

Claims

1. A construct comprising:

- a promoter;
- 5 - at least one non-mammalian or synthetic miRNA is expressed within an intron, wherein the synthetic miRNA is a sequence which is not naturally occurring;
- a transgene;
- 10 - at least one non-mammalian or synthetic miRNA binding site(s) which provides for control of the expression of the transgene, wherein the synthetic miRNA binding site(s) is a sequence which is not naturally occurring; and
- a polyadenylation signal.

2. A construct of claim 1 wherein the miRNA binding site(s) which provide for control of the expression of the transgene is provided within the 3' UTR, or the 5' UTR.

3. A construct of claim 1 wherein the non-mammalian or synthetic miRNA binding site(s) is provided within the transgene.

4. A construct as claimed in any preceding claim wherein the construct provides a single gene circuit to provide a relatively fixed level of expression of the transgene across cells receiving different levels of vector-derived transgene (i.e. dosage-insensitivity).

5. A construct as claimed in any preceding claim, wherein the at least one synthetic or non-mammalian miRNA exhibits no off-target binding effects.

6. A construct as claimed in any preceding claim wherein the non-mammalian or synthetic miRNA is expressed in an intron provided by

human_EF1a_intron_A (SEQ ID NO: 5)

gtaagtgccgtgtgtgtgttcccgcgggctggcctctttacgggttatggcccttgcgtgcctgaattacttccacgcccc
 tggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggag
 ccccttcgcctcgctgcttgagttgaggcctggcttgggcgctggggccgcccgtgcgaatctggtggcaccttcgcgc
 ctgtctcgctgctttcgataagtcttagccatttaaaattttgatgacctgctgcgacgctttttctggcaagatagcttgt

aaatgcgggccaagatctgcacactggtatttcggttttggggccgcgggcgacggggcccgtgctcccagc
 gcacatgttcggcgaggcggggcctgcgagcgcgccaccgagaatcggacgggggtagtctcaagctggccgg
 cctgctctggtgcctggcctcgcgccgccgtgtatcgccccgccctgggcggaaggctggcccggtcggcaccagt
 tgcgtgagcggaaagatggccgctcccggccctgctgcagggagctcaaaatggaggacgcggcgctcgggag
 5 agcggggcgggtgagtcacccacacaaaggaaaagggccttccgtcctcagccgtcgcttcatgtgactccacgga
 gtaccggggcgccgtccaggcacctcgattagttctcgagcttttgagtagctcgtcttaggtggggggaggggttta
 tgcgatggagtttccccacactgagtggtggagactgaagttaggccagcttggcacttgatgaattctccttgaatt
 tgcccttttgagtttggtccttggtcattctcaagcctcagacagtggttcaaagtttttcttcatttcag

>MINIX_artificial_intron (SEQ ID NO: 6)

10 Gtaagagcctagcatgtagaactggtacctgcagcccaagcttgctgcacgtctagggctcaccgggttccttgatg
 aggtaccgacatacttatcctgtccctttttccacag

7. A construct as claimed in any preceding claim wherein the miRNA is non-
 mammalian miRNA derived from an insect miRNA, optionally wherein the miRNA is
 15 capable of specifically binding to firefly luciferase (ffluc1) miRNA binding site.

8. A construct as claimed in any preceding claim wherein there are a plurality of
 miRNA binding sites provided in the construct, optionally three miRNA binding sites,
 at least four miRNA binding sites, at least five miRNA binding sites, at least six
 20 miRNA binding sites.

9. A construct as claimed in any preceding claims wherein there are a plurality of
 non-mammalian or synthetic miRNAs expressed in a construct.

25 10. A construct as claimed in any preceding claim wherein the non-mammalian firefly
 luciferase miRNA is a sequence selected from

>ffluc1 (SEQ ID NO:9)

tgtttgaatgaggcttcagtagttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa
 cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaacgatatgggctgaatacaatagtagc
 30 cacagatgtattgtattcagccatcgtgtgcctactgcctcggacttcaaggggctagaattcgagcaattatctgtt
 tactaaaactgaataccttgctatctcttgatacttttcaaaagctgaattaaaatgtataaattaaatcacttt

