CLAIMS

What is claimed is:

1. A polynucleotide comprising

a) a segment encoding a multi-hairpin amiRNA sequence, wherein the segment comprises:

i) a first guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 21 nucleotides that is perfectly complementary to a first target site ~~of~~ in a natural mammalian cellular mRNA ~~of~~ encoded by SEQ ID NO:21 and a first passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78% complementary to the first guide strand sequence, wherein the first guide strand and first passenger strand sequence are separated by between 5 and 35 nucleotides;

ii) a second guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 21 nucleotides that is perfectly complementary to a second target site~~r~~e different than the first target site in the same natural mammalian cellular mRNA as the first guide strand sequence and a second passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78% complementary to the second guide strand sequence, wherein the second guide strand and second passenger strand sequences are separated by between 5 and 35 nucleotides, and wherein the first and second guide strand sequences are different from each other; and

b) a eukaryotic promoter that is active in a mammalian cell and is transcribed by RNA polymerase II or RNA polymerase III operably linked to the segment encoding the amiRNA sequence, wherein the amiRNA sequence can be expressed and fold into multiple hairpins;

wherein the first and second guide strand sequences are selected ~~from~~ to comprise any one of SEQ ID NOs: 114-129.

2. The polynucleotide of claim 1, wherein the first guide strand sequence is a ~~19~~ 21 or 22 nucleotide sequence perfectly complementary to the natural mammalian cellular mRNA and the first passenger strand sequence has the same length as the first guide sequence.

3. The polynucleotide of claim 1, wherein the first guide strand sequence is a ~~19~~ 21 or 22 nucleotide sequence perfectly complementary to the natural mammalian cellular mRNA and the first passenger strand sequence is shorter than the first guide sequence.
4. The polynucleotide of claim 1, wherein the first and second target sites do not overlap.

5. The polynucleotide of claim 1, wherein the segment encoding the multi-hairpin amiRNA sequence further comprises a third guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is perfectly complementary to the same natural mammalian cellular mRNA as the first and second guide strand sequences and a third passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78% complementary to the third guide strand sequence, wherein the third guide strand and third passenger strand sequences are separated by between 5 and 35 nucleotides, and wherein the first, second and third guide strand sequences are different from each other.

6. The polynucleotide of claim 1, further comprising two transposon ends flanking the segment and the promoter, wherein the segment and the promoter are transposable by a corresponding transposase.

7. The polynucleotide of claim 6, wherein each transposon end comprises a sequence selected from SEQ ID ~~NOs~~NOS:~~-~~1004 and 1005, or from SEQ ID ~~NOs~~NOS:~~-~~1010 and 1011, or from SEQ ID ~~NOs~~NOS:~~-~~1014 and 1015, or from SEQ ID ~~NOs~~NOS: 1016 and 1017, or from SEQ ID ~~NOs~~NOS:~~-~~1022 and 1023, or from SEQ ID ~~NOs~~NOS:~~-~~1026 and 1027, or from SEQ ID ~~NOs~~NOS:~~-~~1030 and 1031, or from SEQ ID ~~NOs~~NOS:~~-~~1036 and 1037, or from SEQ ID ~~NOs~~NOS:~~-~~1040 and 1041, or from SEQ ID ~~NOs~~NOS:~~-~~1044 and 1045, or from SEQ ID ~~NOs~~NOS:~~-~~1112 and 1113.

8. The polynucleotide of claim 1, wherein the polynucleotide comprises SEQ ID NO: 741.

9. The polynucleotide of claim 1, wherein each of the guide strand sequences and its passenger strand sequence are selected from any of the following sequence pairs: SEQ ID NOS:114 and 418 respectively, SEQ ID NOS:115 and 419 respectively, SEQ ID NOS:117 and 421 respectively, SEQ ID NOS:118 and 422 respectively, SEQ ID NOS:119 and 423 respectively, SEQ ID NOS:120 and 424 respectively, SEQ ID NOS:121 and 425 respectively, SEQ ID NOS:122 and 426 respectively, SEQ ID NOS:123 and 427 respectively, SEQ ID NOS:124 and 428 respectively, SEQ ID NOS:125 and 429 respectively, SEQ ID NOS:126 and 430 respectively, SEQ ID NOS:127 and 431 respectively, SEQ ID NOS:128 and 432 respectively, SEQ ID NOS:129 and 433 respectively, or SEQ ID NOS:116 and 420 respectively.

