WHAT IS CLAIMED IS:

1. A method of modifying an organism or a non-human organism by manipulation of a hepatic target sequence in a genomic locus of interest comprising

delivering a non-naturally occurring or engineered composition comprising :

A) - I. a CRISPR-Cas system RNA polynucleotide sequence, wherein the polynucleotide sequence comprises:

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell,

(b) a tracr mate sequence, and

(c) a tracr sequence, and

II. a polynucleotide sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences,

wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA,

or

(B) I. polynucleotides comprising:

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell, and

(b) at least one or more tracr mate sequences,

II. a polynucleotide sequence encoding a CRISPR enzyme, and

III. a polynucleotide sequence comprising a tracr sequence,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate

sequence that is hybridized or hybridizable to the tracr sequence, and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA.

2. The method of claim 1, wherein any or all of the polynucleotide sequence encoding a CRISPR enzyme, guide sequence, tracr mate sequence or tracr sequence, is/are RNA.

3. The method of claim 1 or 2, wherein the polynucleotides comprising the sequence encoding a CRISPR enzyme, the guide sequence, tracr mate sequence or tracr sequence is/are RNA and are delivered via liposomes, nanoparticles, exosomes, microvesicles, or a gene-gun.

4. The method of any of claims 1 to 3, wherein the polynucleotides are comprised within a vector system comprising one or more vectors.

5. A method of modifying an organism or a non-human organism by manipulation of a hepatic target sequence in a genomic locus of interest comprising

delivering a non-naturally occurring or engineered composition comprising a viral vector system comprising one or more viral vectors operably encoding a composition for expression thereof, wherein the composition comprises:

(A) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising

I. a first regulatory element operably linked to a CRISPR-Cas system RNA polynucleotide sequence, wherein the polynucleotide sequence comprises

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell,

(b) a tracr mate sequence, and

(c) a tracr sequence, and

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences,

wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,

wherein components I and II are located on the same or different vectors of the system,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence,

or

(B) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising

I. a first regulatory element operably linked to

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell, and

(b) at least one or more tracr mate sequences,

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and

III. a third regulatory element operably linked to a tracr sequence,

wherein components I, II and III are located on the same or different vectors of the system,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence.

6. The method of claim 5, wherein one or more of the viral vectors are delivered via liposomes, nanoparticles, exosomes, microvesicles, or a gene-gun.

7. A method of treating or inhibiting a condition caused by a defect in a hepatic target sequence in a genomic locus of interest in a subject or a non-human subject in need thereof comprising modifying the subject or a non-human subject by manipulation of the hepatic target sequence and wherein the condition is susceptible to treatment or inhibition by manipulation of the hepatic target sequence comprising providing treatment comprising:

delivering a non-naturally occurring or engineered composition comprising an AAV or lentivirus vector system, comprising one or more AAV or lentivirus vectors operably encoding a composition for expression thereof, wherein the hepatic target sequence is manipulated by the composition when expressed, wherein the composition comprises:

(A) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising

I. a first regulatory element operably linked to a CRISPR-Cas system RNA polynucleotide sequence, wherein the polynucleotide sequence comprises

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell,

(b) a tracr mate sequence, and

(c) a tracr sequence, and

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising at least one or more nuclear localization sequences,

wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,

wherein components I and II are located on the same or different vectors of the system,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence,

or

(B) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising

I. a first regulatory element operably linked to

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell, and

(b) at least one or more tracr mate sequences,

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and

III. a third regulatory element operably linked to a tracr sequence,

wherein components I, II and III are located on the same or different vectors of the system,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence.

8. The method of any preceding claim, wherein the method is carried out in vitro, and/or ex vivo.

9. The method of any preceding claim including inducing expression.

10. The method of any preceding claim wherein the organism or subject is a eukaryote.

11. The method of claim 10 wherein the organism or subject is a non-human eukaryote.

12. The method of any of claims 1 to 11 wherein the organism or subject is a mammal or a non-human mammal.

13. The method of any of claims 4 to 8 wherein the viral vector is an AAV or lentiviral vector.

14. The method according to any preceding claim wherein the CRISPR enzyme is a Cas9.

15. The method according to any preceding claim wherein expression of the guide sequence is under the control of the T7 promoter and is driven by the expression of T7 polymerase.

