

CLAIMS

What is claimed is:

1. A nucleic acid comprising an adeno-associated virus (AAV) expression cassette, wherein the AAV expression cassette comprises, from 5' to 3':

a 5' inverted terminal repeat (ITR);

a ~~synthetic, activity-dependent~~ human Arc gene promoter; (hArcMin)

a Rett Syndrome-associated gene; and

a 3' ITR;

wherein the hArcMin comprises a nucleic acid sequence of SEQ ID NO: 12, or a sequence at least 95% identical thereto.

2. The nucleic acid of claim 1, wherein the promoter drives expression of the Rett syndrome-associated gene.

3. The nucleic acid of claim 1 ~~or 2~~, wherein the promoter ~~is a MECP2-independent promoter.~~

~~4. The nucleic acid of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence derived from a promoter of a neuronal immediate early gene.~~

~~5. The nucleic acid of claim 4, wherein the neuronal immediate early gene is selected from the group consisting of the Arc gene, the c fos gene, and the egr 1 gene.~~

~~6. The nucleic acid of any one of claims 1-5, wherein the promoter comprises a minimal Arc gene promoter (ArcMin).~~

~~7. The nucleic acid of claim 6, wherein the ArcMin is a human ArcMin (hArcMin).~~

~~8. The nucleic acid of claim 7, wherein the hArcMin comprises a nucleic acid sequence of SEQ ID NO: 12, or a sequence at least 90% identical thereto.~~

~~9. The nucleic acid of any one of claims 1-8, wherein the promoter~~further ~~comprises a cyclic AMP response element (CRE), a serum response element (SRE), a synaptic activity response element (SARE), a MEF2 response element, or a combination thereof.~~

~~10.4.~~ The nucleic acid of claim ~~93~~, wherein the promoter ~~further~~ comprises ~~at least one~~ synaptic activity response element (SARE).

~~11.5.~~ The nucleic acid of claim ~~104~~, wherein the synaptic activity response element (SARE) is a human synaptic activity response element (hSARE).

~~12.~~ The nucleic acid of claim ~~11~~, wherein the hSARE) and comprises a nucleic acid sequence of SEQ ID NO: 11, or a sequence at least ~~90~~95% identical thereto.

~~13.6.~~ The nucleic acid of ~~any one of claims 1-12~~claim ~~5~~, wherein the promoter comprises ~~a human ArcMin (hArcMin) and at least one hSARE.~~

~~14.~~ The nucleic acid of claim ~~13~~, wherein the promoter comprises ~~hArcMin and one hSARE.~~

~~15.~~ The nucleic acid of claim ~~14~~, wherein the promoter comprises ~~a~~the nucleic acid sequence of SEQ ID NO: 6, or a sequence at least ~~90~~95% identical thereto.

~~16.7.~~ The nucleic acid of claim ~~135~~, wherein the promoter comprises ~~hArcMin and five hSAREs.~~

~~17.~~ The nucleic acid of claim ~~16~~, wherein the promoter comprises ~~a~~ nucleic acid sequence of SEQ ID NO: 16, or a sequence at least ~~90~~95% identical thereto.

~~18.~~ The nucleic acid of any one of claims 1-17, wherein the promoter binds to a neuronal activity dependent transcription factor.

~~19.~~ The nucleic acid of claim ~~18~~, wherein the neuronal activity dependent transcription factor is ~~cAMP-responsive element binding protein (CREB), myocyte enhancer factor 2 (MEF2), serum response factor (SRF), or Elk 1.~~

~~20.~~ The nucleic acid of any one of claims 1-198. The nucleic acid of claim ~~1~~, wherein the Rett-syndrome-associated gene encodes brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (~~IGF4IGFI~~), methyl-CpG-binding protein 2 (MECP2), Huntingtin protein,

Huntington-associated protein 1, Orthodenticle homeobox 2 (OTX-2), FXYD Domain Containing Ion Transport Regulator 1 (~~FXYD~~FXYDI), Neurexin-2-alpha (NRXN2), or Protein Kinase C Gamma (PRKCG).

~~21.9.~~ The nucleic acid of claim ~~20~~1, wherein ~~the Rett-syndrome-associated gene encodes brain-derived neurotrophic factor (BDNF).~~

~~22.~~ The nucleic acid of claim ~~21~~, wherein the BDNF is a human BDNF.

~~23.~~ The nucleic acid of claim ~~22~~, wherein the BDNF is encoded by a nucleic acid sequence of SEQ ID NO: 7, or a sequence at least 90% identical thereto.

~~24.~~ The nucleic acid of any one of claims ~~1-23~~, wherein at least one of the ~~5'~~5' ITR and the ~~3'3'~~ ITR is about ~~110~~ to about 160 nucleotides in length.

