

SUMMARY

[0003a] In one aspect, provided herein is an expression cassette comprising a nucleic acid sequence encoding a non-naturally occurring DNA binding protein comprising a DNA binding domain, wherein the DNA binding domain comprises SEQ ID NO: 22, SEQ ID NO: 92, SEQ ID NO: 131, or SEQ ID NO: 409, and wherein the DNA binding domain binds a target site comprising SEQ ID NO: 35.

[0003b] In another aspect, provided herein is an expression vector comprising the expression cassette as described herein.

[0003c] In another aspect, provided herein is a viral particle comprising the expression cassette as described herein or the expression vector as described herein.

[0003d] In another aspect, provided herein is a host cell comprising the expression cassette as described herein or the expression vector as described herein.

[0003e] In another aspect, provided herein is a pharmaceutical composition comprising (i) the expression cassette as described herein, the expression vector as described herein, or the viral particle as described herein, and (ii) one or more pharmaceutically acceptable excipients.

[0003f] In another aspect, provided herein is a use of the expression cassette as described herein, in the manufacture of a medicament for treating a central nervous system disorder.

[0003g] In another aspect, provided herein is the expression cassette as described herein for use in therapy.

[0004] In one example, the application provides an expression cassette, comprising a sequence encoding a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell. In certain examples, the transcription factor which increases expression of SCN1A binds to a target site that is capable of increasing SCN1A expression by at least 20% in a transcriptional activation assay. In certain examples, the transcription factor which increases expression of SCN1A is capable of reducing the seizure frequency in a mouse model of Dravet syndrome by at least 20% in a hyperthermic seizure assay. In certain examples, the transcription factor which increases expression of SCN1A binds to a target site that is capable of increasing SCN1A expression by at least 20% in a transcriptional activation assay and the transcription factor is

capable of reducing the seizure frequency in a mouse model of Dravet syndrome by at least 20% in a hyperthermic seizure assay.

[0005] In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2. In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within 110 kb upstream or downstream of the transcriptional start site of the SCN1A gene. In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within positions 166179652-165989571 (with reference to GRCh38.p12). In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within positions 166128050-166127958 (with reference to GRCh38.p12). In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within positions 166155414-166140590 (with reference to GRCh38.p12). In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within positions 166179652-1661777272 (with reference to GRCh38.p12). In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic location on chromosome 2 that is within positions 1659990246-165989592 (with reference to GRCh38.p12). In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic region within 200 bp of a genomic location having a sequence of any one of SEQ ID NOs: 35-37, 105-111, 136, 195-211, 224-238, 240-267. In certain examples, the transcription factor which increases expression of SCN1A binds to a genomic region that is at least partially overlapping with a genomic location having a sequence of any one of SEQ ID NOs: 35-37, 105-111, 136, 195-211, 224-238, 240-267. In certain examples, the transcription factor which increases expression of

CLAIMS

WHAT IS CLAIMED IS:

1. An expression cassette comprising a nucleic acid sequence encoding a non-naturally occurring DNA binding protein comprising a DNA binding domain, wherein the DNA binding domain comprises SEQ ID NO: 22, SEQ ID NO: 92, SEQ ID NO: 131, or SEQ ID NO: 409, and wherein the DNA binding domain binds a target site comprising SEQ ID NO: 35.
2. The expression cassette of claim 1, wherein the DNA binding domain comprises SEQ ID NO: 22.
3. The expression cassette of claim 1, wherein the DNA binding domain comprises SEQ ID NO: 92.
4. The expression cassette of claim 1, wherein the DNA binding domain comprises SEQ ID NO: 131.
5. The expression cassette of claim 1, wherein the DNA binding domain comprises SEQ ID NO: 409.
6. The expression cassette of any one of claims 1-5, wherein the non-naturally occurring DNA binding protein further comprises a transcription activating domain.
7. The expression cassette of claim 6, wherein the transcription activating domain comprises one or more of SEQ ID NO: 95, 96, 97, 98, 100, or 114.
8. The expression cassette of claim 6 or 7, wherein the non-naturally occurring DNA binding protein further comprises a nuclear localization sequence (NLS).

9. The expression cassette of claim 8, wherein the NLS is a SV40 large T-antigen NLS or a nucleoplasmin NLS.
10. The expression cassette of claim 2, wherein the non-naturally occurring DNA binding protein comprises SEQ ID NO: 57, 58, 61, 62, 69, 295, 313, 315, or 316.
11. The expression cassette of claim 10, wherein:
- (i) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 313 and the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 445;
 - (ii) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 315 and the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 448; or
 - (iii) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 316 and the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 446.
12. The expression cassette of claim 3, wherein the non-naturally occurring DNA binding protein comprises SEQ ID NO: 13, 70, 71, 74, or 75.
13. The expression cassette of claim 4, wherein the non-naturally occurring DNA binding protein comprises SEQ ID NO: 305 or 306.
14. The expression cassette of claim 13, wherein:
- (i) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 305 and the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 442; or
 - (ii) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 306 and the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 443.

