

We claim:

1. A use of a Cas9 protein or a nucleic acid encoding the Cas9 protein and first guide RNA or a DNA encoding the first guide RNA in the manufacture of a medicament for treating a human subject having or at risk of developing chronic liver disease by modifying or altering expression of an *HSD17B13* gene in a cell in the human subject,

wherein the first guide RNA comprises a first CRISPR RNA (crRNA) portion and a first trans-activating CRISPR RNA (tracrRNA) portion, wherein the first guide RNA forms a complex with the Cas9 protein and targets a first guide RNA target sequence within the *HSD17B13* gene, wherein the first guide RNA target sequence includes or is proximate to the start codon for the *HSD17B13* gene, and

wherein the Cas9 protein cleaves the first guide RNA target sequence to generate a targeted genetic modification in the *HSD17B13* gene or the Cas9 protein binds the first guide RNA target sequence and alters expression of the *HSD17B13* gene, wherein the expression of the *HSD17B13* gene is decreased.

2. The use of claim 1, wherein:

(a) the Cas9 protein is a nuclease-active Cas9 protein, and the *HSD17B13* gene does not have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1; or

(b) the Cas9 protein is a nuclease-inactive Cas9 protein fused to a transcriptional repressor domain, and the *HSD17B13* gene does not have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

3. The use of claim 1 or 2, wherein:

(a) the first guide RNA target sequence comprises any one of SEQ ID NOS: 20-81; and/or

(b) the first guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1423-1484; and/or

(c) the first guide RNA comprises any one of SEQ ID NOS: 500-561, 730-791, 960-1021, 1190-1251.

4. The use of any one of claims 1-3, wherein the first guide RNA target sequence is within a region corresponding to exon 1 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2.

5. The use of any one of claims 1-4, wherein the first guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon for the *HSD17B13* gene or comprises the start codon for the *HSD17B13* gene.

6. The use of any one of claims 1-5, wherein the medicament further comprises a second guide RNA or a DNA encoding the second guide RNA,
wherein the second guide RNA comprises a second CRISPR RNA (crRNA) portion and a second trans-activating CRISPR RNA (tracrRNA) portion,
wherein the second guide RNA forms a complex with the Cas9 protein and targets a second guide RNA target sequence within the *HSD17B13* gene, and
wherein the second guide RNA target sequence includes or is proximate to the start codon for the *HSD17B13* gene.

7. The use of claim 6, wherein the second guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon for the *HSD17B13* gene or comprises the start codon for the *HSD17B13* gene.

8. The use of any one of claims 1-7, wherein the medicament results in disruption of the start codon of the *HSD17B13* gene.

9. The use of any one of claims 1-5, wherein the medicament further comprises a second guide RNA or a DNA encoding the second guide RNA,
wherein the second guide RNA comprises a second CRISPR RNA (crRNA) portion and a second trans-activating CRISPR RNA (tracrRNA) portion,
wherein the second guide RNA forms a complex with the Cas9 protein and targets a second guide RNA target sequence within the *HSD17B13* gene,
wherein the second guide RNA target sequence includes or is proximate to the stop codon for the *HSD17B13* gene, and

wherein the cell is modified to comprise a deletion of an *HSD17B13* coding region between the first guide RNA target sequence and the second guide RNA target sequence.

10. The use of claim 9, wherein:

(a) the second guide RNA target sequence comprises any one of SEQ ID NOS: 82-225; and/or

(b) the second guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1485-1628; and/or

(c) the second guide RNA comprises any one of SEQ ID NOS: 562-705, 792-935, 1022-1165, and 1252-1395.

11. The use of claim 9 or 10, wherein the second guide RNA target sequence is within a region corresponding to exon 7 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2.

12. The use of any one of claims 9-11, wherein the second guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the stop codon for the *HSD17B13* gene or comprises the stop codon for the *HSD17B13* gene.

