What is claimed

1. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising:

(i) a first strand comprising a nucleotide sequence from 5' to 3' which corrects or induces a mutation in a genomic DNA (gDNA) molecule in a cell and comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

2. The donor polynucleotide of claim 1, wherein the mutation is a substitution, missense, nonsense, insertion, deletion or frameshift mutation.

3. The donor polynucleotide of claim 1 or 2, wherein the mutation is in an exon.

4. The donor polynucleotide of claim 1 or 2, wherein the mutation is a substitution, insertion or deletion, and wherein the mutation is in an intron.

5. The donor polynucleotide of any one of claims 1-3, wherein the mutation is proximal to a splicing signal in a gDNA.

6. The donor polynucleotide of claim 5, wherein the mutation is proximal to a 3' splice site in a gDNA.

7. The donor polynucleotide of claim 5, wherein the mutation is proximal to a 5' splice site in a gDNA.

8. The donor polynucleotide of claim 4, wherein the mutation is in a splicing signal in a gDNA.

9. The donor polynucleotide of claim 8, wherein the mutation is in a 3' splice site in a gDNA.

10. The donor polynucleotide of claim 8, wherein the mutation is in a 5' splice site in a gDNA.

11. The donor polynucleotide of claim 8, wherein the mutation is in a polypyrimidine tract.

12. The donor polynucleotide of claim 8, wherein the mutation is in a branch point sequence.

13. The donor polynucleotide of any one of claims 1-3, or 5-7, wherein the mutation is protein-coding mutation.

14. The donor polynucleotide of any one of claims 1-13, wherein the mutation is associated with or causes a disease.

15. The donor polynucleotide of any one of claims 1-14, comprising an intronic sequence.

16. The donor polynucleotide of any one of claims 1, 2, 4, 8-12, or 14, comprising an intronic sequence which corrects the mutation.

17. The donor polynucleotide of any one of claims 1-16, comprising an exonic sequence.

18. The donor polynucleotide of any one of claims 1-17, wherein the one or more splicing signals is selected from the group consisting of:

(a) a natural or enhanced 3' splice site;
(b) a natural or enhanced 5' splice site;
(c) a polypyrimidine tract;
(d) a branch point;
(e) an exon splicing enhancer (ESE);
(f) an intron splicing enhancer (ISE);
(g) an exon splicing silencer (ESS);
(h) an intron splicing silencer (ISS); and
(i) a combination of any of (a)-(h).

19. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a natural or enhanced 3' splice site.

20. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a natural or enhanced 5' splice site.

21. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a polypyrimidine tract.

22. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a branch point.

23. The donor polynucleotide of claim 18, wherein the one or more splicing signals is an exon splicing enhancer (ESE).

24. The donor polynucleotide of claim 18, wherein the one or more splicing signals is an intron splicing enhancer (ISE).

25. The donor polynucleotide of claim 18, wherein the one or more splicing signals is an exon splicing silencer (ESS).

26. The donor polynucleotide of claim 18, wherein the one or more splicing signals is an intron splicing silencer (ISS).

27. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a combination comprising a natural or enhanced 3' splice site and a polypyrimidine tract.

28. The donor polynucleotide of claim 18, wherein the one or more splicing signals is a combination comprising a natural or enhanced 3' splice site, a polypyrimidine tract, and a branch point.

29. A donor polynucleotide comprising a non-replicative dsDNA molecule which corrects or induces a mutation in a gDNA molecule in a cell and comprising one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the donor polynucleotide is configured for bi-directional insertion into a DSB, wherein the donor polynucleotide comprises:

(i) a first strand comprising a nucleotide sequence from 5' to 3' comprising a first branch point sequence and a first nucleotide sequence which corrects the mutation in the gDNA; and

(ii) a second strand comprising a nucleotide sequence from 5' to 3' comprising a second branch point sequence and a second nucleotide sequence which corrects the mutation in the gDNA, wherein the second strand is complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, wherein when the donor polynucleotide is inserted into the DSB in a first orientation, (i) comprises a sense strand, thereby correcting the mutation and providing the one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in a second orientation, (ii) comprises a sense strand, thereby correcting the mutation and providing the one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA.

30. The donor polynucleotide of claim 29, wherein the first and second branch point sequences conforms to a branch point consensus sequence on either strand, wherein the nucleotide sequences of the first branch point sequence and second branch point sequence are complementary.

31. The donor polynucleotide of claim 30, wherein the branch point consensus sequence is YTNAY (SEQ ID NO: 49), wherein Y is a nucleotide comprising either a cytosine (C) or thymine (T) nucleobase, and wherein N is a nucleotide comprising a nucleobase selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C).

32. The donor polynucleotide of any one of claims 29-31, wherein the first branch point sequence is TATTAAC (SEQ ID NO: 50).

33. The donor polynucleotide of any one of claims 29-32, wherein the second branch point sequence is GTTAATA (SEQ ID NO: 51).

34. The donor polynucleotide of claims 29 or 31, wherein the second branch point sequence is TACTGAC (SEQ ID NO: 52).

35. The donor polynucleotide of any one of claims 29-34, comprising a second splicing signal comprising a polypyrimidine tract, wherein the first strand comprises a first polypyrimidine tract located

downstream of the first branch point sequence; and the second strand comprises a second polypyrimidine tract located downstream of the second branch point sequence.

36. The donor polynucleotide of claim 35, wherein the nucleotide sequence comprising the first and second polypyrimidine tracts each comprise nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), and wherein the nucleotide sequence is about 100%, about 90%-100%, or about 80%-90% pyrimidine nucleobases.