~~25.~~ The nucleic acid of any one of claims ~~1-24~~, wherein the ~~5' ITR~~ is the same length as the ~~3' ITR~~.

~~26.~~ The nucleic acid of any one of claims ~~1-24~~, wherein the ~~5' ITR~~ and the ~~3' ITR~~ have different lengths.

~~27.~~ The nucleic acid of any one of claims ~~1-26~~, wherein at least one of the ~~5' ITR~~ and the ~~3' ITR~~ is isolated or derived from the genome of ~~AAV1~~AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, ~~AAV10~~AAV10, ~~AAV11~~AAV11, AAV12, AAVrh8, ~~AAVrh10~~AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.

~~28.10.~~ The nucleic acid of ~~any one of claims~~claim ~~1-23~~, wherein the ~~5'5'~~ ITR comprises the sequence of SEQ ID NO: 1.

~~29.~~ The nucleic acid of any one of claims ~~1-23~~, wherein the ~~3'~~3', or the 3' ITR comprises the sequence of SEQ ID NO: 2, or a combination thereof.

~~30.11.~~ The nucleic acid of ~~any one of claims~~claim ~~1-29~~, wherein the AAV cassette comprises ~~a brain-derived one of the following located between the Rett-syndrome-associated gene~~

and the 3' ITR: (a) a brain- derived neurotrophic factor (BDNF) short ~~3'3'~~ UTR, ~~or (b)~~ a BDNF long ~~3' UTR~~3' UTR, or (c) a polyadenylation signal.

~~31.12.~~ The nucleic acid of claim ~~30, wherein the BDNF short 3' UTR, or the BDNF long 3' UTR is located between the Rett syndrome-associated gene and the 3' ITR.~~

~~32.~~The nucleic acid of claim ~~30 or claim 31~~11, wherein the AAV cassette comprises a BDNF short ~~3'3'~~ UTR.

~~33.~~The nucleic acid of claim ~~32, and~~ wherein the BDNF short ~~3'3'~~ UTR comprises a nucleic acid sequence of SEQ ID NO: 8, or a sequence at least ~~90~~95% identical thereto.

~~34.13.~~ The nucleic acid of claim ~~30 or claim 31~~11, wherein the AAV cassette comprises a BDNF long ~~3'3'~~ UTR.

~~35.~~The nucleic acid of claim ~~34, and~~ wherein the BDNF long ~~3'3'~~ UTR comprises a nucleic acid sequence of SEQ ID NO: 10, or a sequence at least ~~90~~95% identical thereto.

~~36.~~The nucleic acid of any one of claims 1-35, wherein the AAV cassette comprises a polyadenylation signal.

~~37.14.~~ The nucleic acid of claim ~~36~~11, wherein the polyadenylation signal is a polyadenylation signal isolated or derived from one or more of the following genes: simian virus 40 (SV40), rBG, ~~α~~-globin, ~~β~~-globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) or bovine growth hormone (bGH).

~~38.15.~~ The nucleic acid of claim ~~36~~14, wherein the AAV cassette comprises a bGH polyadenylation signal.

~~39.~~The nucleic acid of claim ~~38, and~~ wherein the bGH polyadenylation signal comprises a nucleic acid sequence of SEQ ID NO: 9, or a sequence at least 90% identical thereto.

~~40. The nucleic acid of any one of claims 1-39, wherein the AAV cassette comprises at least one stuffer sequence.~~

~~41.16. The nucleic acid of claim 40, wherein the at least one stuffer sequence comprises a nucleic acid sequence of SEQ ID NO: 13, or a sequence at least 90% identical thereto.~~

~~42. The nucleic acid of any one of claims 1-41, wherein the AAV expression cassette comprises a Kozak sequence, wherein one of the Kozak sequence overlaps with the start codon of the Rett syndrome associated gene.~~

~~43. The nucleic acid of claim 42, wherein the Kozak sequence comprises following: (a) a nucleic acid sequence of SEQ ID NO: 14, or a sequence at least 90% identical thereto; or a nucleic acid sequence of SEQ ID NO: 15, or a sequence at least 90% identical thereto.~~

~~44. The nucleic acid of any one of claims 1-43, wherein the AAV expression cassette comprises a nucleic acid sequence SEQ ID NO: 3, or a sequence at least 9095% identical thereto; (b) a nucleic acid sequence of SEQ ID NO: 4 or a sequence at least 9095% identical thereto; or (c) a nucleic acid sequence of SEQ ID NO: 5 or a sequence at least 9095% identical thereto.~~

~~45. A plasmid comprising the nucleic acid of any one of claims 1-44.~~

~~46. A cell comprising the nucleic acid of any one of claims 1-44 or the plasmid of claim 45.~~

