

cassettes, vectors, and pharmaceutical compositions for the purpose of providing transgene expression in target tissues that is capable of endogenous regulation for treating disorders such as dose-sensitive intellectual ability disorders.

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SUMMARY OF THE INVENTION

The present ~~invention~~disclosure is based, in part, on the development of feedback-enabled synthetic genes, polynucleotide target cassettes, vectors, and pharmaceutical compositions for the purpose of providing transgene expression in target tissues that is capable of endogenous regulation for treating disorders such as dose-sensitive intellectual ability disorders such as Rett Syndrome (RTT). This ~~invention~~disclosure was made in part with support from the Rett Syndrome Research Trust.

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Thus, one aspect of the ~~invention~~disclosure relates to a synthetic gene comprising a polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory regions, wherein the polynucleotide further comprises ~~three or~~ three or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and a 5' flanking sequence and a 3' flanking sequence neighboring said seed match, wherein said one or more nucleic acid segments are inserted into a regulatory region of said polynucleotide such that expression of said protein or nucleic acid of interest when said synthetic gene is delivered to a cell expressing the endogenous miRNA is reduced relative to expression of a protein or nucleic acid of interest when a synthetic gene that does not comprise the one or more nucleic acid segments is delivered to a cell expressing the endogenous miRNA, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

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A further aspect of the ~~invention~~disclosure relates to vectors and pharmaceutical compositions comprising the synthetic gene of the present ~~invention~~disclosure.

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Another ~~aspect~~example of the ~~invention~~disclosure relates to a polynucleotide target cassette for providing dose dependent inhibitory feedback to the synthetic gene of the present ~~invention~~disclosure.

An additional ~~aspect~~example relates to a method of preparing a synthetic gene, comprising the step of inserting the polynucleotide target cassette into a regulatory region of the synthetic gene.

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A further ~~aspect~~example of the ~~invention~~disclosure relates to a method of making a synthetic gene comprising the steps of inserting one or more nucleic acid segments comprising a seed match and 5' and 3' flanking sequences into a regulatory region of the polynucleotide of the synthetic gene.

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In one aspect of the present disclosure refers to a polynucleotide target cassette for providing dose dependent inhibitory feedback to a synthetic gene, the cassette comprising one or more nucleic acid segments comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

Another aspect of the ~~invention~~disclosure relates to a method of identifying one or more seed matches and flanking sequences to be inserted into a synthetic gene, comprising the steps of identifying a seed match and flanking sequences, and inserting said seed match and flanking sequences into a regulatory region of a synthetic gene, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p; or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

An additional ~~aspect~~example of the ~~invention~~disclosure relates to a method of delivering a synthetic gene to a subject, comprising administering to the subject the synthetic gene, vectors, or pharmaceutical composition of the present ~~invention~~disclosure. A further ~~aspect~~example of the ~~invention~~disclosure relates to a method of treating a disease associated with abnormal expression of an endogenous gene, comprising administering the synthetic genes, vectors, or pharmaceutical compositions of the present ~~invention~~disclosure, thereby treating the disease. In yet another aspect, the present disclosure refers to a use of the synthetic gene, the vector, or the pharmaceutical composition as disclosed herein encoding a protein or nucleic acid of interest encoded by the endogenous gene in the manufacture of a medicament for treating a disease associated with abnormal expression of an endogenous gene or expression of a mutant protein encoded by an endogenous gene in a subject.

Another ~~aspect~~example of the ~~invention~~disclosure relates to a method of treating a disease associated with abnormal expression of an endogenous gene or expression of a mutant protein encoded by an endogenous gene in a subject, comprising genetically knocking down the endogenous gene in a cell of the subject, and administering the synthetic genes, vectors, or pharmaceutical compositions of the present ~~invention~~disclosure, thereby treating the disease.

These and other ~~aspects~~examples of the ~~invention~~disclosure are set forth in more detail in the description of the ~~invention~~disclosure below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a feedback loop depicting how MeCP2-driven miRNAs may attenuate toxic overexpression of exogenous MeCP2.

FIG. 2 shows sample microarray data. The selected data quantifies miRNA expression levels in MeCP2-null versus wild-type (WT) tissue.

FIG. 3 shows miRNAs with increased mean expression levels in saline-treated WT mice (compared to saline-treated KO mice). miRNAs with statistically significant increases in expression (in at least 1 of the 3 tissue types) are indicated (*). False positive hits that are also upregulated in response to AAV9/*EGFP* (in any tissue) are indicated (**). n=2 screening replicates per biological sample; n=3 mice per treatment group.

FIG. 4 shows miRNAs with increased expression in either cervical cord (CC) or medulla in correlation with endogenous MeCP2 expression. Each data point is the average of 2 screening replicates for a single mouse. Groups that are significantly different from each other are boxed in red. n=3 mice per treatment group.