37. The donor polynucleotide of claims 35 or 36, wherein the nucleotide sequence comprising the polypyrimidine tract is TTTTTTTCT (SEQ ID NO: 53).

38. The donor polynucleotide of claims 35 or 36, wherein the nucleotide sequence comprising the polypyrimidine tract is TTTTTTTCTTTTT (SEQ ID NO: 54).

39. The donor polynucleotide of claims 35 or 36, wherein the nucleotide sequence comprising the polypyrimidine tract is CTTCTTCTCTTCTTCC (SEQ ID NO: 55).

40. The donor polynucleotide of any one of claims 35-39, wherein the first branch point sequence and the first polypyrimidine tract are adjacent to each other.

41. The donor polynucleotide of any one of claims 35-40, wherein the second branch point sequence and the second polypyrimidine tract are adjacent to each other.

42. The donor polynucleotide of any one of claims 35-41, comprising a third splicing signal, wherein the third splicing signal comprises a 3' splice site, wherein the first strand comprises a nucleotide sequence comprising a first 3' splice site located downstream of the first polypyrimidine tract; and wherein second strand comprises a nucleotide sequence comprising a second 3' splice site located downstream of the second polypyrimidine tract.

43. The donor polynucleotide of claim 42, wherein the first and second 3' splice sites comprise the nucleotide sequence YAG, and wherein Y is a nucleotide comprising a nucleobase selected from the group consisting of: thymine (T) and cytosine (C).

44. The donor polynucleotide of claims 42-43, comprising a coding sequence, wherein the first strand comprises a first coding sequence, wherein the second strand comprises a second coding sequence, wherein the first nucleotide sequence that corrects the mutation in the gDNA comprises the first coding sequence, wherein the second nucleotide sequence that corrects the mutation in the gDNA comprises the second coding sequence, wherein the first coding sequence is located downstream of the first 3' splice site, and wherein the second coding sequence is located downstream of the second 3' splice site.

45. The donor polynucleotide of claim 44, wherein the nucleotide sequence comprising the first and second coding sequences comprise nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C).

46. The donor polynucleotide of claims 44 or 45, wherein the coding sequence and/or splicing signals comprising (i) and (ii) are not identical or complementary to reduce self-annealing.

47. The donor polynucleotide of any one the preceding claims, comprising one or more delimiter sequences comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein the nucleotide sequence is about 1-40, about 1-30, about 1-20, about 1-15, about 1-10, about 30, about 20, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2 or 1 nucleotide(s) in length.

48. The donor polynucleotide of claim 47, wherein the one or more delimiter sequences is located between the first branch point sequence and the second branch point sequence.

49. The donor polynucleotide of claim 47, wherein the one or more delimiter sequences is located between the first branch point sequence and the first polypyrimidine tract.

50. The donor polynucleotide of claim 47, wherein the one or more delimiter sequences is located between the second branch point and the second polypyrimidine tract.

51. The donor polynucleotide of any one of claims 29-50, wherein the donor polynucleotide is configured for bi-directional insertion into the DSB, wherein, when the donor polynucleotide is inserted into the DSB in either orientation, the first splicing signal, optionally, the second splicing signal, optionally, the third splicing signal and coding sequence comprise a sense strand, thereby correcting the mutation and providing one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA.

52. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) which corrects or induces a mutation in a genomic DNA (gDNA) molecule in a cell and comprising one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the donor polynucleotide is configured for bi-directional insertion into a DSB, wherein the donor polynucleotide comprises:

(i) a first strand comprising from 5' to 3' a nucleotide sequence comprising a first 5' splice site and a first nucleotide sequence which corrects the mutation in the gDNA; and

(ii) a second strand comprising from 5' to 3' a nucleotide sequence comprising a second 5' splice site and a second nucleotide sequence which corrects the mutation in the gDNA, wherein the second strand is complementary to the first strand,

the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein, when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, wherein when the donor polynucleotide is inserted into the DSB in a first orientation, (i) comprises a sense strand, thereby correcting the mutation and providing the one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in a second orientation, (ii) comprises a sense strand, thereby correcting the mutation and providing the one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA.

53. The donor polynucleotide of claim 52, wherein the first strand comprises a first coding sequence, wherein the second strand comprises a second coding sequence, wherein the first coding sequence is located upstream of the first 5' splice site, and wherein the second coding sequence is located upstream of the second 5' splice site, and wherein the coding sequences in the first and second strand are not complementary (or comprise one, two, three, four or more mismatches) to reduce self-annealing.

54. The donor polynucleotide of claim 52 or 53, comprising a delimiter sequence comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein the nucleotide sequence is about 1-40, about 1-30, about 1-20, about 1-15, about 1-10, about 30, about 20, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2 or 1 nucleotide(s) in length.

55. The donor polynucleotide of claim 54, wherein the delimiter sequence is located between the first and second 5' splice sites.

56. The donor polynucleotide of any one of claims 52-55, wherein the donor polynucleotide is configured for bi-directional insertion into the DSB, wherein when the donor polynucleotide is inserted into the DSB in a first orientation, the first 5' splice site and first coding sequence comprise a sense strand, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, and wherein when the donor polynucleotide is inserted into the DSB in a second orientation, the second 5' splice site and second coding sequence comprise a sense strand, thereby correcting the mutation and providing one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA.

