

CLAIMS

What is claimed is:

1. A method of producing an induced pluripotent stem cell (iPSC), the method comprising contacting a blood cell in suspension with one or more circular RNAs encoding at least one reprogramming factor selected from Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc, or a fragment or variant thereof, and maintaining the cell under conditions under which the iPSC is obtained, optionally wherein the blood cell is selected from a T cell, a B cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a peripheral blood mononuclear cell (PBMC), and a cord blood mononuclear cell (CBMC).
2. A method of producing an induced pluripotent stem cell (iPSC), the method comprising contacting a CD34+ cell in suspension with one or more circular RNAs encoding at least one reprogramming factor selected from Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc, or a fragment or variant thereof, and maintaining the cell under conditions under which the iPSC is obtained.
3. The method of claim 1 or 2, wherein:
 - (a) the at least one reprogramming factor is a human or a humanized reprogramming factor;
 - (b) the at least one reprogramming factor is Oct3/4, and wherein the Oct3/4 has the amino acid sequence of SEQ ID NO: 7, or a sequence at least 90% or at least 95% identical thereto;
 - (c) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 8, or a sequence at least 90% or at least 95% identical thereto;
 - (d) the at least one reprogramming factor is Klf4, and wherein the Klf4 has the amino acid sequence of SEQ ID NO: 9 or 10, or a sequence at least 90% or at least 95% identical thereto;
 - (e) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 11, or a sequence at least 90% or at least 95% identical thereto;

(f) the at least one reprogramming factor is Sox2, and wherein Sox2 has the amino acid sequence of SEQ ID NO: 12, or a sequence at least 90% or at least 95% identical thereto;

(g) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 13, or a sequence at least 90% or at least 95% identical thereto;

(h) the at least one reprogramming factor is Nanog, and wherein the Nanog has the amino acid sequence of SEQ ID NO: 14 or 15, or a sequence at least 90% or at least 95% identical thereto;

(i) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 16, or a sequence at least 90% or at least 95% identical thereto;

(j) the at least one reprogramming factor is Lin28A, and wherein the Lin28A has the amino acid sequence of SEQ ID NO: 17, or a sequence at least 90% or at least 95% identical thereto;

(k) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 18, or a sequence at least 90% or at least 95% identical thereto;

(l) the at least one reprogramming factor is c-Myc, and wherein the c-Myc has the amino acid sequence of SEQ ID NO: 19 or 20, or a sequence at least 90% or at least 95% identical thereto;

(m) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 21, or a sequence at least 90% or at least 95% identical thereto;

(n) the at least one reprogramming factor is L-Myc, and wherein the L-Myc has the amino acid sequence of any one of SEQ ID NO: 22-24, or a sequence at least 90% or at least 95% identical thereto;

(o) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 25, or a sequence at least 90% or at least 95% identical thereto;

(p) the circular RNA is substantially non-immunogenic;

(q) the circular RNA comprises one or more M-6-methyladenosine (m⁶A) residues;

(r) the circular RNA comprises from about 200 nucleotides to about 5,000 nucleotides;

(s) the circular RNA comprises an internal ribosome entry site (IRES) operably linked to the protein-coding sequence; and/or

(t) the circular RNA encodes two or more reprogramming factors selected from Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc.

4. The method of any one of claims 1-3, wherein the method comprises:

(a) contacting the cell with a first circular RNA encoding Oct4 or a fragment or variant thereof, a second circular RNA encoding Sox2 or a fragment or variant thereof, a third circular RNA encoding Klf4 or a fragment or variant thereof, a fourth circular RNA encoding C-Myc or a fragment or variant thereof, a fifth circular RNA encoding Lin28 or a fragment or variant thereof, and a sixth circular RNA encoding Nanog or a fragment or variant thereof;

(b) contacting the cell with three circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, and a third circular RNA encoding Klf4, wherein the method does not comprise contacting the cell with a circular RNA encoding any of Nanog, Lin28, and c-Myc;

(c) contacting the cell with four circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, a third circular RNA encoding Klf4, and a fourth circular RNA encoding C-Myc, wherein the method does not comprise contacting the cell with a circular RNA encoding either of Nanog or Lin28; or

(d) contacting the cell with five circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, a third circular RNA encoding Klf4, a fourth circular RNA encoding Nanog, and a fifth circular RNA encoding Lin 28, wherein the method does not comprise contacting the CD34+ cell with a circular RNA encoding c-Myc.

