

WHAT IS CLAIMED IS:

- 1 1. A method of editing the genome of a cell, wherein the cell is a primary
2 hematopoietic cell or a primary hematopoietic stem cell, the method comprising:
3 a) providing a reaction mixture comprising a Cas9 ribonucleoprotein complex
4 and the cell, wherein the Cas9 ribonucleoprotein complex comprises a Cas9 nuclease domain
5 and a guide RNA, wherein the guide RNA specifically hybridizes to a target region of the
6 genome of the cell; and
7 b) introducing the Cas9 ribonucleoprotein complex inside the cell.
- 1 2. The method of claim 1, wherein the method provides an efficiency of
2 genome editing of at least about 20%.
- 1 3. The method of claim 1, wherein the cell does not contain a nucleic acid
2 encoding the Cas9 and/or a DNA nucleic acid encoding a guide RNA.
- 1 4. The method of claim 1, wherein prior to the providing of a) the cell is
2 not immortalized or transformed.
- 1 5. The method of claim 4, wherein after the introducing of b) the cell is
2 not immortalized or transformed.
- 1 6. The method of claim 1, wherein the cell has not been passaged prior to
2 the providing of a).
- 1 7. The method of claim 6, wherein prior to the providing of a), the cell
2 has been directly isolated from a host organism or tissue and cultured.
- 1 8. The method of claim 6, wherein prior to the providing of a), the cell
2 has been directly isolated from a host organism or tissue and has not been cultured.
- 1 9. The method of claim 1, wherein the introducing comprises
2 electroporation.
- 1 10. The method of claim 1, wherein the introducing comprises:
2 coating a nanowire or nanotube with the Cas9 ribonucleoprotein complex;
3 contacting the cell with the nanowire or nanotube coated with the Cas9
4 ribonucleoprotein complex; and

5 piercing a cell membrane of the cell with the nanowire or nanotube coated
6 with the Cas9 ribonucleoprotein complex.

1 11. The method of claim 1, wherein the introducing comprises:
2 forcing the reaction mixture through a cell deforming constriction that is
3 smaller than the diameter of the cell, wherein the forcing introduces transient pores into a cell
4 membrane of the cell; and
5 allowing the Cas9 ribonucleoprotein complex to enter the cell through the
6 transient pores.

1 12. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a ligand for an extracellular receptor on the cell, and the introducing comprises
3 receptor mediated internalization of the Cas9 ribonucleoprotein complex.

1 13. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a cell penetrating peptide, and the introducing comprises contacting the cell
3 penetrating peptide to the cell.

1 14. The method of claim 9, wherein the electroporation comprises
2 positioning the reaction mixture into a chamber between a cathode and an anode, and
3 applying a voltage potential between the cathode and the anode of from about 20 kV/m to
4 about 100 kV/m.

1 15. The method of claim 14, wherein the voltage potential is applied as a
2 pulse having a length of from about 5 ms to about 100 ms.

1 16. The method of claim 15, wherein the method further comprises
2 repeating the application of the voltage potential pulse from 2 to 10 times.

1 17. The method of claim 14, wherein:
2 the chamber is a hollow member having a longitudinal length and a horizontal
3 cross sectional area;
4 the chamber comprises a first and second distal end separated by the
5 longitudinal length; and
6 the chamber has:
7 a first electrode at the first distal end; and

8 a reservoir containing an electrolytic solution in fluid communication
9 with the second distal end of the chamber, said reservoir having a second electrode.

1 18. The method of claim 17, wherein the chamber has a ratio of
2 longitudinal length to horizontal cross-sectional area in the range of 50 to 10,000.

1 19. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 in the reaction mixture is at a concentration of from about 0.25 μM to about 5 μM .

1 20. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 in the reaction mixture is at a concentration of from about 0.9 μM to about 1.8 μM .

1 21. The method of claim 1, wherein the reaction mixture contains from
2 about 1×10^5 to about 4×10^5 primary hematopoietic cells or primary hematopoietic stem
3 cells.

1 22. The method of claim 1, wherein the reaction mixture contains from
2 about 2×10^5 to about 2.5×10^5 primary hematopoietic cells or primary hematopoietic stem
3 cells.

1 23. The method of claim 1, wherein the cell is a primary hematopoietic
2 cell.

1 24. The method of claim 23, wherein the primary hematopoietic cell is an
2 immune cell.

1 25. The method of claim 24, wherein the immune cell is a T cell.

1 26. The method of claim 25, wherein the T cell is a regulatory T cell, an
2 effector T cell, or a naïve T cell.

1 27. The method of claim 26, wherein the regulatory T cell, effector T cell,
2 or naïve T cell is a CD4^+ T cell.

1 28. The method of claim 25, wherein the T cell is a $\text{CD4}^+\text{CD25}^{\text{hi}}\text{CD127}^{\text{lo}}$
2 regulatory T cell.

1 29. The method of claim 25, wherein the T cell is a FOXP3^+ T cell.