>ffluc9 (SEQ ID NO: 10)

tgtttgaatgaggcttcagtagttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa
 cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaacgtgaattgctcaacagtatagtagc

acagatgtatactgttgagcaattcacgttgctactgcctcggacttcaaggggctagaattcgagcaattatctgtt
tactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>ffluc18 (SEQ ID NO: 11)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
5 cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgacccgacgatgacgccggtgaatagtgaag
ccacagatgtattcaccggcgatcgtcggtgcctactgcctcggacttcaaggggctagaattcgagcaattatc
ttgttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>ffluc22 (SEQ ID NO: 12)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
10 cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgattcgatagggacaagacaatttagtgaagcc
acagatgtaaattgtctgtccctatcgaagtcctactgcctcggacttcaaggggctagaattcgagcaattatctgtt
tactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt.

11. A construct as claimed in any of claims 1 to 6 or 8 to 9 wherein the synthetic
15 miRNA is a sequence selected from

>novel_seq_1 (SEQ ID NO: 13)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgagcatgttacgggacttctattagtgaagcca
cagatgtataagaagtcggtaacatgcgtgcctactgcctcggacttcaaggggctagaattcgagcaattatcttg
20 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>novel_seq_2 (SEQ ID NO: 14)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgagtgaggagcagcgatcttaatagtgaagc
cacagatgtattaagatccgctgctcctcacgtgcctactgcctcggacttcaaggggctagaattcgagcaattatctt
25 gttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>novel_seq_3 (SEQ ID NO: 15)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgagtcacgtcacggaacttatagtgaagcc
acagatgtataagttccgtgacgacatgacgtgcctactgcctcggacttcaaggggctagaattcgagcaattatctt
30 gttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>novel_seq_4 (SEQ ID NO: 16)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttctaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgagagaagtcggatttcgtatttagtgaagcc
acagatgtaaatacgaatccgcacttctcgtgcctactgcctcggacttcaaggggctagaattcgagcaattatcttg
35 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggtataaattaaatcacttt

>novel_seq_5 (ran1g) (SEQ ID NO: 17)

tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaagcgccaaaggagctgtgatagtgaagc
cacagatgtatcacagactccttggcgcttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatcttg
5 ttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt

>novel_seq_6 (ran2g) (SEQ ID NO: 18)

tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaagtgcggaatttcgtatttgctagtgaagcca
cagatgtagcaaatacgaaatccgcacttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatcttg
10 ttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt

>novel_seq_7 (SEQ ID NO: 19)

tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaagtggaatgcatgcatgattgctagtgaagcc
acagatgtagcaatcgcatcgcatccacttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatcttg
15 ttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt

>novel_seq_8 (SEQ ID NO: 20)

Tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaacggatccgcaacttgcatagtgaagc
cacagatgtatcgcaagttgaggataccgttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatctt
20 gtttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt.

12. A construct as claimed in any one of the preceding claims wherein the synthetic
miRNA is targeted against the coding sequence of a target gene and is selected
from

>MECP2_coding_1 (SEQ ID NO: 21)

tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaagtgatgtttacttgatctagtgaagcca
cagatgtagatcaagtaaacatcgacttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatcttg
tactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt

>MECP2_coding_2 (SEQ ID NO: 22)

tgtttgaatgaggcttcagactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgacttcttaa
cccaacagaaggctcgagaaggatatattgctgttgacagtgagcgaaagccgctcttggtctctacctagtgaagcca
cagatgtaggtagagaccaagagcggttgctgcctactgcctcggaactcaaggggctagaattcgagcaattatctt
gtttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaattaaatcacttt

>MECP2_coding_3 (SEQ ID NO: 23)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgaaagtccgaagatcaagacctgtagtgaagc
 cacagatgtacaggtcttgatcttcggacttgctgcctactgcctcggacttcaaggggctagaattcgagcaattatctgt
 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

5 >MECP2_coding_4 (SEQ ID NO: 24)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgagagtccagtatacgagtgatagtgaagcc
 acagatgtatacactgcgtatactggactcgctgcctactgcctcggacttcaaggggctagaattcgagcaattatctgt
 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

10 > MECP2_coding_5 (SEQ ID NO: 25)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgagagtccagtatacgagtgatagtgaagcc
 acagatgtatgtacactgcgtatactggacgtgcctactgcctcggacttcaaggggctagaattcgagcaattatctgt
 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