57. A donor polynucleotide comprising a non-replicative dsDNA molecule comprising

(i) a first strand comprising a nucleotide sequence from 5' to 3' which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand, wherein the one or more splicing signals is selected from the group consisting of:

(a) a natural or enhanced 3' splice site;

(b) a polypyrimidine tract;

(c) a branch point;

(d) an exon splicing enhancer (ESE);

(e) an intron splicing enhancer (ISE);

(f) an exon splicing silencer (ESS);

(g) an intron splicing silencer (ISS); and

(h) a combination of any of (a)-(g),

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA

break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

58. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA (gDNA) molecule in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 5' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the one or more splicing signals is selected from the group consisting of:

(a) a natural or enhanced 5' splice site;

(b) an exon splicing enhancer (ESE);

(c) an intron splicing enhancer (ISE);

(d) an exon splicing silencer (ESS);

(e) an intron splicing silencer (ISS), and

(f) a combination of any of (a)-(e),

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein, when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

59. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein at least one splicing signal is a natural or enhanced 3' splice site; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

60. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the one or more splicing signals is a combination of a natural or enhanced 3' splice site and a polypyrimidine tract; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein, when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

61. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the one or more splicing signals is a combination of a natural or enhanced 3' splice site, a polypyrimidine tract, and a branch point; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

62. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is in a 3' splice site, wherein the first strand comprises an intronic sequence, optionally, an exonic sequence, wherein the intronic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the one or more splicing signals is selected from the group consisting of:

(a) a polypyrimidine tract;

(b) a branch point;

(c) an exon splicing enhancer (ESE);

(d) an intron splicing enhancer (ISE);

(e) an exon splicing silencer (ESS);

(f) an intron splicing silencer (ISS); and

(g) a combination of any of (a)-(f),

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

63. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 5' splice site, wherein the donor polynucleotide comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein at least one splicing signal is a natural or enhanced 5' splice site; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

64. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is in a 5' splice site, wherein the first strand comprises an intronic sequence, optionally, an exonic sequence, wherein the intronic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand, wherein the one or more splicing signals is selected from the group consisting of:

(a) an exon splicing enhancer (ESE);

(b) an intron splicing enhancer (ISE);

(c) an exon splicing silencer (ESS);

(d) an intron splicing silencer (ISS); and

(e) a combination of any of (a)-(d),

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

65. The donor polynucleotide of any one of claims 57-61, 63, or 64, wherein insertion of the donor polynucleotide into the DSB results in the formation of an exon in the gDNA comprising the exonic sequence.

66. The donor polynucleotide of claim 65, wherein the one or more splicing signals directs the inclusion of the exon comprising the exonic sequence which corrects the mutation into an mRNA.

67. The donor polynucleotide of claim 64, wherein the insertion of the donor polynucleotide results in the formation of an intron comprising the intronic sequence, wherein the intronic sequence corrects the mutation.

68. A donor polynucleotide comprising a non-replicative dsDNA molecule comprising a first strand and a second strand complementary to the first strand, wherein the first strand comprises a nucleotide sequence from 5' to 3' which corrects a disease-causing mutation in a gDNA molecule in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the first strand comprises the formula:

5'-$[B]_a$-$[S1]_b$-$[P]_c$-$[S2]_d$-X-E-3', wherein

(i) B, if present, is a branch point sequence comprising a nucleotide sequence that conforms to a branch point consensus sequence on each strand of the donor polynucleotide, wherein B comprises a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein a is an integer whose value indicates the number of nucleotides comprising B, wherein a =0 or 5-7;

(ii) P is a polypyrimidine tract comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein c is an integer whose value indicates the number of nucleotides comprising P, wherein c=9-20, wherein the nucleotide sequence comprising P is about 100%, about 90%-100%, about 80%-90% pyrimidine nucleobases;

(iii) E is an exonic sequence comprising a nucleotide sequence which corrects the mutation, wherein the nucleotide sequence comprises nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C);

(iv) X is a nucleotide sequence comprising a 3' splice site; and

(v) S1 and S2, if either is present, are each delimiter sequences comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein b and d are each integers whose value indicates the number of nucleotides comprising the delimiter sequence, respectively, wherein b and d = 0-20,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for directional insertion into a DSB, wherein when the donor polynucleotide is inserted into the DSB, B, if present, P, X, if present, and E, if present, comprise a sense strand, wherein B, if present, P, and X, if present, comprise the one or more splicing signals, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a NHEJ DNA repair pathway inserts the donor polynucleotide into the DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

69. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising a first strand and a second strand, wherein the second strand is complementary to the first strand, wherein the first strand from 5' to 3' comprises a nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 5' splice site, wherein the first strand comprises an intronic sequence and an exonic sequence, wherein the exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA,

wherein the first strand comprises the formula:

5'-E-Y-I-3', wherein

(i) E is an exonic sequence comprising a nucleotide sequence which corrects the mutation, wherein the nucleotide sequence comprises nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C);

(ii) Y is a nucleotide sequence comprising a 5' splice site; and

(iii) I, if present, comprises an intronic sequence comprising nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C),

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for directional insertion into a double-stranded DNA break (DSB), wherein

when the donor polynucleotide is inserted into the DSB, E, if present, Y, and I, if present, comprise a sense strand, wherein Y comprises the one or more splicing signals, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

70. A donor polynucleotide comprising a non-replicative dsDNA molecule comprising

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is proximal to a 3' splice site, wherein the first strand comprises a first intronic sequence, wherein the first intronic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising from 5' to 3' a second nucleotide sequence which corrects a disease-causing mutation in a gDNA in a cell, wherein the disease-causing mutation is proximal to a 3' splice site, wherein the second strand comprises a second intronic sequence, wherein the second intronic sequence corrects the mutation, wherein the second strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the second strand is complementary to the first strand, and wherein the first strand and the second strand each comprise the formula:

5'-$[P1]_a$-$[S1]_b$-$[B]_c$-$[S2]_d$-$[P2]_e$-3', wherein

(a) B comprises a branch point sequence comprising a nucleotide sequence that conforms to a branch point consensus sequence on each strand of the donor polynucleotide, wherein B comprises a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein c is an integer whose value indicates the number of nucleotides comprising B, wherein c = 5-7;

(b) P1 and P2 are polypyrimidine tracts each comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein a and e are integers whose value indicates the number of nucleotides comprising P1 and P2, respectively, wherein a=9-20 and c=9-20, wherein the nucleotide sequence comprising P1 and P2 are each about 100%, about 90%-100%, about 80%-90% pyrimidine nucleobases, and wherein P1 is in the reverse orientation and on the opposite strand of the donor polynucleotide relative to P2; and

(c) S1 and S2, if either is present, are each delimiter sequences comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein b and d are each integers whose value indicates the number of nucleotides comprising the delimiter sequence, respectively, wherein b and d = 0-20,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for bi-directional insertion into a double-stranded DNA break (DSB), wherein when the donor polynucleotide is inserted into the DSB in the first orientation, B and P2 comprise a sense strand and B and P2 comprise the first and second splicing signals, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein, when the donor polynucleotide is inserted into the DSB in the second orientation, B and P1 comprise a sense strand and B and P2 comprise the first and second splicing signals, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

71. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is proximal to a 3' splice site, wherein the first strand comprises a first intronic sequence, wherein the first intronic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising from 5' to 3' a second nucleotide sequence which corrects a disease-causing mutation in a gDNA in a cell, wherein the disease-causing mutation is proximal to a 3' splice site, wherein the second strand comprises a second intronic sequence, wherein the second intronic sequence corrects the mutation, wherein the second strand comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the second strand is complementary to the first strand, and wherein the first strand and the second strand each comprise the formula:

5'-$[P1]_a$-$[S1]_b$-$[B1]_c$-$[S2]_d$-$[B2]_e$-$[S3]_f$-$[P2]_g$-3', wherein

(a) B1, if present, and B2 are each branch point sequences, wherein B2 comprises a nucleotide sequence that conforms to a branch point consensus sequence on each strand of the donor polynucleotide, wherein the branch point sequence comprising B1, if present, is in a reverse orientation and on the opposite strand of the donor polynucleotide relative to the branch point sequence comprising B2, wherein B1 and B2 each comprise a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein c and e are integers whose value indicates the number of nucleotides comprising B1 and B2, respectively, wherein c = 0 or 5-7, wherein e = 5-7;

(b) P1 and P2 are polypyrimidine tracts each comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine, guanine, thymine and cytosine, wherein a and g are integers whose value indicates the number of nucleotides comprising P1 and P2, respectively, wherein a=9-20 and g=9-20, wherein the nucleotide sequence comprising P1 and P2 are each about 100%, about 90%-100%, about 80%-90% pyrimidine nucleobases, wherein P1 is in the reverse orientation and on the opposite strand of the donor polynucleotide relative to P2; and

(c) S1, S2 and S3, if any are present, are each delimiter sequences comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein b, d and f are each integers whose value indicates the number of nucleotides comprising the delimiter sequence, respectively, wherein b and f = 1-20, wherein d = 1-40,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for bi-directional insertion into a double-stranded DNA break (DSB), wherein when the donor polynucleotide is inserted into the DSB in the first orientation, B2 and P2 comprise a sense strand and B2 and P2 comprise the first and second splicing signals, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in the second orientation, B1 and P1 comprise a sense strand and B1 and P1 provide the first and second splicing signals, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

72. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises a first intronic sequence and a first exonic sequence, wherein the first exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising from 5' to 3' a second nucleotide sequence which corrects a disease-causing mutation in a gDNA in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the second strand comprises a second intronic sequence and a second exonic sequence, wherein the second exonic sequence corrects the mutation, wherein the second strand comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the second strand is complementary to the first strand, and wherein the first strand and the second strand each comprise the formula:

5'-$[E1]_a$-X1-$[P1]_b$-$[S1]_c$-$[B]_d$-$[S2]_e$-$[P2]_f$-X2-$[E2]_g$-3', wherein

(a) B is a branch point sequence comprising a nucleotide sequence that conforms to a branch point consensus sequence on each strand of the donor polynucleotide, wherein B comprises a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine, guanine, thymine and cytosine, wherein d is an integer whose value indicates the number of nucleotides comprising B, wherein d = 5-7;

(b) P1 and P2 are polypyrimidine tracts each comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein b and f are integers whose value indicates the number of nucleotides comprising P1 and P2, respectively, wherein b=9-20 and f=9-20, wherein the nucleotide sequence comprising P1 and P2 are each about 100%, about 90%-100%, about 80%-90% pyrimidine nucleobases, wherein P1 is in the reverse orientation and on the opposite strand of the donor polynucleotide relative to P2;

(c) E1 and E2 are each exonic sequences each comprising a nucleotide sequence which corrects the mutation, wherein the nucleotide sequence comprises nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein a and g are integers whose value indicates the number of nucleotides comprising E1 and E2, respectively, wherein the exonic sequence comprising E1 is in a reverse orientation and on the opposite strand of the donor polynucleotide relative to the exonic sequence comprising E2, wherein the nucleotide sequences comprising E1 and E2 are not complementary;

(d) X1 and X2 are each nucleotide sequences comprising a 3' splice site, wherein the nucleotide sequence comprising X1 is in the reverse orientation and on the opposite strand relative to the nucleotide sequence comprising X2; and