What is claimed is:

1. A synthetic gene comprising:
 - a polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory regions;
 - the polynucleotide further comprising ~~three~~one or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and a 5' flanking sequence and a 3' flanking sequence neighboring said seed match;
 - wherein said one or more nucleic acid segments are inserted into a regulatory region of said polynucleotide such that expression of said protein or nucleic acid of interest when said synthetic gene is delivered to a cell expressing the endogenous miRNA is reduced relative to expression of a protein or nucleic acid of interest when a synthetic gene that does not comprise the one or more nucleic acid segments is delivered to a cell expressing the endogenous miRNA, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.
2. The synthetic gene of claim 1, wherein the coding region encoding a protein or nucleic acid of interest comprises the coding region of a gene selected from *TCF4*, *UBE3A*, *DYRK1A*, *MEF2C*, *NSD1*, *ZEB2*, *MBD5*, *RPS6KA3*, *ATRX*, *MECP2*, *SLC6A1*, *FOXG1*, *AKT3*, or an active fragment thereof.
3. The synthetic gene of claim 1, wherein the coding region encoding a protein or nucleic acid of interest comprises the coding region of the gene *MECP2* or an active fragment thereof.
4. ~~The synthetic gene of any one of claims 1-3, wherein the seed matches and 5' and 3' flanking sequences bind to one or more miRNAs selected from miR-690, miR-124-3p, miR-451a, miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, let-7-5p/98-5p, miR-29-3p, miR-338-3p, miR-98-5p, miR-7-5p, miR-494-3p, or any combination thereof.~~

~~54.~~ The synthetic gene of any one of claims 1-3, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

~~65.~~ The synthetic gene of any one of claims 1-3, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

~~76.~~ The synthetic gene of any one of claims 1-~~65~~, wherein the seed match is about 5 to about 10 nucleotides in length.

~~87.~~ The synthetic gene of any one of claims 1-~~65~~, wherein the seed match is about 6 to about 8 nucleotides in length.

~~98.~~ The synthetic gene of any one of claims 1-~~65~~, wherein the 5' and 3' flanking sequences neighboring the seed match are each about 9 to about 13 nucleotides in length.

~~109.~~ The synthetic gene of any one of claims 1-~~65~~, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.

~~1110.~~ The synthetic gene of any one of claims 1-~~65~~, wherein the polynucleotide comprises at least two seed matches and the seed matches are separated by about 7 to about 40 nucleotides.

~~1211.~~ The synthetic gene of claim ~~1110~~, wherein the at least two seed matches are separated by about 20 to about 25 nucleotides.

~~1312.~~ The synthetic gene of claim ~~1110~~, wherein the at least two seed matches are separated by 22 nucleotides.

~~14.~~ ~~The synthetic gene of any one of claims 11-13, wherein the polynucleotide comprises 3-8 seed matches.~~

~~1513.~~ The synthetic gene of any one of claims 1-~~1412~~ wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 70% identical to SEQ ID NO:1.

14. The synthetic gene of any one of claims 1-12 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.

~~16~~15. The synthetic gene of any one of claims 1-14 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 70% identical to SEQ ID NO:2.

~~17~~16. A vector comprising the synthetic gene of any one of claims 1-~~16~~15.

~~18~~17. The vector of claim ~~17~~16, wherein the vector is a plasmid, a viral vector, an expression cassette, a transformed cell or a nanoparticle.

~~19~~18. A pharmaceutical composition comprising the synthetic gene of any one of claims 1-~~16~~14 or the vector of claim ~~17~~16 or ~~18~~17 and a pharmaceutically acceptable carrier.

~~20~~19. A polynucleotide target cassette for providing dose dependent inhibitory feedback to a synthetic gene, the cassette comprising one or more nucleic acid segments comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

~~21. The polynucleotide target cassette of claim 20, wherein the seed matches and 5' and 3' flanking sequences bind to one or more miRNAs selected from miR-690, miR-124-3p, miR-451a, miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, let-7-5p/98-5p, miR-29-3p, miR-338-3p, miR-98-5p, miR-7-5p, miR-494-3p, or any combination thereof.~~

~~22~~20. The polynucleotide target cassette of claim ~~20~~19, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

~~23~~21. The polynucleotide target cassette of claim ~~20~~19, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

~~24~~22. The polynucleotide target cassette of any one of claims ~~20-23~~19-21, wherein the seed match is about 5 to about 10 nucleotides in length.

~~25~~23. The polynucleotide target cassette of any one of claims ~~20-23~~19-21, wherein the seed match is about 6 to about 8 nucleotides in length.