15 > MECP2_coding_6 (SEQ ID NO: 26)

Tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgagactcgggaaaccgttagtattagtgaagcc
 acagatgtaataactaacggttcccgagtcgtgcctactgcctcggacttcaaggggctagaattcgagcaattatctgt
 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

20 >SYNGAP1_coding_1 (SEQ ID NO: 27)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgagcgccctaagtccagcataaatagtgaagc
 cacagatgtattatgctggacttaaggcgctgcctactgcctcggacttcaaggggctagaattcgagcaattatctt
 gttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

25 > SYNGAP1_coding_2 (SEQ ID NO: 28)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgagaggttctgtttcgaagtacttagtgaagccac
 agatgtaagtaacttcgaaacagaactcgctgcctactgcctcggacttcaaggggctagaattcgagcaattatctgtt
 tactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

30 > SYNGAP1_coding_3 (SEQ ID NO: 29)

tgtttgatgaggcttcagtactttacagaatcggtgcctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaagggtatattgctgttgacagtgagcgaggcgagcacttcgagttcaattagtgaagcc
 acagatgtaattgaactcgaagtgcctgcctactgcctcggacttcaaggggctagaattcgagcaattatctgtt
 ttactaaaactgaataccttgctatctctttgatacattttacaaagctgaattaaaatggataaaattaatcacttt

35 > SYNGAP1_coding_4 (SEQ ID NO: 30)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaagatccgaatctagcagaaatagtgaaagc
 cacagatgtatttctgctagattcggatcttgtgcctactgcctcggacttcaaggggctagaattcgagcaattatcttgt
 tactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt

5 > SYNGAP1_coding_5 (SEQ ID NO: 31)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaaggctgaaacagccactgttagtgaaagc
 cacagatgtaacagtggctgtttacgaccttgtgcctactgcctcggacttcaaggggctagaattcgagcaattatcttg
 ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt

10 > SYNGAP1_coding_6 (SEQ ID NO: 32)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttgaaacacttgctgggattacttcgacttcttaa
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaaggagaaatacactctctgtagtgaaagc
 cacagatgtatcagagagtgtatttcttctgtgcctactgcctcggacttcaaggggctagaattcgagcaattatcttgt
 ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt

15

13. A construct as claimed in any one of the preceding claims wherein the non-mammalian or synthetic miRNA binding site is selected from

>ffluc1_x1_binding_site (SEQ ID NO: 33)

gctatgaaacgatatgggctgaatacaaatcacag

20 >ffluc1_x 3_binding_sites (SEQ ID NO: 34)

gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg
 aaacgatatgggctgaatacaaatcacag

>ffluc1_x6_binding_sites (SEQ ID NO: 35)

gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg
 25 aaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatgaaacg
 atatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacag

>ffluc1_x3_binding_sites_1bp_central_mismatch (SEQ ID NO: 36)

gctatgaaacgatatgggcggaatacaaatcacaggctatgaaacgatatgggcggaatacaaatcacaggctat
 gaaacgatatgggcggaatacaaatcacag

30 > ffluc1_x3_binding_sites_3bp_central_mismatch (SEQ ID NO: 37)

gctatgaaacgatatgttatgaatacaaatcacaggctatgaaacgatatgttatgaatacaaatcacaggctatgaa
 acgatatgttatgaatacaaatcacag

>ffluc1_x3_binding_sites_3'_mismatch (SEQ ID NO: 38)

gctatgacccaaactgtgaagaatacaaatcacaggctatgagtgctatcacccgaatacaaatcacaggctatga
 35 ctaggcccgtttcgaatacaaatcacag

>ffluc1_x3_binding_sites_mutant_1 (SEQ ID NO: 39)
gctatgaaacgatatgcgctgaatacaaatcacaggctatgaaacgatatgcgctgaatacaaatcacaggctatga
aacgatatgcgctgaatacaaatcacag

>ffluc1_binding_sites_mutant_2 (SEQ ID NO: 40)
5 gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg
aaacgatatgggctgaatacaaatcacag

>ffluc1_x3_binding_sites_mutant_3 (SEQ ID NO: 41)
gctatgaaacgatatgggctcaatacaaatcacaggctatgaaacgatatgggctcaatacaaatcacaggctatga
aacgatatgggctcaatacaaatcacag