(e) S1 and S2, if either is present, are each delimiter sequences comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine, guanine, thymine and cytosine, wherein c and e are each integers whose value indicates the number of nucleotides comprising the delimiter sequence, respectively, wherein c and e = 1-20

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for bi-directional insertion into a double-stranded DNA break (DSB), wherein when the donor polynucleotide is inserted into the DSB in the first orientation, B, P2, E2 and X2 and comprise a sense strand, wherein B, P2 and X2 comprise the first, second and third splicing signal, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in a second orientation, B, P1, E1 and X1 comprise a sense strand, wherein B, P1 and X1 comprise the first, second and third splicing signal, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein, when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

73. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises a first intronic sequence and a first exonic sequence, wherein the first exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising from 5' to 3' a second nucleotide sequence which corrects a disease-causing mutation in a gDNA in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the second strand comprises a second intronic sequence and a second exonic sequence, wherein the second exonic sequence corrects the mutation, wherein the second strand

comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the second strand is complementary to the first strand, and

wherein the first strand and the second strand each comprise the formula:

5'-$[E1]_a$-X1-$[P1]_b$-$[S1]_c$-$[B1]_d$-$[S2]_e$-$[B2]_f$-$[S3]_g$-$[P2]_h$-X2-$[E2]_i$-3', wherein

(a) B1, if present, and B2 are each branch point sequences, wherein B2 comprises a nucleotide sequence that conforms to a branch point consensus sequence on each strand of the donor polynucleotide, wherein the branch point sequence comprising B1, if present, is in a reverse orientation and on the opposite strand of the donor polynucleotide relative to the branch point sequence comprising B2, wherein B1 and B2 each comprise a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein d and f are integers whose value indicates the number of nucleotides comprising B1 and B2, respectively, wherein d and f = 5-7;

(b) P1 and P2 are polypyrimidine tracts each comprising a nucleotide sequence comprising nucleobases selected from the group consisting of: adenine, guanine, thymine and cytosine, wherein b and h are integers whose value indicates the number of nucleotides comprising P1 and P2, respectively, wherein b=9-20 and h=9-20, wherein the nucleotide sequence comprising P1 and P2 are each about 100%, about 90%-100%, about 80%-90% pyrimidine nucleobases, wherein P1 is in the reverse orientation and on the opposite strand of the donor polynucleotide relative to P2;

(c) E1 and E2 are each exonic sequences each comprising a nucleotide sequence which corrects the mutation, wherein the nucleotide sequence comprises nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein a and i are integers whose value indicates the number of nucleotides comprising E1 and E2, respectively, wherein the exonic sequence comprising E1 is in the reverse orientation and on the opposite strand relative to the exonic sequence comprising E2, wherein the nucleotide sequences comprising E1 and E2 are not complementary;

(d) X1 and X2 each comprise a nucleotide sequence comprising a 3' splice site, wherein the nucleotide sequence of X1 is in a reverse orientation and on the opposite strand of the donor polynucleotide relative to the nucleotide sequence of X2; and

(e) S1, S2 and S3, if any present, are each delimiter sequences comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein c, e and g are each integers whose value indicates the number of nucleotides comprising the delimiter sequence, respectively, wherein c and g = 1-20, wherein e = 1-40,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for bi-directional insertion into a double-stranded DNA break (DSB), wherein when the donor polynucleotide is inserted into the DSB in a first orientation, B2, P2, E2 and X2 comprise

a sense strand, wherein B2, P2 and X2 comprise the first, second and third splicing signal, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in a second orientation, B1, P1, E1 and X1 comprise a sense strand, wherein B1, P1 and X1 comprise the first, second and third splicing signal, respectively, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA,

and wherein, when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

74. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects a disease-causing mutation in a genomic DNA molecule (gDNA) in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the first strand comprises a first intronic sequence and a first exonic sequence, wherein the first exonic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA; and

(ii) a second strand comprising from 5' to 3' a second nucleotide sequence which corrects a disease-causing mutation in a gDNA in a cell, wherein the disease-causing mutation is a protein-coding mutation proximal to a 3' splice site, wherein the second strand comprises a second intronic sequence and a second exonic sequence, wherein the second exonic sequence corrects the mutation, wherein the second strand comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the second strand is complementary to the first strand, and

wherein the first strand and the second strand each comprise the formula:

5'-$[E1]_a$-Y1-$[S1]_b$-Y2-$[E2]_c$-3', wherein

(a) E1 and E2 each are exonic sequences each comprising a nucleotide sequence which corrects the mutation, wherein the nucleotide sequence comprises nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (T), wherein a and c are integers whose value indicates the number of nucleotides comprising E1 and E2, respectively, wherein the exonic sequence comprising E1 is in the reverse orientation and on the opposite strand relative to the exonic sequence comprising E2, wherein the nucleotide sequences comprising E1 and E2 are not complementary;

(b) Y1 and Y2 each comprise a nucleotide sequence comprising a 5' splice site, wherein the nucleotide sequence of Y1 is in a reverse orientation and on the opposite strand of the donor polynucleotide relative to the nucleotide sequence of Y2; and

(c) S1, if present, is a delimiter sequence comprising one or more nucleotides comprising nucleobases selected from the group consisting of: adenine (A), guanine (G), thymine (T) and cytosine (C), wherein b is an integers whose value indicates the number of nucleotides comprising the delimiter sequence, wherein b = 1=50,

wherein the donor polynucleotide is about 10-500, about 10-400, about 10-300, about 10-200, is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length, wherein the donor polynucleotide is configured for bi-directional insertion into a double-stranded DNA break (DSB), wherein when the donor polynucleotide is inserted into the DSB in a first orientation, E2 and Y2 comprise a sense strand and E2 comprises the splicing signal, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein when the donor polynucleotide is inserted into the DSB in a second orientation, E1 and Y1 comprise a sense strand and E1 comprises the splicing signal, thereby correcting the mutation and providing splicing signals to control processing of a pre-mRNA transcribed from the gDNA

and wherein when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

75. The donor polynucleotide of any one of claims 68, 69, 72-74, wherein the exonic sequence comprising the sense strand corrects the mutation.