5. The method of any one of claims 1-4, wherein the cell is not contacted with one or more factors selected from E3, K3, and B18R; one or more micro RNAs (miRs) selected from miR302a, miR302b, miR302c, miR302d, and miR367; or a combination thereof.

6. The method of any one of claims 1-5, wherein the cell is contacted with each of the one or more circular RNA (a) once; (b) two, three, four, or more times; (c) fewer than four times; or (d) two to four times, optionally wherein the concentration of each of the

one or more circular RNAs is at least 3 pg RNA/cell or from 5 pg RNA/cell to 15 pg RNA/cell.

7. The method of any one of claims 1-6, 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; and/or

(ii) a decrease in cell death at one or more timepoints during reprogramming compared to a method of producing an iPSC with one or more linear RNAs.

8. A method for reprogramming and editing the genome of a cell, the method comprising:

(i) contacting the cell with one or more circular RNAs comprising a protein-coding sequence, wherein the protein-coding sequence encodes at least one reprogramming factor; and

(ii) contacting the cell with an enzyme capable of editing the DNA or RNA of the cell, or a nucleic acid encoding the same,

wherein (i) occurs before, after, or approximately at the same time as (ii).

9. The method of claim 8, wherein:

(a) the at least one reprogramming factor is a human or a humanized reprogramming factor;

(b) the at least one reprogramming factor is Oct3/4, and wherein the Oct3/4 has the amino acid sequence of SEQ ID NO: 7, or a sequence at least 90% or at least 95% identical thereto;

(c) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 8, or a sequence at least 90% or at least 95% identical thereto;

(d) the at least one reprogramming factor is Klf4, and wherein the Klf4 has the amino acid sequence of SEQ ID NO: 9 or 10, or a sequence at least 90% or at least 95% identical thereto;

(e) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 11, or a sequence at least 90% or at least 95% identical thereto;

(f) the at least one reprogramming factor is Sox2, and wherein Sox2 has the amino acid sequence of SEQ ID NO: 12, or a sequence at least 90% or at least 95% identical thereto;

(g) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 13, or a sequence at least 90% or at least 95% identical thereto;

(h) the at least one reprogramming factor is Nanog, and wherein the Nanog has the amino acid sequence of SEQ ID NO: 14 or 15, or a sequence at least 90% or at least 95% identical thereto;

(i) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 16, or a sequence at least 90% or at least 95% identical thereto;

(j) the at least one reprogramming factor is Lin28A, and wherein the Lin28A has the amino acid sequence of SEQ ID NO: 17, or a sequence at least 90% or at least 95% identical thereto;

(k) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 18, or a sequence at least 90% or at least 95% identical thereto;

(l) the at least one reprogramming factor is c-Myc, and wherein the c-Myc has the amino acid sequence of SEQ ID NO: 19 or 20, or a sequence at least 90% or at least 95% identical thereto;

(m) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 21, or a sequence at least 90% or at least 95% identical thereto;

(n) the at least one reprogramming factor is L-Myc, and wherein the L-Myc has the amino acid sequence of any one of SEQ ID NO: 22-24, or a sequence at least 90% or at least 95% identical thereto;

(o) the circular RNA comprises a nucleic acid sequence of SEQ ID NO: 25, or a sequence at least 90% or at least 95% identical thereto;

(p) the circular RNA is substantially non-immunogenic;

(q) the circular RNA comprises one or more M-6-methyladenosine (m⁶A) residues;

(r) the circular RNA comprises from about 200 nucleotides to about 5,000 nucleotides;

(s) the circular RNA comprises an internal ribosome entry site (IRES) operably linked to the protein-coding sequence; and/or

(t) the circular RNA encodes two or more reprogramming factors selected from Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc.