- 1 30. The method of claim 25, wherein the T cell is a $CD4^{+}CD25^{lo}CD127^{hi}$
2 effector T cell.
- 1 31. The method of claim 25, wherein the T cell is a
2 $CD4^{+}CD25^{lo}CD127^{hi}CD45RA^{hi}CD45RO^{-}$ naïve T cell.
- 1 32. The method of claim 25, wherein the T cell is a $CD8^{+}$ T cell.
- 1 33. The method of claim 25, wherein the T cell is a $CD4^{+}CD8^{+}$ T cell.
- 1 34. The method of claim 25, wherein prior to the providing of a), the T cell
2 is pre-activated.
- 1 35. The method of claim 25, wherein prior to the providing of a), the T cell
2 is unstimulated.
- 1 36. The method of claim 25, wherein the T cell comprises a recombinant
2 antigen receptor.
- 1 37. The method of claim 1, wherein the reaction mixture further comprises
2 a single-stranded oligonucleotide DNA template, and wherein the method comprises
3 introducing the single-stranded oligonucleotide DNA template inside the cell.
- 1 38. The method of claim 37, wherein the single-stranded oligonucleotide
2 DNA template is at a concentration of from about 9 μ M to about 180 μ M.
- 1 39. The method of claim 37, wherein the single-stranded oligonucleotide
2 DNA template is at a concentration of about 45 μ M.
- 1 40. The method of any one of claims 37-39, wherein the method provides
2 an efficiency of primary hematopoietic cell or primary hematopoietic stem cell genome
3 editing of at least about 30%.
- 1 41. The method of any one of claims 37-39, wherein the method provides
2 an efficiency of primary hematopoietic cell or primary hematopoietic stem cell template
3 directed genome editing of at least about 10%, or at least about 14%.

- 1 42. The method of any one of claims 37-39, wherein the single stranded
2 oligonucleotide DNA template encodes a recombinant antigen receptor, a portion thereof, or
3 a component thereof.
- 1 43. The method of any one of claims 1 to 41, wherein the cell is a T cell,
2 and the method further comprises:
3 c) after the introducing of b), transferring the reaction mixture to a culture
4 medium containing a CD3 agonist and a CD28 agonist and culturing the cells.
- 1 44. The method of claim 43, wherein the CD3 agonist and the CD28
2 agonist are immobilized on a solid surface.
- 1 45. The method of claim 43, wherein the CD3 agonist is an anti-CD3
2 antibody.
- 1 46. The method of claim 43, wherein the CD28 agonist is an anti-CD28
2 antibody.
- 1 47. The method of claim 43, wherein the method further comprises:
2 c) after the culturing of c), transferring the reaction mixture to a culture
3 medium that does not contain a CD3 agonist or a CD28 agonist and culturing the cells.
- 1 48. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a Cas9 nuclease.
- 1 49. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a Cas9 nickase.
- 1 50. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a Cas9 nuclease domain fused to a restriction endonuclease or nickase.
- 1 51. The method of claim 1, wherein the Cas9 ribonucleoprotein complex
2 comprises a Cas9 nuclease domain fused to a transcriptional modulator or a chromatin
3 modifier.
- 1 52. The method of claim 1, wherein the reaction mixture comprises at least
2 two structurally different Cas9 ribonucleoprotein complexes.

- 1 53. The method of claim 52, wherein the at least two structurally different
2 Cas9 ribonucleoprotein complexes contain structurally different sgRNAs.
- 1 54. The method of claim 52, wherein the at least two structurally different
2 Cas9 ribonucleoprotein complexes contain structurally different Cas9 domains.
- 1 55. A method of editing the genome of a cell, wherein the cell is a primary
2 hematopoietic cell or a primary hematopoietic stem cell, the method comprising:
3 a) providing a reaction mixture comprising a Cas9 nuclease domain and the
4 cell; and
5 b) introducing the Cas9 nuclease domain inside the cell,
6 wherein the Cas9 nuclease domain forms a complex with a guide RNA inside the cell.
- 1 56. The method of claim 55, wherein the guide RNA inside the cell is
2 encoded by a guide RNA gene inside the cell, wherein the guide RNA gene comprises DNA.
- 1 57. The method of claim 55 or 56, wherein the cell does not contain a
2 nucleic acid encoding the Cas9 nuclease domain.
- 1 58. The method of claim 55, wherein the efficiency of Cas9 delivery is at
2 least about 20% or 30%.
- 1 59. A plurality of primary hematopoietic cells or primary hematopoietic
2 stem cells, wherein the plurality of cells do not contain a nucleic acid encoding Cas9 and/or a
3 DNA nucleic acid encoding a guide RNA, and wherein at least 20% of the plurality of cells
4 contains a Cas9 ribonucleoprotein complex.
- 1 60. The plurality of cells of claim 59, wherein at least 30% of the plurality
2 of cells contains a Cas9 ribonucleoprotein complex.
- 1 61. The plurality of cells of claim 59, wherein at least 20% of the plurality
2 of cells contains a Cas9 ribonucleoprotein complex and a single stranded oligonucleotide
3 DNA template.
- 1 62. The plurality of cells of claim 59, wherein the plurality of cells have
2 not enriched for the presence of the Cas9 ribonucleoprotein complex.

- 1 63. The plurality of cells of claim 59, wherein at least 20% or 30% of the
2 plurality of cells contains a double stranded break, or an NHEJ or HDR repaired double
3 stranded break at a target genomic region.