

CLAIMS

What is claimed is:

1. A method of producing an induced pluripotent stem cell (iPSC), the method comprising contacting a somatic cell with a circular RNA comprising a protein-coding sequence, wherein the protein-coding sequence encodes at least one reprogramming factor, wherein the at least one reprogramming factor is Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc, or a fragment or variant thereof, and maintaining the somatic cell under conditions under which a reprogrammed iPSC is obtained.
2. The method of claim 1, wherein the at least one reprogramming factor is a human or a humanized reprogramming factor.
3. The method of claim 1 or 2, wherein:
 - (a) the at least one reprogramming factor is Oct3/4, and wherein the Oct3/4 has the sequence of SEQ ID NO: 1, or a sequence at least 90% or at least 95% identical thereto;
 - (b) the at least one reprogramming factor is Klf4, and wherein the Klf4 has the sequence of SEQ ID NO: 2 or 3, or a sequence at least 90% or at least 95% identical thereto;
 - (c) the at least one reprogramming factor is Sox2, and wherein Sox2 has the sequence of SEQ ID NO: 4, or a sequence at least 90% or at least 95% identical thereto;
 - (d) the at least one reprogramming factor is Nanog, and wherein the Nanog has the sequence of SEQ ID NO: 5 or 6, or a sequence at least 90% or at least 95% identical thereto;
 - (e) the at least one reprogramming factor is Lin28, and wherein the Lin28 has the sequence of SEQ ID NO: 7, or a sequence at least 90% or at least 95% identical thereto;
 - (f) the at least one reprogramming factor is c-Myc, and wherein the c-Myc has the sequence of SEQ ID NO: 8 or 9, or a sequence at least 90% or at least 95% identical thereto; and/or

(g) the at least one reprogramming factor is L-Myc, and wherein the L-Myc has the sequence of any one of SEQ ID NO: 10-12, or a sequence at least 90% or at least 95% identical thereto.

4. The method of claim 3, wherein

(a) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 33, or a sequence at least 90% or at least 95% identical thereto;

(b) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 37, or a sequence at least 90% or at least 95% identical thereto;

(c) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 34, or a sequence at least 90% or at least 95% identical thereto;

(d) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 36, or a sequence at least 90% or at least 95% identical thereto;

(e) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 35, or a sequence at least 90% or at least 95% identical thereto; and/or

(f) the recombinant circular RNA comprises a nucleic acid sequence of SEQ ID NO: 38, or a sequence at least 90% or at least 95% identical thereto.

5. The method of any one of claims 1-4, wherein the circular RNA is substantially non-immunogenic, optionally wherein the circular RNA comprises one or more M-6-methyladenosine (m⁶A) residues.

6. The method of claim 5, wherein the circular RNA comprises from about 200 nucleotides to about 5,000 nucleotides, optionally wherein the circular RNA comprises an internal ribosome entry site (IRES) operably linked to the protein-coding sequence.

7. The method of any one of claims 1-6, wherein the circular RNA is complexed with a lipid nanoparticle (LNP), wherein optionally the LNP comprises a cationic lipid.

8. The method of claim 7, wherein the recombinant circular RNA and the LNP are conjugated.

9. The method of claim 8, wherein the recombinant circular RNA and the LNP are covalently conjugated or are non-covalently conjugated.
10. The method of any one of claims 1-9, wherein the method comprises contacting the somatic cell with at least 2, at least 3, at least 4, at least 5, at least 6 circular RNAs encoding Oct4, Sox2, Klf4, C-Myc, L-Myc, Lin28, or Nanog, optionally wherein a first circular RNA encodes Oct4, a second circular RNA encodes Sox2, a third circular RNA encodes Klf4, a fourth circular RNA encodes C-Myc or L-Myc, and a fifth circular RNA encodes Lin28.
11. The method of any one of claims 1-9, wherein the method comprises contacting the somatic cell with at least 2, at least 3, at least 4, at least 5, at least 6 circular RNAs encoding Oct4, Sox2, Klf4, C-Myc, L-Myc, Lin28, or Nanog, optionally wherein a first circular RNA encodes Oct4, a second circular RNA encodes Sox2, a third circular RNA encodes Klf4, a fourth circular RNA encodes C-Myc or L-Myc, a fifth circular RNA encodes Lin28, and a sixth circular RNA encodes Nanog.
12. The method of any one of claims 1-11, wherein the somatic cell is a fibroblast, a peripheral blood-derived cell, an endothelial progenitor cell, a cord-blood derived cell, a keratinocyte, a melanocyte, an adipose-tissue derived cell, or a urine-derived cell.
13. The method of any one of claims 1-12, wherein the somatic cell is a CD34+ cell.
14. The method of any one of claims 1-13, wherein the somatic cell is an adherent cell or is in suspension.
15. The method of any one of claims 1-12, wherein the somatic cell is a CD34+ cell in suspension.

16. The method of any one of claims 1-15, comprising contacting the somatic cell one or more times with at least one of the recombinant circular RNAs, optionally wherein the somatic cell is contacted two, three, or four times.
17. The method of any one of claims 1-16, wherein the method results in one or more of:
- (i) an increase in the number of reprogrammed iPSC present at the end of culture compared to a method of producing an iPSC with one or more linear RNAs;
 - (ii) an increase in the rate of reprogrammed iPSC maturation compared to a method of producing an iPSC with one or more linear RNAs; and/or
 - (iii) a decrease in cell toxicity at one or more timepoints during reprogramming compared to a method of producing an iPSC with one or more linear RNAs.
18. An iPSC produced using the method of any one of claims 1-17.
19. A differentiated cell derived from the iPSC of claim 18.
20. The differentiated cell of claim 19, wherein the differentiated cell is a muscle cell, a neuron, a cardiomyocyte, a hepatocyte, an islet cell, a keratinocyte, a T-cell, or a NK-cell.