13. The use of any one of claims 9-12, wherein the coding sequence of the *HSD17B13* gene is deleted.

14. The use of any one of claims 1-13, wherein the targeted genetic modification is generated by repair of the cleaved first guide RNA target sequence by non-homologous end-joining.

15. The use of any one of claims 1-14, wherein the medicament results in loss of function of the *HSD17B13* gene.

16. The use of any one of claims 1-15, wherein the medicament further comprises an expression vector, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

17. The use of claim 16, wherein the recombinant *HSD17B13* gene is a human gene.

18. The use of claim 16 or 17, wherein the recombinant *HSD17B13* gene is an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene, wherein the deleted segments comprise one or more intronic sequences, and wherein the *HSD17B13* minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

19. The use of any one of claims 1-15, wherein the medicament further comprises an expression vector, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), and optionally wherein the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

20. The use of any one of claims 1-15, wherein the medicament further comprises an HSD17B13 protein or fragment thereof, wherein the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

21. The use of any one of claims 1-15, wherein the medicament further comprises an exogenous donor sequence, wherein the exogenous donor sequence recombines with a target genomic locus in the *HSD17B13* gene to generate the targeted genetic modification.

22. The use of claim 21, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via non-homologous-end-joining-mediated insertion.

23. The use of claim 21, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via homology-directed repair.

24. The use of claim 23, wherein the exogenous donor sequence comprises a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position

12666 of SEQ ID NO: 2 and a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, wherein the exogenous donor sequence recombines with the *HSD17B13* gene.

25. The use of claim 24, wherein the exogenous donor sequence further comprises a nucleic acid insert flanked by the 5' homology arm and the 3' homology arm.

26. The use of claim 25, wherein the nucleic acid insert comprises a thymine, and wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

27. The use of any one of claims 21-26, wherein the exogenous donor sequence is between about 50 nucleotides to about 1 kb in length.

28. The use of claim 27, wherein the exogenous donor sequence is between about 80 nucleotides to about 200 nucleotides in length.

29. The use of any one of claims 21-28, wherein the exogenous donor sequence is a single-stranded oligodeoxynucleotide.

30. The use of any one of claims 1-29, wherein the first guide RNA is a single-molecule guide RNA in which the first crRNA portion is linked to the first tracrRNA portion.

31. The use of claim 30, wherein the first guide RNA comprises the sequence set forth in SEQ ID NO: 1420, 256, 257, or 258.

32. The use of any one of claims 1-29, wherein the first crRNA portion and the first tracrRNA portion are separate RNA molecules.

33. The use of claim 32, wherein the first crRNA portion comprises the sequence set forth in SEQ ID NO: 1421 and/or the first tracrRNA portion comprises the sequence set forth in SEQ ID NO: 1422.

34. The use of any one of claims 1-33, wherein the first guide RNA comprises a modification providing for modified or regulated stability.

35. The use of any one of claims 1-34, wherein the medicament comprises the nucleic acid encoding the Cas9 protein, wherein the nucleic acid encoding the Cas9 protein comprises DNA.

36. The use of any one of claims 1-34, wherein the medicament comprises the nucleic acid encoding the Cas9 protein, wherein the nucleic acid encoding the Cas9 protein comprises RNA.

37. The use of any one of claims 1-36, wherein the medicament comprises the first guide RNA in the form of RNA.

38. The use of any one of claims 1-36, wherein the medicament comprises the DNA encoding the first guide RNA.

39. The use of any one of claims 1-38, wherein the Cas9 protein or the nucleic acid encoding the Cas9 protein and/or the first guide RNA or the DNA encoding the first guide RNA are in a lipid nanoparticle.

40. The use of any one of claims 1-39, wherein the Cas9 protein or the nucleic acid encoding the Cas9 protein and/or the first guide RNA or the DNA encoding the first guide RNA are in an adeno-associated virus vector.

