includes, an adeno-associated virus (AAV) vector comprising a neurogenic differentiation 1 (NeuroD1) nucleic acid coding sequence encoding a NeuroD1 protein, where the coding sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal sequence.

[0009a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising a neurogenic differentiation 1 (NeuroD1) nucleic acid coding sequence encoding a NeuroD1 protein, wherein said coding sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal sequence.

[0010] In an example, this disclosure provides, and includes, a composition comprising an adeno-associated virus (AAV) vector for converting glial cells to functional neurons in a human, where the AAV vector comprises a human neurogenic differentiation 1 (hNeuroD1) sequence having a nucleic acid sequence of SEQ ID NO: 6, and where the sequence is operably linked to regulatory

elements comprising: (a) a human glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0010a] In one aspect, the present disclosure refers to use of a composition comprising an adeno-associated virus (AAV) vector in the manufacture of a medicament for converting glial cells to functional neurons in a human, wherein said AAV vector comprises a human neurogenic differentiation 1 (hNeuroD1) sequence having a nucleic acid sequence of SEQ ID NO: 6, and wherein said sequence is operably linked to regulatory elements comprising: (a) a human glial fibrillary acidic protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0011] In an example, this disclosure provides, and includes, a composition comprising an adeno-associated-virus (AAV) vector for converting glial cells to functional neurons in a human, where the AAV vector comprises a nucleic acid coding sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid sequence of SEQ ID NO: 10, and where the coding sequence is operably linked to regulatory elements comprising: (a) a human glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the

group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0011a] In one aspect, the present disclosure refers to use of a composition comprising an adeno-associated-virus (AAV) vector in the manufacture of a medicament for converting glial cells to functional neurons in a human, wherein said AAV vector comprises a nucleic acid sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid coding sequence of SEQ ID NO: 10, and wherein said coding sequence is operably linked to regulatory elements comprising: (a) a human glial fibrillary acidic protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0012] In an example, this disclosure provides, and includes, a composition comprising an adeno-associated virus (AAV) vector for the treatment of a subject in need thereof, where the AAV vector comprises a neurogenic differentiation 1 (NeuroD1) sequence operably linked to expression control elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal.

[0012a] In one aspect, the present disclosure refers to use of a composition comprising an adeno-associated virus (AAV) vector in the manufacture of a medicament for the treatment of a neurological condition in a subject in need thereof, wherein said AAV vector comprises a neurogenic differentiation 1 (NeuroD1) sequence operably linked to expression control elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal.

[0013] In an example, this disclosure provides, and includes, a method of converting reactive astrocytes to functional neurons in a brain a living human comprising: injecting an adeno-associated virus (AAV) into a subject in need thereof, where the AAV comprises a DNA vector construct comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the sequence is operably linked to regulatory elements comprising: (a) a human glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NO: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0014] In an example, this disclosure provides, and includes, a method of converting reactive astrocytes to functional neurons in the brain of a living human brain comprising: injecting an adeno-associated virus (AAV) into a subject in need thereof, where the AAV comprises a DNA vector construct comprising a nucleic acid coding sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid sequence of SEQ ID NO: 10, where the coding sequence is operably linked to expression control elements comprising: (a) a human glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH

polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0014a] In one aspect, the present disclosure refers to use of a composition in the manufacture of a medicament for converting reactive astrocytes to functional neurons in a brain of a living human, the composition comprising an adeno-associated virus (AAV) comprising a DNA vector construct comprising a nucleic acid sequence encoding a human neurogenic differentiation 1 (hNeuroD1) protein comprising the amino acid coding sequence of SEQ ID NO: 10, wherein said coding sequence is operably linked to expression control elements comprising: (a) a human glial fibrillary acid protein (GFAP) promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 15; (b) an enhancer from the human elongation factor-1 alpha (EF-1 alpha) promoter comprising the nucleic acid sequence of SEQ ID NO: 2 or a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 5 or 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7 and 18; and (e) a SV40 polyadenylation signal sequence comprising the nucleic acid sequence of SEQ ID NO: 8, a hGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 13, or a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14, wherein the composition is to be injected into said brain of a living human.

[0015] In an example, this disclosure provides, and includes, a method of converting glial cells to neurons in a subject in need thereof comprising: delivering an adeno-associated virus (AAV) to the subject in need thereof, where the AAV comprises a DNA vector construct comprising a neurogenic differentiation 1 (NeuroD1) sequence operably linked to expression control elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) and a polyadenylation signal sequence, where the vector is capable of converting at least one glial cell to a neuron in the subject in need thereof.