10 >ffluc1_x3_binding_sites_mutant_4 (SEQ ID NO: 42)
gctatgaaacgatatgggctgattacaaatcacaggctatgaaacgatatgggctgattacaaatcacaggctatga
aacgatatgggctgattacaaatcacag

>ffluc1_x3_binding_sites_mutant_5 (SEQ ID NO: 43)
gctatgaaacgatatgggctgaattcaaatcacaggctatgaaacgatatgggctgaattcaaatcacaggctatga
15 aacgatatgggctgaattcaaatcacag

>ffluc1_x3_binding_sites_mutant_6 (SEQ ID NO: 44)
Gctatgaaacgatatgggctgaataactaatcacaggctatgaaacgatatgggctgaataactaatcacaggctatga
aacgatatgggctgaataactaatcacag

>ffluc9_x3_binding_sites (SEQ ID NO: 45)
20 gctatgaaacgtgaattgctcaacagtaatcacaggctatgaaacgtgaattgctcaacagtaatcacaggctatgaa
acgtgaattgctcaacagtaatcacag

>ffluc18_x3_binding_sites (SEQ ID NO: 46)
gctatgacccgacgatgacgccggtgaaatcacaggctatgacccgacgatgacgccggtgaaatcacaggctat
gacccgacgatgacgccggtgaaatcacag

25 >ffluc22_x3_binding_sites (SEQ ID NO: 47)
gctatgattcgatagggacaagacaattatcacaggctatgattcgatagggacaagacaattatcacaggctatgat
tcgatagggacaagacaattatcacag

>Novel_seq_1_3x_binding_sites (SEQ ID NO: 48)
gctatgagcatgttacgggacttcttatatcacaggctatgagcatgttacgggacttcttatatcacaggctatgagcat
30 gttacgggacttcttatatcacag

>Novel_seq_2_3x_binding_sites (SEQ ID NO: 49)
gctatgagtgaggagcagcggtcttaaatcacaggctatgagtgaggagcagcggtcttaaatcacaggctatg
agtgaggagcagcggtcttaaatcacag

>Novel_seq_3_3x_binding_sites (SEQ ID NO: 50)

gctatgagtcatgtcgtcacggaacttaatcacaggctatgagtcatgtcgtcacggaacttaatcacaggctatgagtc
catgtcgtcacggaacttaatcacag

>Novel_seq_4_3x_binding_sites (SEQ ID NO: 51)

gctatgagagaagtgcggtatttcgtattatcacaggctatgagagaagtgcggtatttcgtattatcacaggctatgaga
5 gaagtgcggtatttcgtattatcacag

>Novel_seq_5_3x_binding_sites (ran1g binding sites) (SEQ ID NO: 52)

gctatgaaagcgccaaaggagtgctgtgaatcacaggctatgaaagcgccaaaggagtgctgtgaatcacaggctatg
aaagcgccaaaggagtgctgtgaatcacag

>Novel_seq_6_3x_binding_sites (ran2g binding sites) (SEQ ID NO: 53)

10 gctatgaaagtgcggtatttcgtatttgcacacaggctatgaaagtgcggtatttcgtatttgcacacaggctatgaaagt
gcggtatttcgtatttgcacacag

>Novel_seq_7_3x_binding_sites (SEQ ID NO: 54)

gctatgaaagtggtgcatgcatgcatcacaggctatgaaagtggtgcatgcatgcatcacaggctatga
aagtggtgcatgcatgcatcacag

15 >Novel_seq_8_3x_binding_sites (SEQ ID NO: 55)

gctatgaaacggtatccgcaacttgcaatcacaggctatgaaacggtatccgcaacttgcaatcacaggctatga
aacggtatccgcaacttgcaatcacag

20 14. A construct as claimed in claim 12 wherein the synthetic miRNA is targeted
against the coding sequence of a target gene and the synthetic miRNA binding site
is selected from

>MECP2_coding_1_binding_site (SEQ ID NO: 56)

aagtacgatgtttacttgatc

> MECP2_coding_2_binding_site (SEQ ID NO: 57)