76. The donor polynucleotide of any one of claims 68, 72 or 73, wherein the nucleotide sequence comprising a 3' splice site comprising the sense strand corrects the mutation.

77. The donor polynucleotide of any one of claims 69 or 74, wherein the nucleotide sequence comprising a 5' splice site comprising the sense strand corrects the mutation.

78. The donor polynucleotide of any one of claims 68, 70-72, or 73, wherein the nucleotide sequence comprising a polypyrimidine tract comprising the sense strand corrects the mutation.

79. The donor polynucleotide of any one of claims 68, 70-72, or 73, wherein the nucleotide sequence comprising a branch point sequence comprising the sense strand corrects the mutation.

80. The donor polynucleotide of any one of the preceding claims, wherein the 5' most nucleotide of each strand of the donor polynucleotide comprises a 5' phosphate group.

81. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide is about 10-400, about 10-300, or about 10-200 nucleotides in length.

82. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide is about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length.

83. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide is about 40-70 nucleotides or about 50-60 nucleotides in length.

84. The donor polynucleotide of claim 83, wherein the donor polynucleotide is about 50-60 nucleotides in length.

85. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 nucleotides in length.

86. The donor polynucleotide of claim 85, wherein the donor polynucleotide is 50 nucleotides in length.

87. The donor polynucleotide of any one of the preceding claims, wherein the site-directed nuclease comprises a CRISPR/Cas system.

88. The donor polynucleotide of claim 87, wherein the CRISPR/Cas system comprises one or more guide RNAs (gRNAs).

89. The donor polynucleotide of claim 88, wherein the site-directed nuclease comprises a Cas9 polypeptide derived from *Streptococcus pyogenes* (SpCas9).

90. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide comprises natural nucleotides.

91. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide comprises one or more non-natural and/or modified nucleotides.

92. The donor polynucleotide of claim 91, wherein the one or more non-natural and/or modified nucleotides is a 2'-O-methyl nucleotide.

93. The donor polynucleotide of any one of the preceding claims, wherein the donor polynucleotide comprises one or more backbone modifications.

94. The donor polynucleotide of claim 93, wherein the one or more backbone modification is a phosphorothioate.

95. The donor polynucleotide of any one of claims 1-94, wherein the donor polynucleotide comprises two blunt ends.

96. The donor polynucleotide of any one of claims 1-94, wherein the donor polynucleotide comprises one blunt end and comprises one end comprising an overhang (e.g. a 5' or 3' overhang).

97. The donor polynucleotide of any one of the preceding claims, wherein the nucleotide sequence comprises one or more nucleotides that prevent the site-directed nuclease from recognizing and cleaving the donor polynucleotide.

98. The donor polynucleotide of any one of the preceding claims, wherein the disease is Glycogen Storage Disease 1a (GSD1a).

99. The donor polynucleotide of claim 98, wherein the mutation is located in the human G6PC gene on human chromosome 17q21.

100. The donor polynucleotide of claim 99, wherein the mutation in the G6PC gene results in an R83C, an R83H, or an E110K amino acid substitution in the human G6PC protein.

101. The donor polynucleotide of claim 100, wherein the mutation in the G6PC gene results in an R83C amino acid substitution in the human G6PC protein.

102. The donor polynucleotide of claim 100, wherein the mutation in the G6PC gene results in an R83H amino acid substitution in the human G6PC protein.

103. The donor polynucleotide of claim 100, wherein the mutation in the G6PC gene results in an E110K amino acid substitution in the human G6PC protein.

104. A donor polynucleotide comprising a non-replicative dsDNA molecule comprising a nucleotide sequence which corrects a mutation that causes Glycogen Storage Disease 1a in a gDNA molecule in a cell, wherein the mutation is located in the human G6PC gene on human chromosome 17q21 and results in the amino acid substitution R83C or R83H, the donor polynucleotide comprising:

(i) a first strand comprising from 5' to 3' a nucleotide sequence comprising an exonic sequence which corrects the mutation, wherein the exonic sequence comprises a codon encoding arginine (R) corresponding to the codon at position 83 in the G6PC gene, and a nucleotide sequence comprising one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA molecule, wherein the one or more splicing signals is a combination of a 3' splice site, a polypyrimidine tract, and a branch point sequence; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand,

wherein the donor polynucleotide is about 40-70 nucleotides in length and comprises two blunt ends, wherein the 5' most nucleotide of each strand of the donor polynucleotide comprises a 5' phosphate moiety, wherein when the donor polynucleotide is introduced into the cell in combination with an site-directed nuclease a NHEJ DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, wherein the insertion of the donor polynucleotide forms an exon in the gDNA comprising the exonic sequence that corrects the mutation, wherein the splicing signals direct the inclusion of the exon into an mRNA, thereby correcting the mutation.

105. The donor polynucleotide of claim 104, wherein the branch point sequence comprises the nucleotide sequence TTCAT, wherein the polypyrimidine tract comprises the nucleotide sequence CTTGTTCTGTTTTTTT, wherein the 3' splice site comprises the nucleotide sequence TAG, and wherein the exonic sequence comprises the nucleotide sequence GATTCTCTTTGGACAGCGCCCTTACT.