41. The use of any one of claims 1-40, wherein the human cell is a human liver cell.

42. The use of any one of claims 1-41, wherein:

(a) the first guide RNA target sequence comprises any one of SEQ ID NOS: 20-41, any one of SEQ ID NOS: 21-23, 33, and 35, or any one of SEQ ID NOS: 33 and 35; and/or

(b) the first guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1447-1468, any one of SEQ ID NOS: 1448-1450, 1460, and 1462; or any one of SEQ ID NOS: 1460 and 1462; and/or

(c) the first guide RNA comprises any one of SEQ ID NOS: 524-545, 754-775, 984-1005, and 1214-1235, or any one of SEQ ID NOS: 295-297, 525-527, 755-757, 985-987, 1215-1217, 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229, or any one of SEQ ID NOS: 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229.

43. The use of any one of claims 1-42, wherein the chronic liver disease is a nonalcoholic fatty liver disease, an alcoholic fatty liver disease, a cirrhosis, or a hepatocellular carcinoma, and optionally wherein the subject has or is at risk for steatohepatitis, fibrosis, cirrhosis, and/or hepatocellular carcinoma.

44. An *in vitro* or *ex vivo* method of modifying or altering expression of an *HSD17B13* gene in a human cell, comprising introducing into the human cell:

- (a) a Cas9 protein or a nucleic acid encoding the Cas9 protein; and
- (b) a first guide RNA or a DNA encoding the first guide RNA, wherein the first guide RNA forms a complex with the Cas9 protein and targets a first guide RNA target sequence within the *HSD17B13* gene, wherein the first guide RNA target sequence includes or is proximate to the start codon for the *HSD17B13* gene,

wherein the Cas9 protein cleaves or alters expression of the *HSD17B13* gene, and wherein the method results in disruption of the start codon of the *HSD17B13* gene or loss of function of the *HSD17B13* gene.

45. The method of claim 44, wherein the human cell is a human liver cell.

46. The method of claim 44 or 45, wherein:

- (a) the first guide RNA target sequence comprises any one of SEQ ID NOS: 20-81; and/or
- (b) the first guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1423-1484; and/or
- (c) the first guide RNA comprises any one of SEQ ID NOS: 500-561, 730-791, 960-1021, and 1190-1251,
optionally wherein:

(a) the first guide RNA target sequence comprises any one of SEQ ID NOS: 20-41, any one of SEQ ID NOS: 21-23, 33, and 35, or any one of SEQ ID NOS: 33 and 35; and/or

(b) the first guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1447-1468, any one of SEQ ID NOS: 1448-1450, 1460, and 1462; or any one of SEQ ID NOS: 1460 and 1462; and/or

(c) the first guide RNA comprises any one of SEQ ID NOS: 524-545, 754-775, 984-1005, and 1214-1235, or any one of SEQ ID NOS: 295-297, 525-527, 755-757, 985-987, 1215-1217, 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229, or any one of SEQ ID NOS: 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229.

47. A method of modifying or altering expression of an *HSD17B13* gene in a non-human animal cell, comprising introducing into the non-human animal cell:

(a) a Cas9 protein or a nucleic acid encoding the Cas9 protein; and

(b) a first guide RNA or a DNA encoding the first guide RNA, wherein the first guide RNA forms a complex with the Cas9 protein and targets a first guide RNA target sequence within the *HSD17B13* gene, wherein the first guide RNA target sequence includes or is proximate to the start codon for the *HSD17B13* gene,

wherein the Cas9 protein cleaves or alters expression of the *HSD17B13* gene, and wherein the method results in disruption of the start codon of the *HSD17B13* gene or loss of function of the *HSD17B13* gene.

48. The method of claim 47, wherein the non-human animal cell is a mouse cell or a rat cell.

49. The method of claim 48, wherein the non-human animal cell is a mouse liver cell, a rat liver cell, a mouse pluripotent cell, or a rat pluripotent cell.

