

Claims

1. A method for producing an enriched population of circular polyribonucleotides (circRNA), the method comprising:
 - (a) providing a sample comprising a population of polyribonucleotides comprising circRNA and linear polyribonucleotides (linRNA); and
 - (b) separating the circRNA from the linRNA under denaturing conditions that do not comprise the use of gel electrophoresis, thereby producing an enriched population of circRNA.
2. A method for producing an enriched population of circRNA, the method comprising:
 - (a) providing a sample comprising a population of polyribonucleotides comprising circRNA and linRNA; and
 - (b) exposing the population of polyribonucleotides to denaturing conditions that do not comprise the use of gel electrophoresis, thereby enriching the population of circRNA.
3. The method of claim 1 or 2, wherein:
 - (i) the total weight of polyribonucleotides in the population of polyribonucleotides is at least 1 μg ;
 - (ii) the total volume of the sample comprising the population of polyribonucleotides is at least 500 μL ; or
 - (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 $\text{ng}/\mu\text{L}$.
4. The method of claim 2 or 3, wherein the enriched population of circRNA is substantially free of one or more impurities or by-products, wherein the one or more impurities or by-products comprise polyacrylamide, boric acid, magnesium, or ethylenediaminetetraacetic acid (EDTA).
5. The method of any one of claims 1-4, wherein the denaturing conditions comprise a temperature of at least 50°C; or wherein the denaturing conditions comprise a temperature of at least 50°C followed by a temperature of not greater than 8°C within a time period of no greater than 30 seconds.
6. The method of any one of claims 1-5, wherein the denaturing conditions comprise a pH of less than 5 or greater than 9.
7. The method of any one of claims 1-6, wherein the denaturing conditions comprise a chemical treatment comprising treatment with an acid, base, organic solvent, chaotropic agent, crowding agent, chelator, detergent, or salt solution.

8. The method of any one of claims 1-7, wherein step (b) comprises performing column chromatography on the population of polyribonucleotides, wherein performing column chromatography comprises an equilibration step, sample loading step, column washing step, and elution step; wherein the separating is performed during the sample loading step, the column washing step, and/or during the elution step, wherein the column chromatography comprises fast protein liquid chromatography (FPLC), high-pressure liquid chromatography (HPLC), hydrophobic interaction chromatography (HIC), anion exchange chromatography (AEC), mixed mode chromatography, or affinity chromatography.
9. The method of any one of claims 1-8, wherein the circRNA and the linRNA have the same ribonucleotides sequence; and/or wherein the circRNA and the linRNA have the same mass; and/or wherein the circRNA and the linRNA lack a poly(A) tail.
10. The method of any one of claims 1-9, wherein the method comprises exonuclease digestion of the linRNA or wherein the method does not comprise exonuclease digestion of the linRNA; and/or wherein the method does not comprise a selective modification to the circRNA or to the linRNA that improves enrichment of the circRNA.
11. The method of any one of claims 1-10, wherein the percent (w/w) of the circRNA in the enriched population of circRNA is 2-fold greater than the percent (w/w) of the circRNA in the polyribonucleotide population; and/or wherein the percent (w/w) of the circRNA in the enriched population of circRNA is at least 65%; and/or wherein the percent (w/w) of the linRNA in the enriched population of circRNA is less than 35%.
12. A composition comprising a population of polyribonucleotides comprising circRNA and linRNA, wherein the population of polyribonucleotides is in solution under denaturing conditions, and wherein:
- (i) the total mass of polyribonucleotides in the population of polyribonucleotides is at least 1 μg ;
 - (ii) the total volume of the sample comprising the population of polyribonucleotides is at least 500 μL ; or
 - (iii) the concentration of the population of polyribonucleotides in the sample is at least 500 $\text{ng}/\mu\text{L}$.
13. A composition comprising an enriched population of circRNA, wherein:
- (a) the composition is obtained from a sample comprising a population of polyribonucleotides comprising circRNA and linRNA;
 - (b) the composition has been exposed to one or more denaturing conditions; and
 - (c) the composition is substantially free of one or more impurities or by-products.

14. The composition of claim 12 or 13, wherein the denaturing conditions comprise a temperature of at least 50°C; or wherein the denaturing conditions comprise a temperature of at least 50°C followed by a temperature of not greater than 8°C.
15. The composition of any one of claims 12-14, wherein the denaturing conditions comprise a pH of less than 5 or greater than 9.
16. The composition of any one of claims 12-15, wherein the denaturing conditions comprise a chemical treatment comprising treatment with an acid, base, organic solvent, chaotropic agent, crowding agent, chelator, detergent, or salt solution.
17. The composition of any one of claims 12-16, wherein the percent (w/w) of the circRNA in the enriched population of circRNA is 2-fold greater than the percent (w/w) of the circRNA in the polyribonucleotide population; and/or wherein the percent (w/w) of the circRNA in the enriched population of circRNA is at least 65%; and/or wherein the percent (w/w) of the linRNA in the enriched population of circRNA is less than 35%.
18. The composition of any one of claims 12-17, wherein the circRNA and the linRNA have the same ribonucleotides sequence; and/or wherein the circRNA and the linRNA have the same mass; and/or wherein the circRNA and the linRNA lack a poly(A) tail.
19. A method of determining the purity of a circRNA, the method comprising:
- (a) providing a sample comprising a population of polyribonucleotides comprising circRNA and linRNA;
 - (b) separating the circRNA from the linRNA under denaturing conditions by chromatography; and
 - (c) collecting a chromatogram of the sample comprising a peak for the circRNA and a peak for the linRNA;
 - (d) calculating the area under each peak to determine the purity of the circRNA in the sample.
20. The method of claim 19, wherein the denaturing conditions do not comprise the use of gel electrophoresis.