16. A method of delivering a CRISPR enzyme of any preceding claim, comprising delivering to a cell mRNA encoding the CRISPR enzyme.

17. The method of any one of claims 1 to 16, wherein the polynucleotide or enzyme coding sequence encoding the CRISPR enzyme is delivered to the cell by delivering mRNA encoding the CRISPR enzyme to the cell.

18. A method of preparing the AAV or lentivirus vector of claim 7 comprising transfecting plasmid(s) containing or consisting essentially of nucleic acid molecule(s) coding for the AAV or lentivirus into AAV-infected or lentivirus-infected cells, and supplying AAV AAV or lentivirus rep and/or cap and/or helper nucleic acid molecules obligatory for replication and packaging of the AAV or lentivirus.

19. A method of preparing an AAV or lentivirus vector for use in the method of claim 7, comprising transfecting plasmid(s) containing or consisting essentially of nucleic acid molecule(s) coding for the AAV or lentivirus into AAV-infected or lentivirus-infected cells, and supplying AAV AAV or lentivirus rep and/or cap and/or helper nucleic acid molecules obligatory for replication and packaging of the AAV or lentivirus.

20. The method of claim 18 or 19 wherein the AAV or lentivirus rep and/or cap obligatory for replication and packaging of the AAV or lentivirus are supplied by transfecting the cells with helper plasmid(s) or helper virus(es).

21. The method of claim 20 wherein the helper virus is a poxvirus, adenovirus, lentivirus, herpesvirus or baculovirus.

22. The method of claim 21 wherein the poxvirus is a vaccinia virus.

23. The method of any of claims 18 to 22 wherein the cells are mammalian cells.

24. The method of any of claims 18 to 22 wherein the cells are insect cells and the helper virus (where present) is baculovirus.

25. The method of any of claims 1 to 15 wherein the hepatic target sequence is flanked at its 3' end or followed by 5'-NRG (where N is any Nucleotide), and where the CRISPR enzyme is (or is derived from) *S. pyogenes* or *S. aureus* Cas9.

26. A composition as defined in any of claims 1-25 for use in medicine or in therapy.

27. A composition as defined in any of claims 1-25 for use in a method of modifying an organism or a non-human organism by manipulation of a hepatic target sequence in a genomic locus of interest or in a method of treating or inhibiting a condition caused by a defect in a hepatic target sequence in a genomic locus of interest.

28. Use of a composition as defined in any of claims 1-25 in *ex vivo* gene or genome editing.

29. Use of a composition as defined in any of claims 1-25 in the manufacture of a medicament for *ex vivo* gene or genome editing or for use in a method of modifying an organism or a non-human organism by manipulation of a hepatic target sequence in a genomic locus of interest or in a method of treating or inhibiting a condition caused by a defect in a hepatic target sequence in a genomic locus of interest.

30. A composition comprising:

A) - I. a CRISPR-Cas system RNA polynucleotide sequence, wherein the polynucleotide sequence comprises:

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell,

(b) a tracr mate sequence, and

(c) a tracr sequence, and

II. a polynucleotide sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences,

wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA,

or

(B) I. polynucleotides comprising:

(a) a guide sequence capable of hybridizing to a hepatic target sequence in a eukaryotic cell, and

(b) at least one or more tracr mate sequences,

II. a polynucleotide sequence encoding a CRISPR enzyme, and

III. a polynucleotide sequence comprising a tracr sequence,

wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the hepatic target sequence, and

wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized or hybridizable to the hepatic target sequence, and (2) the tracr mate sequence that is hybridized or hybridizable to the tracr sequence, and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA;

for use in medicine or therapy; or for use in a method of modifying an organism or a non-human organism by manipulation of a hepatic target sequence in a genomic locus of interest; or for use in a method of treating or inhibiting a condition caused by a defect in a hepatic target sequence in a genomic locus of interest; or for use in *ex vivo* gene or genome editing.

31. The composition of claim 30, wherein the polynucleotides are comprised within a vector system comprising one or more vectors.

32. The method, use or composition of any of the preceding claims, wherein the CRISPR-Cas system RNA is a chimeric RNA (chiRNA).

33. The method, use or composition of any of the preceding claims, wherein the CRISPR-Cas system is a multiplexed CRISPR enzyme system further comprising multiple chimeras and/or multiple multiguide sequences and a single tracr sequence.