106. The donor polynucleotide of any one of claims 98-105, wherein the nucleotide sequence of the donor polynucleotide is set forth in SEQ ID NO: 30 (CH34 54-0)

107. The donor polynucleotide of any one of claims 98-105, wherein the nucleotide sequence of the donor polynucleotide is set forth in SEQ ID NO: 20. (CH32 50-0)

108. The donor polynucleotide of any one of claims 1-97, wherein the disease is Pompe's Disease.

109. The donor polynucleotide of claim 108, wherein the mutation is located in the human glucosidase alpha (GAA) gene on human chromosome 17q25.3.

110. The donor polynucleotide of claim 109, wherein the mutation in a splicing signal of GAA results in mRNA transcripts of the GAA gene lacking exon2 and/or activation of one or more cryptic splice sites.

111. A donor polynucleotide comprising a non-replicative dsDNA molecule comprising a nucleotide sequence which corrects a mutation that causes Pompe's Disease in a gDNA molecule in a cell, wherein the mutation in a splicing signal of GAA results in mRNA transcripts of the GAA gene lacking exon2 and/or activation of one or more cryptic splice sites, the donor polynucleotide comprising:

(i) a first strand comprising from 5' to 3' a first nucleotide sequence which corrects the mutation, wherein the first strand comprises a first intronic sequence, wherein the first intronic sequence corrects the mutation, wherein the first strand comprises one or more splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the one or more splicing signals comprises a combination of a 3' splice site, a polypyrimidine tract, and a branch point sequence; and

(ii) a second strand comprising from 5' to 3' a second intronic sequence, wherein the second intronic sequence corrects the mutation, wherein the second strand comprises one or more splicing signals to control processing of a pre-mRNA transcribed from the gDNA, wherein the one or more splicing signals comprises a combination of a 3' splice site, a polypyrimidine tract, and a branch point sequence, wherein the second strand is complementary to the first strand

wherein the donor polynucleotide about 10-100, about 20-80, about 30-70, or about 40-60 nucleotides in length and comprises two blunt ends, wherein the 5' most nucleotide of each strand of the donor polynucleotide comprises a 5' phosphate moiety, wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease a NHEJ DNA repair pathway inserts the donor polynucleotide into a DSB introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, wherein the donor polynucleotide is configured for bi-directional insertion into a DSB break, wherein insertion in either direction forms a 3' splice site, a polypyrimidine tract, and a branch

point sequence that corrects the mutation, wherein the splicing signals direct the inclusion of exon2 into an mRNA, thereby correcting the mutation.

112. The donor polynucleotide of any one of claims 108-111, wherein the nucleotide sequence of the donor polynucleotide is set forth in SEQ ID NO: 63 (GAA_50-0).

113. A system to correct a mutation in a genomic DNA molecule (gDNA) in a cell, the system comprising the components:

(a) the donor polynucleotide according to any one of claims 1-112;

(b) one or more gRNA molecules; and

(c) a site-directed nuclease,

wherein when the system is introduced into a cell, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation.

114. The system of claim 113, wherein the site-directed nuclease is encoded by an mRNA.

115. The system of claim 113, wherein the site-directed nuclease is a polypeptide.

116. The system of claim 115, wherein the one or more gRNA molecules and the site-directed nuclease comprise a ribonucleoprotein.

117. The system of any one of claims 113-116, wherein the site-directed nuclease is a Cas nuclease.

118. The system of claim 117, wherein the Cas nuclease is *S. pyogenes* Cas9 (SpCas9) or a homolog, derivative or modified version thereof.

119. The system of any one of claims 113-118, wherein the one or more gRNA molecules comprises a modification selected from the group consisting of: a backbone modification, a sugar modification, a modified inter-nucleoside linkage, or a modified or non-natural nucleobase.

120. The system of claim 119, wherein the one or more gRNA molecules comprise a backbone modification.

121. The system of claim 120, wherein the backbone modification is a phosphorothioate modification.

122. The system of any one of claims 113-121, wherein the donor polynucleotide, the gRNA molecule and the site-directed nuclease are individually formulated or co-formulated in a liposome or lipid nanoparticle.

123. The system of claim 122, wherein the donor polynucleotide, the gRNA molecule and the site-directed nuclease are individually formulated in a liposome or lipid nanoparticle.

124. The system of claim 122, wherein the donor polynucleotide, the gRNA molecule and the site-directed nuclease are co-formulated in a liposome or lipid nanoparticle.

125. The system of any one of claims 113-124, wherein the nucleotide sequence of the gRNA comprises the sequence set forth in SEQ ID NO: 107 (CH32 Mutant-CTX1 sgRNA)

126. The system of any one of claims 113-124, wherein the nucleotide sequence of the gRNA comprises the sequence set forth in SEQ ID NO: 108 (CH34 Mutant-CTX1 sgRNA)

127. The system of any one of claims 113-124, wherein the nucleotide sequence of the gRNA comprises the sequence set forth in SEQ ID NO: 93 (mutant GAA sgRNA spacer)

128. A cell comprising the donor polynucleotide of any one of claims 1-112 or the system of any one of claims 113-127.

129. The cell of claim 128, wherein the cell is a dividing or non-dividing cell.

130. The cell of claim 129, wherein the cell is a dividing cell.

131. The cell of claim 129, wherein the cell is a non-dividing cell.

132. The cell of claim 128, wherein the cell is a patient-specific induced pluripotent stem cell (iPSC).

133. The cell of claim 128, wherein the cell is a primary hepatocyte.

134. A pharmaceutical composition comprising the donor polynucleotide according to any one of claims 1-112, and a pharmaceutically acceptable carrier.

