

WHAT IS CLAIMED IS:

1. A method of producing a cell comprising edits at two or more target loci:
 - (a) combining two or more guide RNAs (gRNAs) capable of directing CRISPR/Cas9-mediated indel formation at respective target loci with Cas9 protein to form a ribonucleoprotein complex (RNP);
 - (b) serially transfecting a population of cells with the RNP until at least about 50% indel formation is achieved at each target locus; and
 - (c) isolating a cell comprising edits at two or more target loci by single cell cloning of the cell from the population of serially transfected cells.
2. The method of claim 1, wherein the gRNA is an sgRNA.
3. The method of claim 1, wherein the gRNA comprises a crRNA and a tracrRNA.
4. The method of claim 3, wherein the crRNA is an XT-gRNA.
5. The method of any of claims 1-4, wherein the population of cells is serially transfected with the RNP until at least about 60% indel formation is achieved at each target locus.
6. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is between about 0.1 pmol per 10^6 cells to about 5 pmol per 10^6 cells
7. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 0.15 pmol per 10^6 cells.
8. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 0.17 pmol per 10^6 cells.
9. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 0.2 pmol per 10^6 cells.
10. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 1 pmol per 10^6 cells.
11. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 2 pmol per 10^6 cells.

12. The method of claim 1, wherein the ratio of moles of RNP to number of transfected cells is about 3 pmol per 10^6 cells.
13. The method of claim 1, wherein three or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
14. The method of claim 1, wherein four or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
15. The method of claim 1, wherein five or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
16. The method of claim 1, wherein six or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
17. The method of claim 1, wherein seven or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
18. The method of claim 1, wherein eight or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a

population of cells until at least about 50% indel formation is achieved at each target locus.

19. The method of claim 1, wherein nine or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
20. The method of claim 1, wherein ten or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are combined with Cas9 protein to produce RNPs and the RNPs are serially transfecting into a population of cells until at least about 50% indel formation is achieved at each target locus.
21. The method of any one of claims 13-20 wherein the RNPs are serially transfecting into a population of cells until at least about 60% indel formation is achieved at each target locus.
22. The method of any one of claims 1-21, wherein the cell is a T cell, an NK cell, a B cell, a dendritic cell, a CHO cell, a COS-7 cell; an HEK 293 cell, a BHK cells, a TM4 cell, a CV1 cell; a VERO-76 cell; a HELA cells; or an MDCK cell.
23. The method of claim 1, wherein the two or more gRNAs capable of directing CRISPR/Cas9-mediated indel formation at respective target loci are identified via a efficiency screen comprising:
 - (a) transfecting a population of cells with a population of RNPs, where each RNP comprises a gRNA capable of directing CRISPR/Cas9-mediated indel formation at a target locus; and
 - (b) sequencing the target loci to identify gRNAs based on their efficiency in directing CRISPR/Cas9-mediated indel formation.
24. The method of claim 23, wherein the sequencing is performed using Sanger sequencing.