CLAIMS

1. A method of generating a genetically modified non-human mammal, the method comprising

introducing, via electroporation, a Cas9 protein and a guide RNA (gRNA), into a zygote of a non-human mammal to modify the genome of the non-human mammal, wherein said Cas9 protein is electroporated at a final concentration of 150-350 ng/μL; and

transferring the zygote to a pseudopregnant host mammal and allowing the zygote to develop into a live-born genetically modified non-human mammal.

2. The method of claim 1, wherein said electroporation comprises multiple cycles, wherein each cycle includes 1-3 pulses, and each pulse is delivered at 20-40 volts for a duration of 1-2 ms.

3. The method of claim 1 or 2, wherein each cycle of includes 1-3 pulses, the interval between each pulse is 100-1000 ms, and each pulse is delivered at 20-40 volts.

4. The method of claim 3, wherein each cycle includes 2 pulses, the interval between each pulse is 100 ms, and each pulse is delivered at 30 volts for 1.0 ms or 1.5 ms.

5. The method of any one of claims 1-4, wherein the CRISPR-Cas system comprises a single guide RNA (sgRNA) that hybridizes to a target sequence in the genome of the non-human mammal, and wherein the sgRNA is electroporated at a final concentration of 200 ng/μL to400 ng/μL(*e.g.*, 200 ng/μL, 250 ng/μL, 300 ng/μL, 350 ng/μL, or 400 ng/μL).

6. The method of any one of claims 1-5, wherein said Cas9 protein is electroporated into the zygote with a donor polynucleotide.

7. The method of claim 6, wherein said donor polynucleotide is a linear or circular double stranded DNA molecule.

8. The method of any one of claims 1-7, wherein the genome of the non-human mammal is modified by insertion or deletion of one or more base pairs, by insertion of a heterologous

DNA fragment(*e.g.*, the donor polynucleotide), by deletion of an endogenous DNA fragment, by inversion or translocation of an endogenous DNA fragment, or a combination thereof.

9. The method of any one of claims 1-8, wherein the genome of the non-human mammal is modified by non-homologous end joining (NHEJ), homology directed repair (HDR), or homologous recombination (HR).

10. The method of claim 8 or 9, wherein the method achieves a knock-in (KI) efficiency of at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more (*e.g.*, 100%).

11. The method of claim 8 or 9, wherein the method achieves a NHEJ efficiency of at least 80%, 85%, 90%, 95%, 97%, or 100%.

12. The method of claim 8 or 9, wherein the method achieves deletion of an endogenous genomic region of at least 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb or more, in IVF (in vitro fertilization)-generated embryos at a rate of at least 10%, 15%, 20%, 25%, 30%, 35%, 40% or more.

13. The method of any one of claims 1-12, wherein more than one zygotes are simultaneously electroporated.

14. The method of claim 13, wherein 2, 3, 5, 10, 20, 30, 40, 50, 100, 125, 150, 200, 250, 300 zygotes are simultaneously electroporated.

15. The method of claim 13 or 14, wherein all zygotes are electroporated in the same electroporation cuvette.

16. The method of any one of claims 1-15, wherein said electroporation is carried out in a 1-mm electroporation cuvette.

17. The method of any one of claims 1-16, wherein the zygote is pre-treated to weaken Zona Pellucida prior to electroporation.

18. The method of claim 17, wherein the zygote is pre-treated in Acidic Tyrode's solution (AT) for 5-15 seconds, or 10 seconds.

19. The method of any one of claims 1-18, wherein the zygote, after electroporation, is frozen for storage.

20. The method of any one of claims 1-19, wherein said electroporation is carried out using an ECM830 square wave electroporation system (a BTX830 type electroporator).

21. The method of any one of claims 1-20, wherein the non-human mammal is a primate (*e.g.*, marmoset, rhesus monkey, chimpanzee), a rodent (*e.g.*, mouse, rat, gerbil, Guinea pig, hamster, cotton rat, naked mole rat), a rabbit, a livestock mammal (*e.g.*, goat, sheep, pig, cow, cattle, horse, camelid), a pet mammal (*e.g.*, dog, cat), a marsupial, or an outbred or a random bred population thereof.

22. The method of claim 21, wherein the non-human mammal is a mouse or a rat.

23. A method of generating a genetically modified mouse, the method comprising: (a) combining a mouse embryo with a solution that comprises Cas9 protein and a guide RNA (gRNA) specific for a genomic target sequence in the mouse embryo; (b) performing one or multiple cycles of electroporation on the mouse embryo in the solution to produce an electroporated mouse embryo comprising the Cas9 protein and the gRNA, wherein said Cas9 protein is electroporated at a final concentration of 150-350 ng/μL; and (c) transferring the electroporated mouse embryo into a pseudopregnant female mouse, wherein the electroporated mouse embryo develops into a live-born mouse comprising a genetic modification in the genomic target sequence.