135. A pharmaceutical composition comprising the system according to any one of claims 113-127, and a pharmaceutically acceptable carrier.

136. A pharmaceutical composition comprising the cell according to any one of claims 128-133, and a pharmaceutically acceptable carrier.

137. The donor polynucleotide of any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, for use in treating a patient with a disease by correcting a mutation in a genomic DNA molecule (gDNA) in a cell, the treatment comprising: isolating a cell from the patient, contacting the cell with the donor polynucleotide, the system, or the pharmaceutical composition, wherein when the donor polynucleotide, the system, or the pharmaceutical composition contacts the cell, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break introduced into the gDNA at a location proximal to the mutation, thereby correcting the mutation.

138. The donor polynucleotide of any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, for use in treating a patient with a disease by correcting a mutation in a genomic DNA molecule (gDNA) in a cell, the treatment comprising: administering to the patient an effective amount of the donor polynucleotide, the system, or the pharmaceutical composition, wherein, when the donor polynucleotide, system or composition is administered, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break introduced into the gDNA at a location proximal to the mutation, thereby correcting the mutation.

139. The use of the donor polynucleotide of any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, for the manufacture of a medicament for in treating a patient with a disease by correcting a mutation in a genomic DNA molecule (gDNA) in a cell.

140. A method to correct a mutation in a genomic DNA molecule (gDNA) in a cell, the method comprising: contacting the cell with the donor polynucleotide according to any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, wherein when the donor polynucleotide, system or composition contacts the cell, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break introduced into the gDNA at a location proximal to the mutation, thereby correcting the mutation.

141. A method of treating a patient with a disease by correcting a mutation in a genomic DNA molecule (gDNA) in a cell, the method comprising: isolating a cell from the patient, contacting the cell with the donor polynucleotide according to any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, wherein, when the donor polynucleotide, system or composition contacts the cell, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break introduced into the gDNA at a location proximal to the mutation, thereby correcting the mutation.

142. A method of treating a patient with a disease by correcting a mutation in a genomic DNA molecule (gDNA) in a cell, the method comprising: administering to the patient an effective amount of the donor polynucleotide according to any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, wherein, when the donor polynucleotide, system or composition is administered, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break introduced into the gDNA at a location proximal to the mutation, thereby correcting the mutation.

143. The method of claim 140 or 141, wherein the cell is a patient-specific induced pluripotent stem cell (iPSC).

144. The method of claim 140 or 141, wherein the cell is a hepatocyte.

145. The method of claim 143, wherein the method further comprises differentiating the iPSC comprising the corrected mutation into a differentiated cell; and implanting the differentiated cell into a patient.

146. The method of any one of claims 140-144, wherein treatment results in the translation of an mRNA transcribed from the genomic DNA molecule (gDNA) comprising the inserted donor polynucleotide, wherein the translation results in the formation of a translation product (protein) that alleviates the disease or that does not cause or contribute to the disease.

147. A kit comprising a container comprising a donor polynucleotide according to any one of claims 1-112, the system according to any one of claims 113-127, or the pharmaceutical composition according to any one of claims 134-136, for correcting a mutation in a genomic DNA molecule (gDNA) in a cell, and a package insert comprising instructions for use.

Claims

1. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising a nucleotide sequence which corrects a mutation that causes Glycogen Storage Disease 1a in a genomic DNA (gDNA) molecule molecule in a cell, wherein the mutation is a protein-coding mutation proximal to a 3' splice site in the human G6PC gene, the donor polynucleotide comprising,

(i) a first strand comprising from 5' to 3': (a) a nucleotide sequence comprising splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the splicing signals are a branch point, a polypyrimidine tract, and a natural or enhanced 3' splice site; and (b) a nucleotide sequence comprising an exonic sequence which corrects the mutation; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand, wherein the donor polynucleotide is at least 25 and up to 500 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation;

wherein the branch point sequence comprises the nucleotide sequence TTCAT, wherein the polypyrimidine tract comprises the nucleotide sequence CTTGTTCTGTTTTTTT (SEQ ID NO: 109), wherein the 3' splice site comprises the nucleotide sequence TAG.

2. The donor polynucleotide of claim 1, wherein the donor polynucleotide is up to 400, 300, 200, 100, 80, 70, or 60 nucleotides in length.

3. The donor polynucleotide of claim 1, wherein the donor polynucleotide is 30-70 or 40-60 nucleotides in length.

4. The donor polynucleotide of claim 1, wherein the mutation is an amino acid substitution of arginine (R) at position 83 in the G6PC gene, and wherein the exonic sequence comprises a codon encoding arginine (R) corresponding to the codon at position 83 in the G6PC gene.

5. The donor polynucleotide of claim 1, wherein insertion of the donor polynucleotide into the DSB results in the formation of an exon in the gDNA comprising the exonic sequence.

6. The donor polynucleotide of claim 1, wherein the splicing signals direct the inclusion of the exon comprising the exonic sequence which corrects the mutation into an mRNA.

7. The donor polynucleotide of claim 1, wherein the exonic sequence comprises the nucleotide sequence GATTCTCTTTGGACAGCGCCCTTACT (SEQ ID NO: 110).

8. The donor polynucleotide of claim 1, wherein the donor polynucleotide comprises two blunt ends.

9. A pharmaceutical composition comprising the donor polynucleotide according to claim 1 and a pharmaceutically acceptable carrier.