~~47. A method of producing a recombinant AAV vector, the method comprising contacting an AAV producer cell with the nucleic acid of any one of claims 1-44, or the plasmid of claim 45.~~

~~48. A recombinant AAV vector produced by the method of claim 47.~~

~~49. The recombinant AAV vector of claim 48, wherein the vector is of a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV and Bovine AAV.~~

~~50. The recombinant AAV vector of claim 48 or claim 49, wherein the recombinant AAV vector is a single stranded AAV (ssAAV).~~

~~51. The recombinant AAV vector of any one of claims 48-50, wherein the recombinant AAV vector is a self-complementary AAV (scAAV).~~

~~52. The recombinant AAV vector of any one of claims 48-51, wherein the AAV vector comprises a capsid protein of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.~~

~~53. The recombinant AAV vector of any one of claims 48-52, wherein the AAV vector comprises a capsid protein with one or more substitutions or mutations, as compared to a wildtype AAV capsid protein.~~

~~54.17. A composition, comprising: (a) the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, or the recombinant AAV vector of any one of claims 48-53 claim 1; and (b) a pharmaceutically acceptable carrier.~~

~~55. A method for expressing a Rett syndrome-associated gene in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, the recombinant AAV vector of any one of claims 48-53, or the composition of claim 54.~~

~~56. The method of claim 55, wherein the subject has Rett syndrome.~~

~~57. A method for treating or delaying the onset of Rett syndrome in a subject, comprising administering to the subject a therapeutically effective amount of the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, the recombinant AAV vector of any one of claims 48-53, or the composition of claim 54.~~

~~58. A method for expressing brain-derived neurotrophic factor (BDNF) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, the recombinant AAV vector of any one of claims 48-53, or the composition of claim 54.~~

~~59. The method of claim 58, wherein the subject has a cognitive disorder, or a stress-related disorder.~~

~~60. The method of claim 58, wherein the subject has depression, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, and dementia, anorexia nervosa and bulimia nervosa, schizophrenia, epilepsy, post-traumatic stress disorder, obesity, Rett syndrome, or post-chemotherapy cognitive impairment.~~

~~61. A method of treating or delaying the onset of a BDNF-associated disease in a subject, comprising administering to the subject a therapeutically effective amount of the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, the recombinant AAV vector of any one of claims 48-53, or the composition of claim 54.~~

~~62. The method of claim 61, wherein the BDNF-associated disease is a cognitive disorder, and/or a stress-related disorder.~~

~~63. The method of claim 61, wherein the BDNF-associated disease is depression, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, and dementia, anorexia nervosa, bulimia nervosa, schizophrenia, epilepsy, post-traumatic stress disorder, bipolar disease, Rett syndrome, major depressive disorder, or post-chemotherapy cognitive impairment.~~

~~64. A method of treating or delaying the onset of a MECP2-associated disease in a subject, comprising administering to the subject a therapeutically effective amount of the nucleic acid of any one of claims 1-44, the plasmid of claim 45, the cell of claim 46, the recombinant AAV vector of any one of claims 48-53, or the composition of claim 54.~~

~~65. The method of claim 64, wherein the MECP2-associated disease is MECP2 duplication syndrome, MECP2-related severe neonatal encephalopathy, PPM-X syndrome, or Rett syndrome.~~

66.18. The nucleic acid of claim 1, wherein the hArcMin promoter comprises a nucleic acid sequence of SEQ ID NO: 12.

19. The nucleic acid of claim 5, wherein the human synaptic activity response element (hSARE) comprises a nucleic acid sequence of SEQ ID NO: 11.

20. The nucleic acid of claim 6, wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 6.

21. The nucleic acid of claim 7, wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 16.

22. The nucleic acid of claim 12, wherein the BDNF short 3' UTR comprises a nucleic acid sequence of SEQ ID NO: 8.

23. The nucleic acid of claim 13, wherein the BDNF long 3' UTR comprises a nucleic acid sequence of SEQ ID NO: 10.

24. The ~~method of any one~~nucleic acid of claim ~~55-65~~15, wherein the ~~subject is a human~~ subject bGH polyadenylation signal comprises a nucleic acid sequence of SEQ ID NO: 9.

~~67. The method of any one of claims 55-66, wherein the nucleic acid, the plasmid, the cell, the recombinant AAV vector, or composition is administered by injection into the central nervous system.~~

~~68. The method of any one of claims 55-67, wherein the Rett Syndrome associated gene is expressed in the neurons of the subject.~~

~~69. The method of claim 68, wherein the neurons are active neurons.~~25. The nucleic acid of claim 16, wherein the AAV expression cassette comprises one of the following: (a) a nucleic acid sequence of SEQ ID NO: 3; (b) a nucleic acid sequence of SEQ ID NO: 4; or (c) a nucleic acid sequence of SEQ ID NO: 5.