50. The method of claim 48 or 49, wherein the non-human animal cell is the mouse cell, and wherein:

(a) the first guide RNA target sequence comprises any one of SEQ ID NOS: 259-263; and/or

(b) the first guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1643-1647; and/or

(c) the first guide RNA comprises any one of SEQ ID NOS: 720-724, 950-954, 1180-1184, and 1410-1414.

51. The method any one of claims 47-50, wherein the non-human animal cell is *in vivo*.

52. The method of claim 51, wherein the non-human animal cell is a liver cell, and wherein the Cas9 protein or the nucleic acid encoding the Cas9 protein and the first guide RNA or the DNA encoding the first guide RNA are introduced into a liver *in vivo*.

53. The method of any one of claims 47-52, wherein the non-human animal cell is a liver cell in a non-human animal subject having or at risk for a chronic liver disease, optionally wherein the chronic liver disease is a nonalcoholic fatty liver disease, an alcoholic fatty liver disease, a cirrhosis, or a hepatocellular carcinoma, and optionally wherein the subject has or is at risk for steatohepatitis, fibrosis, cirrhosis, and/or hepatocellular carcinoma.

54. The method of any one of claims 44-53, wherein:

(a) the Cas9 protein is a nuclease-active Cas9 protein, and the *HSD17B13* gene does not have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1; or

(b) the Cas9 protein is a nuclease-inactive Cas9 protein fused to a transcriptional repressor domain, and the *HSD17B13* gene does not have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

55. The method of any one of claims 44-54, wherein the first guide RNA target sequence is within a region corresponding to exon 1 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2.

56. The method of any one of claims 44-55, wherein the first guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon for the *HSD17B13* gene or comprises the start codon for the *HSD17B13* gene.

57. The method of any one of claims 44-56, further comprising introducing into the cell a second guide RNA or a DNA encoding the second guide RNA, wherein the second guide RNA comprises a second CRISPR RNA (crRNA) portion and a second trans-activating CRISPR RNA (tracrRNA) portion, wherein the second guide RNA forms a complex with the Cas9 protein and targets a second guide RNA target sequence within the *HSD17B13* gene, and wherein the second guide RNA target sequence includes or is proximate to the start codon for the *HSD17B13* gene.

58. The method of claim 57, wherein the second guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon for the *HSD17B13* gene or comprises the start codon for the *HSD17B13* gene.

59. The method of any one of claims 44-58, wherein the method results in disruption of the start codon of the *HSD17B13* gene.

60. The method of any one of claims 44-56, further comprising introducing into the cell a second guide RNA or a DNA encoding the second guide RNA, wherein the second guide RNA comprises a second CRISPR RNA (crRNA) portion and a second trans-activating CRISPR RNA (tracrRNA) portion, wherein the second guide RNA forms a complex with the Cas9 protein and targets a second guide RNA target sequence within the *HSD17B13* gene, wherein the second guide RNA target sequence includes or is proximate to the stop codon for the *HSD17B13* gene, and wherein the cell is modified to comprise a deletion of an *HSD17B13* coding region between the first guide RNA target sequence and the second guide RNA target sequence.

61. The method of claim 60, wherein:

- (a) the second guide RNA target sequence comprises any one of SEQ ID NOS: 82-225; and/or
- (b) the second guide RNA comprises a DNA-targeting segment that comprises any one of SEQ ID NOS: 1485-1628; and/or

(c) the second guide RNA comprises any one of SEQ ID NOS: 562-705, 792-935, 1022-1165, and 1252-1395.

62. The method of claim 60 or 61, wherein the second guide RNA target sequence is within a region corresponding to exon 7 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2.

63. The method of any one of claims 60-62, wherein the second guide RNA target sequence is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the stop codon for the *HSD17B13* gene or comprises the stop codon for the *HSD17B13* gene.