~~26~~24. The polynucleotide target cassette of any one of claims ~~20-25~~19-23, wherein the 5' and 3' flanking sequences neighboring the seed match are each about 9 to about 13 nucleotides in length.

~~27~~25. The polynucleotide target cassette of any one of claims ~~20-25~~19-23, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.

~~28~~26. The polynucleotide target cassette of any one of claims ~~20-27~~19-25, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 70% identical to SEQ ID NO:1.

27. The polynucleotide target cassette of any one of claims 19-25, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.

~~29~~28. The polynucleotide target cassette of any one of claims ~~20-27~~19-25, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 70% identical to SEQ ID NO:2.

~~30~~29. A method of preparing a synthetic gene comprising a polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory regions, comprising the step of inserting the polynucleotide target cassette of any one of claims ~~20-29~~19-28 into a regulatory region of the synthetic gene.

~~31~~30. A method of making the synthetic gene of any one of claims ~~1-46~~15, comprising the steps of:

inserting ~~three~~one or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match into a regulatory region of the polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory regions,

wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p; or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

~~32~~31. The method of claim ~~31~~30, wherein the coding region encoding a protein or nucleic acid of interest comprises the coding region of a gene selected from *TCF4*, *UBE3A*, *DYRK1A*, *MEF2C*, *NSD1*, *ZEB2*, *MBD5*, *RPS6KA3*, *ATRX*, *MECP2*, *SLC6A1*, *FOXG1*, *AKT3* or an active fragment thereof.

~~33~~32. The method of claim ~~31~~30, wherein the coding region encoding a protein or nucleic acid of interest comprises the coding region of a gene *MECP2* or an active fragment thereof.

~~34. The method of any one of claims 31-33, wherein the seed matches and 5' and 3' flanking sequences bind to one or more miRNAs selected from selected from miR-690, miR-124-3p, miR-451a, miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, let-7-5p/98-5p, miR-29-3p, miR-338-3p, miR-98-5p, miR-7-5p, miR-494-3p, or any combination thereof.~~

~~35~~33. The method of any one of claims ~~31-33~~30-32, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

~~36~~34. The method of any one of claims ~~31-33~~30-32, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

~~37~~35. The method of claim ~~31~~30, further comprising the steps of:

screening for miRNAs with increased expression when the protein or nucleic acid of interest is expressed in a cell relative to when the protein or nucleic acid of interest is not expressed;

identifying a seed match and 5' and 3' flanking sequences for ~~three~~^{one} or more miRNAs having increased expression; and

preparing ~~a~~^{the} nucleic acid segment comprising said seed match and 5' and 3' flanking sequences to be inserted into a regulatory region of said polynucleotide.

36. The method of any one of claims 30-35, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 70% identical to SEQ ID NO:1.

37. The method of any one of claims 30-35, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.

38. ~~A method of identifying one or more seed matches and 5' and 3' flanking sequences to be inserted in a synthetic gene, comprising the steps of:~~

~~identifying a seed match and 5' and 3' flanking sequences for one or more miRNAs having increased expression when a protein or nucleic acid of interest is expressed in a cell relative to when the protein or nucleic acid of interest is not expressed in the cell; and~~

~~inserting said seed match and 5' and 3' flanking sequences into a regulatory region of a synthetic gene comprising a polynucleotide comprising a coding region encoding the protein or nucleic acid of interest and one or more regulatory regions.~~

39. ~~The method of claim 38, further comprising the step of:~~

~~screening a nucleic acid dataset for a validated or putative seed match and 5' and 3' flanking sequences.~~

40. ~~The method of claims 38 or 39, further comprising the step of:~~

identifying miRNAs with increased expression when a protein or nucleic acid of interest is expressed in a cell relative to when the protein or nucleic acid of interest is not expressed in the cell.

41. — The method of any one of claims 38-40, further comprising the step of:
screening a nucleic acid dataset for miRNAs with increased expression when a protein or nucleic acid of interest is expressed in a cell relative to when the protein or nucleic acid of interest is not expressed in the cell.

42. — The method of any one of claims 38-41, further comprising the steps of:
expressing the protein or nucleic acid of interest in a cell;
collecting miRNA from the cell; and
calculating expression levels of said miRNAs when said protein or nucleic acid of interest is expressed in the cell relative to when said protein or nucleic acid of interest is not expressed in the cell, thereby creating a nucleic acid dataset of said miRNAs.

43. — The method of any one of claims 39, 41, or 42, wherein the nucleic acid dataset is a 3' UTR dataset.