15. The expression cassette of claim 5, wherein the non-naturally occurring DNA binding protein comprises SEQ ID NO: 299.
16. The expression cassette of claim 15, wherein the nucleic acid sequence encoding the non-naturally occurring DNA binding protein comprises SEQ ID NO: 452.
17. The expression cassette of any one of claims 6-16, wherein the expression cassette further comprises a parvalbumin (PV) selective regulatory element situated upstream of the sequence encoding the non-naturally occurring DNA binding protein.
18. The expression cassette of claim 17, wherein the PV selective regulatory element comprises SEQ ID NO: 183.
19. The expression cassette of claim 17 or 18, wherein the expression cassette further comprises a polyA sequence positioned downstream of the polynucleotide encoding the non-naturally occurring DNA binding protein.
20. The expression cassette of claim 19, wherein the polyA sequence comprises SEQ ID NO: 326 or 327.
21. The expression cassette of claim 18, wherein:
- (i) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 313, wherein expression cassette comprises SEQ ID NO: 356 or 362;
 - (ii) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 315, wherein the expression cassette comprises SEQ ID NO: 359;
 - (iii) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 316, wherein the expression cassette comprises SEQ ID NO: 357;
 - (iv) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 305, wherein the expression cassette comprises SEQ ID NO: 353;

(v) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 306, wherein the expression cassette comprises SEQ ID NO: 354 or 355; or

(iv) the non-naturally occurring DNA binding protein comprises SEQ ID NO: 299, wherein the expression cassette comprises SEQ ID NO: 363.

22. An expression vector comprising the expression cassette of any one of claims 1 to 21.

23. The expression vector of claim 22, wherein the expression vector is a viral vector.

24. The expression vector of claim 23, wherein the viral vector is an adeno-associated virus (AAV) vector.

25. The expression vector of claim 24, wherein the AAV vector is AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, or scAAV9.

26. The expression vector of claim 24 or 25, wherein the expression cassette further comprises a 5' AAV inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence.

27. The expression vector of claim 26, wherein the 5' AAV ITR sequence and the 3' AAV ITR sequence are selected from the group consisting of AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, and scAAV9 ITR sequences.

28. A viral particle comprising the expression cassette of any one of claims 1 to 21 or the expression vector of any one of claims 22 to 27.

29. The viral particle of claim 28, wherein the viral particle comprises capsid proteins of an AAV.

30. The viral particle of claim 29, wherein the AAV is an AAV1, AAV2, AAV5, AAV8 or AAV9.

31. A host cell comprising the expression cassette of any one of claims 1 to 21 or the expression vector of any one of claims 22 to 27.
32. A pharmaceutical composition comprising the expression cassette of any one of claims 1 to 21, the expression vector of any one of claims 22 to 27, or the viral particle of any one of claims 28 to 30, and one or more pharmaceutically acceptable excipients.
33. Use of the expression cassette of any one of claims 6 to 21, in the manufacture of a medicament for treating a central nervous system disorder.
34. The use of claim 33, wherein the expression cassette is an expression vector.
35. The use of claim 34, wherein the expression vector is a viral vector.
36. The use of claim 35, wherein the viral vector is an adeno-associated virus (AAV) vector.
37. The use of claim 36, wherein the AAV vector is AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, or scAAV9.
38. The use of claim 36 or 37, wherein the expression cassette further comprises a 5' AAV inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence.
39. The use of claim 38, wherein the 5' AAV ITR sequence and the 3' AAV ITR sequence are selected from AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, or scAAV9 ITR sequences.
40. The use of any one of claims 35 to 49, wherein the viral vector comprises a viral particle.
41. The use of claim 40, wherein the viral particle comprises capsid proteins of an AAV.

42. The use of claim 41, wherein the AAV is an AA1, AAV2, AAV5, AAV8 or AAV9.
43. The use of any one of claims 33 to 42, wherein the subject is a mammal.
44. The use of claim 43, wherein the subject is a primate.
45. The use of claim 44, wherein the primate is a non-human primate.
46. The use of claim 45, wherein the non-human primate is an old-world monkey, an orangutan, a gorilla, a chimpanzee, a marmoset, a crab-eating macaque, a rhesus macaque or a pig-tailed macaque.
47. The use of claim 44, wherein the primate is a human.
48. The use of any one of claims 33 to 47, wherein the central nervous system disorder is epilepsy associated with a SCN1A haploinsufficiency.
49. The use of claim 48, wherein the central nervous system disorder is Dravet Syndrome.
50. The use of claim 49, wherein a symptom of the central nervous system disorder is neuronal hyperactivity.
51. The use of claim 50, wherein treatment of the central nervous system disorder comprises reduction of neuronal hyperactivity.
52. The use of any one of claims 33 to 51, wherein a symptom of the central nervous system disorder is seizures.

53. The use of claim 52, wherein treatment of the central nervous system disorder comprises reduction of the frequency and/or severity of seizures.
54. The expression cassette of any one of claims 6 to 21, for use in therapy.
55. The expression cassette of claim 54, wherein the expression cassette is an expression vector.
56. The expression cassette of claim 55, wherein the expression vector is a viral vector.
57. The expression cassette of claim 56, wherein the viral vector is an adeno-associated virus (AAV) vector.
58. The expression cassette of claim 57, wherein the AAV vector is AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, or scAAV9.
59. The expression cassette of claim 57 or 58, wherein the expression cassette further comprises a 5' AAV inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence.
60. The expression cassette of claim 59, wherein the 5' AAV ITR sequence and the 3' AAV ITR sequence are selected from AAV1, AAV2, AAV5, AAV8, AAV9, scAAV1, scAAV2, scAAV5, scAAV8, or scAAV9 ITR sequences.
61. The expression cassette of any one of claims 56 to 60, wherein the viral vector comprises a viral particle.
62. The expression cassette of claim 61, wherein the viral particle comprises capsid proteins of an AAV.
63. The expression cassette of claim 62, wherein the AAV is an AA1, AAV2, AAV5, AAV8 or AAV9.