40. The method, use or composition according any of the preceding claims, wherein the CRISPR enzyme is a nuclease directing cleavage of one or both strands at the location of the target sequence.

41. The method, use or composition according to any of the preceding claims, wherein the CRISPR enzyme comprises one or more mutations.

42 The method, use or composition according to claim 41, wherein the CRISPR enzyme comprises one or more mutations D10A, E762A, H840A, N854A, N863A or D986A.

43. The method, use or composition according to claim 41 wherein the one or more mutations is in a RuvC1 domain of the CRISPR enzyme.

44. The method, use or composition according to claim 40, wherein the CRISPR enzyme is a nickase directing cleavage at the location of the target sequence.

45. The method, use or composition according to claim 44, wherein the nickase is a double nickase.

46. The method, use or composition according to any preceding claim further comprising at least two or more NLS.

47. The method, use or composition according to any preceding claim, wherein the CRISPR enzyme has one or more mutations in a catalytic domain, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the enzyme further comprises a functional domain.

48. The method, use or composition according to claim 47, wherein the functional domain is a transcriptional activation domain.

49. The method, use or composition according to claim 48, wherein the transcriptional activation domain is VP64.

50. The method of any one of claims 1-25 or 32-49 further comprising minimizing off-target modifications by manipulation of a first and a second target sequence on opposite strands of a DNA duplex in a genomic locus of interest in a cell comprising

delivering a non-naturally occurring or engineered composition comprising :

I. a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises:

(a) a first guide sequence capable of hybridizing to the first target sequence,

(b) a first tracr mate sequence,

(c) a first tracr sequence,

(d) a second guide sequence capable of hybridizing to the second target sequence,

(e) a second tracr mate sequence, and

(f) a second tracr sequence, and

optionally, wherein a linker sequence is present between the first tracr sequence and the second guide sequence, whereby the first guide sequence and the second guide sequence are in tandem; and

II. a polynucleotide sequence encoding a CRISPR enzyme comprising at least one or more nuclear localization sequences, wherein (a), (b), (c), (d), (e) and (f) are arranged in a 5' to 3' orientation, wherein the polynucleotide sequence comprises a linker sequence between the first tracr sequence and the second guide sequence, whereby the first guide sequence and the second guide sequence are in tandem, and wherein when transcribed, the first and the second tracr mate sequence hybridize to the first and second tracr sequence respectively and the first and the second guide sequence directs sequence-specific binding of a first and a second CRISPR complex to the first and second target sequences respectively,

or

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and wherein components I and II are located on the same or different vectors of the system, and when transcribed, a first tracr mate sequence hybridizes to a first tracr sequence and the first and the second guide sequence directs sequence-specific binding of a first and a second CRISPR complex to the first and second target sequences respectively;

wherein the first CRISPR complex comprises the CRISPR enzyme complexed with (1) the first guide sequence that is hybridized or hybridizable to the first target sequence, and (2) the first tracr mate sequence that is hybridized or hybridizable to the first tracr sequence,

wherein the second CRISPR complex comprises the CRISPR enzyme complexed with (1) the second guide sequence that is hybridized or hybridizable to the second target sequence, and (2) the second tracr mate sequence that is hybridized or hybridizable to the second tracr sequence,

wherein the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA, and

wherein the first guide sequence directs cleavage of one strand of the DNA duplex near the first target sequence and the second guide sequence directs cleavage of other strand near the second target sequence inducing a double strand break, thereby modifying the organism or the non-human organism by minimizing off-target modifications.

51. The composition, method or use of any one of the preceding claims wherein the hepatic target sequence is proprotein convertase subtilisin kexin 9 (PCSK9).

52. The composition, method or use of any one of the preceding claims wherein the hepatic target sequence is SERPINA1.

53. The composition, method or use of any one of the preceding claims wherein the hepatic target sequence is Hmgcr.

54. The composition, method or use of any one of the preceding claims wherein the hepatic target sequence is Apolipoprotein B (ApoB).

55. The composition, method or use of any one of the preceding claims wherein the hepatic target sequence is low density lipoprotein receptor (LDL-R).

