

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

The present 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 disclosure was made in part with support from the Rett Syndrome Research Trust.

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Thus, one aspect of the disclosure relates to a synthetic gene comprising a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions, wherein the polynucleotide further comprises 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 disclosure relates to vectors and pharmaceutical compositions comprising the synthetic gene of the present disclosure.

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

An additional 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 example of the 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.

What is claimed is:

1. A synthetic gene comprising:
a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions;
the polynucleotide further comprising 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.
2. 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.
3. The synthetic gene of any one of claims 1-2, 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.
4. The synthetic gene of any one of claims 1-2, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.
5. The synthetic gene of any one of claims 1-4, wherein the seed match is 5 to 10 nucleotides in length.
6. The synthetic gene of any one of claims 1-4, wherein the seed match is 6 to 8 nucleotides in length.

7. The synthetic gene of any one of claims 1-4, wherein the 5' and 3' flanking sequences neighboring the seed match are each 9 to 13 nucleotides in length.
8. The synthetic gene of any one of claims 1-4, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.
9. The synthetic gene of any one of claims 1-4, wherein the polynucleotide comprises at least two seed matches and the seed matches are separated by 7 to 40 nucleotides.
10. The synthetic gene of claim 9, wherein the at least two seed matches are separated by 20 to 25 nucleotides.
11. The synthetic gene of claim 9, wherein the at least two seed matches are separated by 22 nucleotides.
12. The synthetic gene of any one of claims 1-11 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 90% identical to SEQ ID NO:1.
13. The synthetic gene of any one of claims 1-11 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.
14. The synthetic gene of any one of claims 1-13 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 90% identical to SEQ ID NO:2.
15. A vector comprising the synthetic gene of any one of claims 1-14.
16. The vector of claim 15, wherein the vector is a plasmid, a viral vector, an expression cassette, a transformed cell or a nanoparticle.
17. A pharmaceutical composition comprising the synthetic gene of any one of claims 1-13 or the vector of claim 15 or 16 and a pharmaceutically acceptable carrier.

18. 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.
19. The polynucleotide target cassette of claim 18, 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.
20. The polynucleotide target cassette of claim 18, wherein 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 any one of claims 18-20, wherein the seed match is 5 to 10 nucleotides in length.
22. The polynucleotide target cassette of any one of claims 18-20, wherein the seed match is 6 to 8 nucleotides in length.
23. The polynucleotide target cassette of any one of claims 18-22, wherein the 5' and 3' flanking sequences neighboring the seed match are each 9 to 13 nucleotides in length.
24. The polynucleotide target cassette of any one of claims 18-22, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.
25. The polynucleotide target cassette of any one of claims 18-24, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 90% identical to SEQ ID NO:1.

26. The polynucleotide target cassette of any one of claims 18-24, 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.
27. The polynucleotide target cassette of any one of claims 18-24, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 90% identical to SEQ ID NO:2.
28. 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 18-27 into a regulatory region of the synthetic gene.
29. A method of making the synthetic gene of any one of claims 1-14, comprising the steps of:
inserting three 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.
30. The method of claim 29, 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.
31. The method of any one of claims 29-30, 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.
32. The method of any one of claims 29-30, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.
33. The method of claim 29, 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 or more miRNAs having increased expression; and preparing the nucleic acid segment comprising said seed match and 5' and 3' flanking sequences to be inserted into a regulatory region of said polynucleotide.

34. The method of any one of claims 29-33, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise a nucleotide sequence at least 90% identical to SEQ ID NO:1.

35. The method of any one of claims 29-33, 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.

36. Use of the synthetic gene of any one of claims 1-14, the vector of claim 15 or 16, or the pharmaceutical composition of claim 17 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.

37. The use of claim 36, wherein the endogenous gene is *MECP2*.

38. The use of claim 36, wherein the disease is Rett syndrome.