[0016] In an example, this disclosure provides, and includes, a method of treating a neurological condition in a subject in need thereof comprising: delivering an adeno-associated virus (AAV) to the subject, where the AAV comprises a DNA vector construct comprising a neurogenic differentiation 1 (NeuroD1) sequence operably linked to expression control elements

comprising: (a) a glial fibrillary acid protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal to the subject in need thereof.

[0016a] In one aspect, the present disclosure refers to a composition comprising an adeno-associated virus (AAV) comprising a DNA vector construct comprising a neurogenic differentiation 1 (NeuroD1) sequence operably linked to expression control elements comprising: (a) a glial fibrillary acid protein (GFAP) promoter; (b) an enhancer; (c) a chimeric intron; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); and (e) a polyadenylation signal, wherein said composition is to be delivered to said subject in need thereof.

[0017] In one example, this disclosure provides, and includes, an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter comprising the nucleic acid sequence of SEQ ID NO: 15; (b) an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid sequence of SEQ ID NO: 2; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence of SEQ ID NO: 18; and (e) a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0017a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: a glial fibrillary acidic protein (GFAP) promoter comprising the nucleic acid sequence of SEQ ID NO: 15; an enhancer from a human elongation factor-1 alpha (EF1- α) promoter comprising the nucleic acid sequence of SEQ ID NO: 2; a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 16; a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence of SEQ ID NO: 18; and a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0018] In one example, this disclosure provides, and includes, an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: (a) a glial fibrillary acidic protein (GFAP) promoter comprising

the nucleic acid sequence of SEQ ID NO: 15; (b) a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; (c) a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 16; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence of SEQ ID NO: 18; and (e) a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

[0018a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising a human neurogenic differentiation 1 (hNeuroD1) sequence comprising the nucleic acid sequence of SEQ ID NO: 6, where the hNeuroD1 sequence is operably linked to regulatory elements comprising: a glial fibrillary acidic protein (GFAP) promoter comprising the nucleic acid sequence of SEQ ID NO: 15; a cytomegalovirus (CMV) enhancer comprising the nucleic acid sequence of SEQ ID NO: 11; a chimeric intron comprising the nucleic acid sequence of SEQ ID NO: 16; a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) comprising the nucleic acid sequence of SEQ ID NO: 18; and a bGH polyadenylation sequence comprising the nucleic acid sequence of SEQ ID NO: 14.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1A depicts a map of a EF-1 α :GfaABC1D:NeuroD1:WPRE:SV40 (P35).

[0020] Figure 1B depicts a map of a EF-1 α :GfaABC1D:NeuroD1:WPRE:hGH.

[0021] Figure 1C depicts a map of a CE:GfaABC1D:NeuroD1:WPRE:SV40 (P11).

[0022] Figure 1D depicts a map of a CE:GfaABC1D:NeuroD1:WPRE:hGH.

[0023] Figure 2A depicts a map of a EF-1 α :Gfa1.6:NeuroD1:WPRE:SV40 (P39).

[0024] Figure 2B depicts a map of a CE:Gfa1.6:NeuroD1:WPRE:hGH.

[0025] Figure 2C depicts a map of a CE:Gfa1.6:NeuroD1:WPRE:SV40 (P38).

[0026] Figure 2D depicts a map of a EF-1 α :Gfa1.6:NeuroD1:WPRE:hGH.

[0027] Figure 3A depicts a map of a EF-1 α :GFA2.2:NeuroD1:WPRE:SV40.

[0028] Figure 3B depicts a map of a EF-1 α :GFA2.2:NeuroD1:WPRE:hGH.

[0029] Figure 3C depicts a map of a CE:GFA2.2:NeuroD1:WPRE:SV40.

[0030] Figure 3D depicts a map of a CE:GFA2.2:NeuroD1:WPRE:hGH.

[0031] Figure 4 is a plot of measurements of AAV virus production of the P35 plasmid. Titer analysis is performed using gene of interest (GOI) primers, ITR region primers, and reverse packaging primers. Virus yield is calculated as vg/cell.

[0032] Figure 5 depicts establishment of rat astrocyte primary culture from 3 day post-natal Sprague-Dawley rat brains. Upper left panel presents an image of GFAP stained cells. Upper right panel presents an image of SOX9 stained cells. Lower left panel presents an image of DAPI stained cells. Lower right panel presents a merge image of GFAP, SOX9, and DAPI stained cells.

[0033] Figure 6 depicts transfection of primary rat astrocytes with plasmid P5 (pEF-1 α :hNeuroD1:GFP). Left panel presents an image of NeuroD1 stained cells. Middle left panel presents an image of GFP expressing cells. Middle right panel represents DAPI stained cells. Right panel represents a merge image of NeuroD1, GFP, and DAPI stained cells.