25 aagccgctcttggtctctacc

> MECP2_coding_3_binding_site (SEQ ID NO: 58)

aagtccgaagatcaagacctg

> MECP2_coding_4_binding_site (SEQ ID NO: 59)

gagtccagtatacgagtgta

30 > MECP2_coding_5_binding_site (SEQ ID NO: 60)

gtccagtatacgaggtaca

> MECP2_coding_6_binding_site (SEQ ID NO: 61)

gactcgggaaaccgtagtat

>SYNGAP1_coding_1_binding_site (SEQ ID NO: 62)

35 gcgccttaagtccagcataaa

> SYNGAP1_coding_2_binding_site (SEQ ID NO: 63)

gagttctgtttcgaagttact

> SYNGAP1_coding_3_binding_site (SEQ ID NO: 64)

ggcgagcacttcgagttcaat

5 > SYNGAP1_coding_4_binding_site (SEQ ID NO: 65)

Aagatccgaatctagcagaaa

> SYNGAP1_coding_5_binding_site (SEQ ID NO: 66)

aaggctgtaaacagccactgt

> SYNGAP1_coding_6_binding_site (SEQ ID NO: 67)

10 aagaggaaatacactctctga

15. A construct as claimed in any preceding claim wherein the promoter is selected from a constitutive or conditional promoter, optionally wherein the promoter is tissue specific.

15

16. A construct as claimed in any preceding claim wherein the promoter is selected from >EF1a_promoter (SEQ ID NO: 68)

Agtaattcatacaaaaaggactcgcccctgccttggggaatcccaggaccgtcgtaaactcccactaacgtagaac

ccagagatcgctgcgttccccgccccctcaccgccccgctctcgtcatcactgaggtggagaagagcatgcgtgaggc

20 tccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttggggggaggggtcggcaattga

accggtgcctagagaaggtggcgcggggtaaactgggaaagtgtgtcgtgtactgggtccgccttttcccgaggggt

gggggagaaccgtatataagtgcagtagtcgctgaacgttcttttcgcaacggggttgccgccagaacacag

>Jet_promoter (SEQ ID NO: 69)

gggcggagttagggcggagccaatcagcgtgcgccgtccgaaagttgcctttatggctgggcgggagaatgggcg

25 gtgaacgccgatgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggatttgggtcgcgg

ttctgtttgt

17. A construct as claimed in any preceding claim wherein the polyA sequence is selected from

30 >sv40 polyA signal (SEQ ID NO: 70)

Aactgtttattgcagcttataatggttacaataaagcaatagcatcacaaattcacaaataaagcattttttcactgc

attctagttgtggtttgtccaaactcatcaatgtatctta

>BGH polyA signal(SEQ ID NO: 71)

Ctgtgccttctagttgccagccatctgtgtttgccccctccccgtgccttccttgaccctggaagggtgccactcccactgtc
ctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggtgggggtggggcagga
cagcaagggggaggattgggaagacaatagcaggcatgtggggatgcggtgggctctatgg, and
>SpA (SEQ ID NO: 72)

5 Aataaagagctcagatgcatcgatcagagtgtgtgtttttgtgtg

18. The construct of claims 1-17, further comprising a stability element, wherein the stability element is located in the 3'UTR.

10 19. The construct of claim 18, wherein the stability element is selected from
> Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE)
(SEQ ID NO: 74)

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATG
TTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTAT
15 TGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT
CTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGT
GTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCC
TTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACATCGCCG
CCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCC
20 GTGGTGTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCC
ACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCC
AGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGT
CTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGC
> a truncated version of WPRE (WPRE3) (SEQ ID NO: 75)

25 ATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTAT
GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTA
TTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTAGTTCTT
GCCACGGCGGAACATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTC
GGCTGTTGGGCACTGACAATTCCGTGGT.

30

20. The construct of any preceding claim, wherein the construct further comprises ,
the Kozak sequence GCCACCATGG (SEQ ID NO: 73).

21. The construct of any preceding claim, wherein the miRNA binding site has been
35 designed to partially ameliorate miRNA binding.

22. A vector comprising a construct of any one of claims 1 to 21.

23. The vector of claim 22, wherein the vector is an AAV or lentiviral vector,
optionally wherein the vector is an AAV vector, optionally wherein the construct is
operably linked to expression control elements, and the expression control elements
and the construct are together flanked by 5' and 3' AAV inverted terminal repeats
(ITR).