64. The method of any one of claims 60-63, wherein the coding sequence of the *HSD17B13* gene is deleted.

65. The method of any one of claims 44-64, wherein the targeted genetic modification is generated by repair of the cleaved first guide RNA target sequence by non-homologous end-joining.

66. The method of any one of claims 44-65, wherein the method results in loss of function of the *HSD17B13* gene.

67. The method of any one of claims 44-66, further comprising introducing an expression vector into the cell, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

68. The method of claim 67, wherein the recombinant *HSD17B13* gene is a human gene.

69. The method of claim 67 or 68, wherein the recombinant *HSD17B13* gene is an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene, wherein the deleted segments comprise one or more intronic sequences, and wherein the *HSD17B13* minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

70. The method of any one of claims 44-66, further comprising introducing an expression vector into the cell, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), and optionally wherein the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

71. The method of any one of claims 44-66, further comprising introducing an HSD17B13 protein or fragment thereof into the cell, wherein the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

72. The method of any one of claims 44-66, further comprising introducing an exogenous donor sequence into the cell, wherein the exogenous donor sequence recombines with a target genomic locus in the *HSD17B13* gene to generate the targeted genetic modification.

73. The method of claim 72, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via non-homologous-end-joining-mediated insertion.

74. The method of claim 72, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via homology-directed repair.

75. The method of claim 74, wherein the exogenous donor sequence comprises a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2 and a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, wherein the exogenous donor sequence recombines with the *HSD17B13* gene.

76. The method of claim 75, wherein the exogenous donor sequence further comprises a nucleic acid insert flanked by the 5' homology arm and the 3' homology arm.

77. The method of claim 76, wherein the nucleic acid insert comprises a thymine, and wherein upon recombination of the exogenous donor sequence with the *HSD17B13*

gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

78. The method of any one of claims 72-77, wherein the exogenous donor sequence is between about 50 nucleotides to about 1 kb in length.

79. The method of claim 78, wherein the exogenous donor sequence is between about 80 nucleotides to about 200 nucleotides in length.

80. The method of any one of claims 72-79, wherein the exogenous donor sequence is a single-stranded oligodeoxynucleotide.

81. The method of any one of claims 44-80, wherein the first guide RNA is a single-molecule guide RNA in which the first crRNA portion is linked to the first tracrRNA portion.

82. The method of claim 81, wherein the first guide RNA comprises the sequence set forth in SEQ ID NO: 1420, 256, 257, or 258.

83. The method of any one of claims 44-80, wherein the first crRNA portion and the first tracrRNA portion are separate RNA molecules.

84. The method of claim 83, wherein the first crRNA portion comprises the sequence set forth in SEQ ID NO: 1421 and/or the first tracrRNA portion comprises the sequence set forth in SEQ ID NO: 1422.

85. The method of any one of claims 44-84, wherein the first guide RNA comprises a modification providing for modified or regulated stability.

86. The method of any one of claims 44-85, wherein the method comprises introducing into the cell the nucleic acid encoding the Cas9 protein, wherein the nucleic acid encoding the Cas9 protein comprises DNA.

87. The method of any one of claims 44-85, wherein the method comprises introducing into the cell the nucleic acid encoding the Cas9 protein, wherein the nucleic acid encoding the Cas9 protein comprises RNA.

88. The method of any one of claims 44-87, wherein the method comprises introducing into the cell the first guide RNA in the form of RNA.

89. The method of any one of claims 44-87, wherein the method comprises introducing into the cell the DNA encoding the first guide RNA.

90. The method of any one of claims 44-89, wherein the Cas9 protein or the nucleic acid encoding the Cas9 and/or the first guide RNA or the DNA encoding the first guide RNA are in a lipid nanoparticle.

91. The method of any one of claims 44-90, wherein the Cas9 protein or the nucleic acid encoding the Cas9 protein and/or the first guide RNA or the DNA encoding the first guide RNA are in an adeno-associated virus vector.