~~9.~~10. A mammalian cell comprising the polynucleotide of claim 1 integrated into its genome.

~~10.~~11. The mammalian cell of claim ~~9~~10, wherein the multi-hairpin amiRNA sequence is expressed and inhibits expression of the natural cellular mRNA, whereby the cell requires exogenous provision of glutamine in order to grow.

~~11.~~12. A mammalian cell comprising (a) the polynucleotide of any one of claims 1-9 integrated into its genome, wherein the multi-hairpin amiRNA sequence is expressed and inhibits expression of the natural cellular mRNA and (b) a second polynucleotide comprising

a gene encoding glutamine synthetase expressible in the mammalian cell, wherein expression of the gene compensates for the inhibition of the expression of the natural cellular mRNA, whereby the cell grows without the exogenous provision of glutamine.

~~12.~~13. The mammalian cell of claim ~~11~~12, wherein the second polynucleotide further comprises a second gene expressible in the mammalian cell.

~~13.~~14. A method of selecting for integration of a nucleic acid encoding a target protein into the genome of a cell comprising;

a) culturing a population of mammalian cells according to claim ~~10~~ 11 in the presence of glutamine required by the cell to grow due to inhibition of expression of the natural cellular mRNA by the multi-hairpin amiRNA sequence;

b) transfecting the population of cells with a second polynucleotide comprising a gene encoding a glutamine synthetase expressible in the mammalian cells and a second gene encoding the target protein, wherein expression of the glutamine synthetase compensates for the inhibition of the expression of the natural cellular mRNA thereby restoring capacity to grow without glutamine;

c) culturing the transfected cells with a reduced concentration or absence of the glutamine,

wherein transfected cells surviving culturing have integrated the second polynucleotide into their genomes and can thereby express the target protein.

~~14.~~15. The method of claim ~~13~~14, wherein the transfected cells are cultured with a downward tapering of the glutamine.

~~15.~~16. A polynucleotide comprising

a) a segment encoding a multi-hairpin amiRNA sequence, wherein the segment comprises

i) a first guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 21 nucleotides that is perfectly complementary to a first target site in a natural mammalian cellular mRNA ~~of~~ encoded by SEQ ID NO: 23 and a first passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78%

complementary to the first guide strand sequence, wherein the first guide strand and first passenger strand sequence are separated by between 5 and 35 nucleotides;

ii) a second guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 21 nucleotides that is perfectly complementary to a second target site different than the first target site in the same natural mammalian cellular mRNA as the first guide strand sequence and a second passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78% complementary to the second guide strand sequence, wherein the second guide strand and second passenger strand sequences are separated by between 5 and 35 nucleotides, and wherein the first and second guide strand sequences are different from each other; and

b) a eukaryotic promoter that is active in a mammalian cell and is transcribed by RNA polymerase II or RNA polymerase III operably linked to the segment encoding the amiRNA sequence, wherein the amiRNA sequence can be expressed and fold into multiple hairpins;

wherein the first and second guide strand sequences are selected ~~from~~ to comprise any one of SEQ ID NOS:130-142.

~~16.~~17. The polynucleotide of claim ~~15~~16, wherein the segment encoding the multi-hairpin amiRNA sequence further comprises a third guide strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is perfectly complementary to the same natural mammalian cellular mRNA as the first and second guide strand sequences and a third passenger strand sequence comprising a contiguous ~~19 nucleotide~~ sequence of at least 19 nucleotides that is at least 78% complementary to the third guide strand sequence, wherein the third guide strand and third passenger strand sequences are separated by between 5 and 35 nucleotides, and wherein the first, second and third guide strand sequences are different from each other.