24. The vector of any one of claims 22-23, packaged into a virion, optionally wherein
the vector when packaged into the virion does not affect the quality of the construct.

25. The vector of any one of claims 22-23, formulated in a nanoparticle.

26. A method of using a construct of any one of claims 1 to 21 or a vector of claim 22
to 25 to express a transgene, optionally to express a transgene in a specific
mammalian cell type or types.

27. A construct of any one of claims 1 to 21 or a vector of any one of claims 22 to 25
for use as a medicament.

28. Use of a composition comprising a construct of any one of claims 1 to 21 or a
vector of any one of claims 22 to 25 in the preparation of a medicament for the
treatment of a disorder caused by insufficient expression of a gene in a subject.

29. The use as claimed in claim 28 wherein the disorder is any monogenic disorder
in which controlled expression of the corrective gene is desired, optionally wherein
the monogenic disorder is selected from the group consisting of Rett Syndrome,
Fragile X syndrome, Angelman syndrome, Syngap-related intellectual disability,
CDKI5 deficiency, Fredrich's ataxia, Spinal muscular dystrophy, Haemophilia and
Diabetes.

30. The use as claimed in claim 28 wherein the disorder is treated by expression of a
gene selected from the list comprising: PRKCZ, TTC34, PRDM16, ARHGEF16,
PARK7, PRDM2, IGSF21, PTCH2, NFIA, ST6GALNAC3, DPYD, COL11A1, PDZK1,

GPR89A, NBPFF11, GPR89B, KCNT2, CFHR2, ASPM, PTPRC, GPATCH2,
 DUSP10, GPR137B, RYR2, CHRM3, RGS7, AKT3, KIF26B, SMYD3, LPIN1,
 EPCAM, MSH2, NRXN1, XPO1, LRP1B, ZEB2, ACVR2A, MBD5, KIF5C, SCN1A,
 COL3A1, PMS1, PLCL1, SATB2, PARD3B, EPHA4, SPHKAP, CHL1, GRM7,
 5 TRANK1, DOCK3, FAM19A1, FOXP1, ROBO1, CADM2, FOXL2, SOX2, LPP,
 RASGEF1B, GRID2, FAT4, NR3C2, LRBA, FGA, GALNTL6, WWC2, TLR3, IRX2,
 IRX1, CDH12, CDH9, NIPBL, HEXB, MEF2C, GRAMD3, FBN2, PRELID2, TCOF1,
 GABRG2, MSX2, NSD1, FOXC1, CDYL, TBC1D7, RUNX2, MUT, RIMS1, NKAIN2,
 LAMA2, ARID1B, PARK2, PACRG, QKI, TNRC18, FBXL18, SUGCT, GLI3, AUTS2,
 10 MLXIPL, COL1A2, PPP1R9A, CFTR, TSPAN12, GRM8, CNTNAP2, MNX1, CSMD1,
 MCPH1, LPL, ANK1, IMPAD1, CHD7, VCP1P1, TRPS1, PARP10, DOCK8, KANK1,
 GLIS3, PTPRD, MLLT3, ROR2, PTCH1, AL162389.1, ARRDC1, EHMT1, PCDH15,
 CTNNA3, ADK, BMPR1A, PAX2, BTRC, INPP5A, MRPL23, ELP4, PAX6, CPT1A,
 DYNC2H1, KIRREL3, WNK1, CACNA1C, PPFIBP1, TBX5, MED13L, NALCN,
 15 CHD8, MYH7, TTC6, DAAM1, NRXN3, MTA1, SNRPN, UBE3A, OCA2, HERC2,
 CHRFAM7A, ARHGAP11B, OTUD7A, FBN1, HEXA, SNUPN, NRG4, AC112693.2,
 IGF1R, LRRC28, HBA2, HBQ1, CREBBP, RBFOX1, CDR2, CDH13, CYBA, NXN,
 YWHAE, SMG6, METTL16, PAFAH1B1, ADORA2B, NT5M, RAI1, NF1, C17orf67,
 PITPNC1, ACOX1, TCF4, DOCK6, CACNA1A, LPHN1, ZSCAN5A, BMP2, MYT1,
 20 PEX26, USP18, DGCR6L, USP41, UBE2L3, NF2, LARGE, BRD1, SHANK3 CDKL5,
 FXN, SMN1, F8 and INS.