44. — The method of any one of claims 38-43, wherein the protein or nucleic acid of interest is a transcription or translation product of a gene selected from *TCF4*, *UBE3A*, *DYRK1A*, *MEF2C*, *NSD1*, *ZEB2*, *MBD5*, *RPS6KA3*, *ATRX*, *SLC6A1*, *FOXG1*, *AKT3*, *MECP2*, or an active fragment thereof.

45. — The method of any one of claims 38-43, wherein the protein or nucleic acid of interest is a transcription or translation product of a gene *MECP2* or an active fragment thereof.

46. — A method of delivering a synthetic gene to a subject, the method comprising the subject to be administered the synthetic gene of any one of claims 1-16, the vector of claim 17 or 18, or the pharmaceutical composition of claim 19, thereby delivering the synthetic gene to the subject.

47.—~~Use of the synthetic gene of any one of claims 1-16, the vector of claim 17 or 18, or the pharmaceutical composition of claim 19 encoding a protein or nucleic acid of interest encoded by the endogenous gene in the manufacture of a medicament for treating a disease associated with abnormal expression of an endogenous gene or expression of a mutant protein encoded by an endogenous gene, the method comprising administering.~~

48.—~~The method of claim 46 or the use of claim 47, wherein the subject is a human.~~

49.—~~The method of claim 46 or the use of any one of claims 47-48, wherein the subject has or is at risk for an intellectual ability gene dose sensitive disorder.~~

50.—~~The method of claim 46 or the use of any one of claims 47-48, wherein the subject has or is at risk for a disorder selected from the group consisting of Rett syndrome, MeCP2 duplication syndrome, Angelman syndrome, dup15Q, DYRK1A haploinsufficiency, Down syndrome, MEF2C haploinsufficiency syndrome, dup5Q14.3, Sotos syndrome, Reverse Sotos syndrome, Alpha-thalassemia X linked intellectual disability syndrome, Xq13.2q21.1 duplication, Coffin Lowry syndrome, Xp22.12 duplication, Pitt Hopkins syndrome, Mowat-Wilson Syndrome, 2q22.3 triplication, 2q23.1 duplication, 2q23.1 microdeletion, FOXP1 syndrome, West Syndrome, megalencephaly polymicrogyria polydactyly hydrocephalus syndrome, AKT3 duplication, Doose syndrome, SLC6A1 duplication and Trisomy 18.~~

51.—~~The method of claim 46 or the use of any one of claims 47-50, wherein the subject has or is at risk for Rett syndrome or MeCP2 duplication syndrome.~~

52.—~~The method of claim 46 or the use of any one of claims 47-51, wherein the synthetic gene, vector, or pharmaceutical composition is to be delivered by a delivery route selected from the group consisting of enteral, parenteral, intrathecal, intracisternal, intracerebral, intraventricular, intranasal, intra-aural, intra-ocular, peri-ocular, intrarectal, intramuscular, intraperitoneal, intravenous, oral, sublingual, subcutaneous and transdermal.~~

53.—~~The method of claim 46 or the use of any one of claims 47-51, wherein the synthetic gene is to be delivered intravenously.~~

~~54. The method of claim 46 or the use of any one of claims 47-51, wherein the synthetic gene is to be delivered intraCSF.~~

~~55. The method of claim 46 or the use of claims 47-54, the method further comprising genetically knocking down an endogenous gene encoding the protein or nucleic acid of interest in the subject.~~

~~56. The method or use of claim 55, wherein the endogenous gene is *MECP2*.~~

~~5738. Use of the synthetic gene of any one of claims 1-1415, the vector of claim 15-16 or 1617, or the pharmaceutical composition of claim 17-18 encoding a protein or nucleic acid of interest encoded by the endogenous gene in the manufacture of a medicament for treating a disease associated with abnormal expression of an endogenous gene or expression of a mutant protein encoded by an endogenous gene in a subject, the use comprising genetically knocking down the endogenous gene in a cell of the subject,.~~

39. The method or use of claim 38, wherein the endogenous gene is *MECP2*.

~~5840. The use of claim 5738, wherein the disease is Rett syndrome, MeCP2 duplication syndrome, Angelman syndrome, dup15Q, DYRK1A haploinsufficiency, Down syndrome, MEF2C haploinsufficiency syndrome, dup5Q14.3, Sotos syndrome, Reverse Sotos syndrome, Alpha-thalassemia X-linked intellectual disability syndrome, Xq13.2q21.1 duplication, Coffin-Lowry syndrome, Xp22.12 duplication, Pitt Hopkins syndrome, Mowat-Wilson Syndrome, 2q22.3 triplication, 2q23.1 duplication, 2q23.1 microdeletion, FOXG1 syndrome, West syndrome, megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome, AKT3 duplication, Doose syndrome, SLC6A1 duplication and/or Trisomy 18.~~

41. The use of claim 38, wherein the disease is Rett syndrome.