~~17.~~18. The polynucleotide of claim ~~15~~16, further comprising two transposon ends flanking the segment and the promoter, wherein the segment and the promoter are transposable by a corresponding transposase.

~~18.~~19. The polynucleotide of claim ~~17~~18, wherein each transposon end comprises a sequence selected from SEQ ID ~~NOs~~NOS:~~-~~1004 and 1005, or from SEQ ID ~~NOs~~NOS:~~-~~1010 and 1011, or from SEQ ID ~~NOs~~NOS:~~-~~1014 and 1015, or from SEQ ID ~~NOs~~NOS: 1016 and 1017, or from SEQ ID ~~NOs~~NOS:~~-~~1022 and 1023, or from SEQ ID ~~NOs~~NOS:~~-~~1026 and 1027, or from SEQ ID ~~NOs~~NOS:~~-~~1030 and 1031, or from SEQ ID ~~NOs~~NOS:~~-~~1036 and 1037, or from SEQ ID ~~NOs~~NOS:~~-~~1040 and 1041, or from SEQ ID ~~NOs~~NOS:~~-~~1044 and 1045, or from SEQ ID ~~NOs~~NOS:~~-~~1112 and 1113.

20. The polynucleotide of claim 16, wherein each of the guide strand sequences and its passenger strand sequence are selected from any of the following sequence pairs: SEQ ID NOS:130 and 434 respectively, SEQ ID NOS:131 and 435 respectively, SEQ ID NOS:132 and 436 respectively, SEQ ID NOS:133 and 437 respectively, SEQ ID NOS:134 and 438 respectively, SEQ ID NOS:135 and 439 respectively, SEQ ID NOS:136 and 440 respectively, SEQ ID NOS:137 and 441 respectively, SEQ ID NOS:138 and 442 respectively, SEQ ID NOS:139 and 443 respectively, SEQ ID NOS:140 and 444 respectively, SEQ ID NOS:141 and 445 respectively, and SEQ ID NOS:142 and 446 respectively.

~~19.~~21. A mammalian cell comprising the polynucleotide of claim ~~15~~ 16 integrated into its genome.

~~20.~~22. The mammalian cell of claim ~~19~~21, wherein the multi-hairpin amiRNA sequence is expressed and inhibits expression of the natural cellular mRNA, wherebythe cell requires exogenous provision of glutamine in order to grow.

~~21.~~23. A mammalian cell comprising (a) the polynucleotide of claim ~~15~~ 16 integrated into its genome, wherein the multi-hairpin amiRNA sequence is expressed and inhibits expression of the natural cellular mRNA, and (b) a second polynucleotide comprising a gene encoding a glutamine synthetase expressible in the mammalian cell, wherein expression of the gene compensates for the inhibition of the expression of the natural cellular mRNA, whereby the cell grows without exogenous provision of glutamine.

~~22.~~24. A mammalian cell of claim ~~21~~23, wherein the second polynucleotide further comprises a second gene expressible in the mammalian cell.

~~23~~25. A method of selecting for integration of a nucleic acid encoding a target protein into the genome of a cell comprising;

a) culturing a population of mammalian cells according to claim ~~19~~ 21 in the presence of an enzyme, growth factor, nutrient or other molecule required by the cell to grow due to inhibition of expression of the natural cellular mRNA by the multi-hairpin amiRNA sequence;

b) transfecting the population of cells with a second polynucleotide comprising a gene encoding a selectable marker expressible in the mammalian cells and a second gene encoding the target protein, wherein expression of the selectable marker gene compensates for the inhibition of the expression of the natural cellular mRNA thereby restoring capacity to grow without the enzyme, growth factor, nutrient or other molecule;

c) culturing the transfected cells with a reduced concentration or absence of the enzyme, growth factor, nutrient or other molecule,

wherein transfected cells surviving culturing have integrated the second polynucleotide into their genomes and can thereby express the target protein.

~~24~~26. The method of claim ~~23~~25, wherein the transfected cells are cultured with a downward tapering of the enzyme, growth factor, nutrient or other molecule.