10. The method of claim 8 or 9, wherein the method comprises:

(a) contacting the cell with a first circular RNA encoding Oct4 or a fragment or variant thereof, a second circular RNA encoding Sox2 or a fragment or variant thereof, a third circular RNA encoding Klf4 or a fragment or variant thereof, a fourth circular RNA encoding C-Myc or a fragment or variant thereof, a fifth circular RNA encoding Lin28 or a fragment or variant thereof, and a sixth circular RNA encoding Nanog or a fragment or variant thereof

(b) contacting the cell with three circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, and a third circular RNA encoding Klf4, wherein the method does not comprise contacting the cell with a circular RNA encoding any of Nanog, Lin28, and c-Myc;

(c) contacting the cell with four circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, a third circular RNA encoding Klf4, and a fourth circular RNA encoding C-Myc, wherein the method does not comprise contacting the cell with a circular RNA encoding either of Nanog or Lin28; or

(d) contacting the cell with five circular RNAs consisting of a first circular RNA encoding Oct4, a second circular RNA encoding Sox2, a third circular RNA encoding Klf4, a fourth circular RNA encoding Nanog, and a fifth circular RNA encoding Lin 28, wherein the method does not comprise contacting the CD34+ cell with a circular RNA encoding c-Myc.

11. The method of claim any one of claims 8-10, wherein the cell is not contacted with one or more factors selected from E3, K3, and B18R; one or more micro RNAs (miRs) selected from miR302a, miR302b, miR302c, miR302d, and miR367; or a combination thereof.

12. The method of any one of claims 8-11, wherein the cell is contacted with each of the one or more circular RNAs (a) once; (b) two, three, four, or more times; (c) fewer than four times; or (d) two to four times, optionally wherein the concentration of each of the one or more circular RNAs is at least 3 pg RNA/cell or from 5 pg RNA/cell to 15 pg RNA/cell.

13. The method of any one of claims 8-12, wherein the enzyme is:

(a) a transcription activator-like effector nuclease (TALEN), an argonaute endonuclease (NgAgo), a structure-guided endonuclease (SGN), an RNA-guided nuclease (RGN), an Adenosine deaminase acting on RNA (ADAR), or modified or truncated variants thereof;

(b) a Cas nuclease selected from Cas9 nuclease, a Cas12(a) nuclease (Cpf1), a Cas12b nuclease, a Cas12c nuclease, a TrpB-like nuclease, a Cas13a nuclease (C2c2), a Cas13b nuclease, a Cas 14 nuclease or a modified or truncated variant thereof;

(c) a Cas9 nuclease isolated or derived from *S. pyogenes* or *S. aureus*; or

(d) any one of APG05083.1, APG07433.1, APG07513.1, APG08290.1, APG05459.1, APG04583.1, and APG1688.1, APG05733.1, APG06207.1, APG01647.1, APG08032.1, APG05712.1, APG01658.1, APG06498.1, APG09106.1, APG09882.1, APG02675.1, APG01405.1, APG06250.1, APG06877.1, APG09053.1, APG04293.1, APG01308.1, APG06646.1, APG09748, APG07433.1, APG00969, APG03128, APG09748, APG00771, APG02789, APG09106, APG02312, APG07386, APG09980, APG05840, APG05241, APG07280, APG09866, and APG00868.

14. The method of any one of claims 8-13, wherein the method further comprises contacting the cell with a guide RNA, or a nucleic acid encoding the same.

15. A suspension culture comprising one or more CD34+ cells, wherein the CD34+ cells comprise one or more exogenous circRNAs encoding a reprogramming factor selected from Oct3/4, Klf4, Sox2, Nanog, Lin28, c-Myc, or L-Myc.