CLAIMS

1. Use of a composition comprising a CRISPR-Cas system comprising:

a Cas9 comprising at least one nuclear localization sequence, a guide sequence capable of hybridizing to a hepatic target sequence expressed in liver and associated with a gene involved in a liver disease or disorder, a tracr mate sequence and a tracr sequence, or

one or more polynucleotides encoding the CRISPR-Cas system

in the preparation of a medicament for treating a liver disease or disorder in an organism.

2. Use according to Claim 1, in which expression of a gene product is altered by the CRISPR-Cas system in liver, thereby producing a phenotypic change.

3. Use according to Claim 1 or 2, in which the composition is to be delivered to liver cells in an organism.

4. Use according to Claim 1, 2 or 3, in which the medicament is for treating or inhibiting a condition caused by a defect in the hepatic target sequence expressed in liver.

5. Use according to Claim 1, in which the composition comprises one or more viral vectors comprising:

(a) a first regulatory element operably linked to a polynucleotide sequence encoding the guide sequence, the tracr mate sequence, and the tracr sequence, and

(b) a second regulatory element operably linked to a polynucleotide sequence encoding the Cas9 comprising at least one nuclear localization sequence,

in which components (a) and (b) are located on same or different vectors.

6. Use according to Claim 1, in which the composition comprises:

(a) a polynucleotide sequence comprising, from 5’ to 3’, the guide sequence, the tracr mate sequence, and the tracr sequence, and

(b) a polynucleotide sequence encoding the Cas9 and at least one nuclear localization sequence, in which the tracr mate sequence is capable of hybridizing to the tracr sequence, in which the guide sequence is capable of directing sequence-specific binding

of a CRISPR complex comprising the Cas9 to the target sequence, and in which the polynucleotide sequence encoding the Cas9 is DNA or RNA.

7. Use according to Claim 1, in which the composition comprises:

(a) a polynucleotide sequence comprising the guide sequence and the tracr mate sequence,

(b) a polynucleotide sequence encoding the Cas9 and at least one nuclear localization sequence, and

(c) a polynucleotide sequence comprising the tracr sequence,

in which the tracr mate sequence is capable of hybridizing to the tracr sequence, in which the guide sequence is capable of directing sequence-specific binding of a CRISPR complex comprising the Cas9 to the target sequence, and in which the polynucleotide sequence encoding a Cas9 is DNA or RNA.

8. Use according to any preceding claim, in which expression of two or more hepatic gene products is altered.

9. Use according to any of Claims 1 to 6, in which the guide sequence is fused to the tracr mate sequence and the tracr sequence.

10. Use according to any one Claims 1 to 7, in which the polynucleotide sequence encoding the Cas9 is codon optimized for expression in the organism.

11. Use according to any of Claims 1 to 7, in which the organism is a mammal.

12. Use according to any of Claims 1 to 7, in which the Cas9 is *S. pyogenes* or *S. aureus* Cas9.

13. Use according Claim 5, in which the one or more viral vectors is an AAV or lentiviral vector.

14. Use according to Claim 13, in which the viral vector is an AAV2/8 vector.

15. Use according to any of Claims 5, 6, or 7, in which (a) and (b) are located on the same viral vector.

16. Use according to Claim 15, in which the viral vector is an AAV vector and the Cas9 is *S. aureus* Cas9.

17. Use according to any of Claims 1 to 7, in which the hepatic target sequence comprises a polynucleotide sequence encoding proprotein convertase subtilisin kexin 9 (PCSK9), Alpha-1 Antitrypsin (A1AT), SERPINA1, Hmgcr, Apolipoprotein B (ApoB), or low density lipoprotein receptor (LDL-R).

18. Use according to any of Claims 1 to 7, in which the polynucleotide sequence encoding the Cas9 is operably linked to a liver-specific promoter.

19. Use according to any of Claims 1 to 7, in which the polynucleotide sequence encoding the Cas9 is operably linked to a TBG promoter.

20. Use according to any of Claims 1 to 7, in which the liver disease or disorder is AAT deficiency.

21. Use according to any of Claims 1 to 7, in which one or more deficient genotypes are corrected.

22. Use according to any of Claims 1 to 7, in which the CRISPR-Cas system is to be delivered as RNA polynucleotides using a stable nucleic acid-lipid particle (SNALP).

23. Use according to any of Claims 1 to 7, in which the CRISPRCas system is to be delivered using a AAV2/8 vector.