

METHODS FOR ENRICHMENT OF CIRCULAR RNA UNDER DENATURING CONDITIONS

Background

Polyribonucleotides are useful for a variety of therapeutic and engineering applications. Thus, new compositions and methods for separating and purifying polyribonucleotides are useful.

Summary of the Invention

The present disclosure is directed, generally, to methods for the enrichment of circular polyribonucleotides (circRNA), e.g., from population of polyribonucleotides containing circRNA and linear polyribonucleotides (linRNA), where the enrichment is performed under denaturing conditions. Also disclosed are compositions including a population of polyribonucleotides containing circRNA and linRNA in a solution under denaturing conditions. Further within the scope of the present disclosure are compositions containing an enriched population of circRNA, such as a composition that was produced by exposing the composition to one or more denaturing conditions. The present disclosure is based, in part, on the inventors' discovery that separation of circRNA under denaturing conditions (e.g., thermal denaturation, pH, or chemical treatment) is a robust method for purification and enrichment of circRNA from a population of mixed polyribonucleotides containing circRNA, linRNA, or other impurities or by-products. Moreover, the disclosed methods facilitate scaling up of circRNA purification processes, thereby allowing for the production and purification of large quantities of circRNA.

In an aspect, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) separating the circRNA from the linRNA under denaturing conditions that do not include the use of gel electrophoresis, thereby producing an enriched population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA. In an aspect, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including 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. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA. In some embodiments, (i) the total weight of polyribonucleotides in the population of polyribonucleotides is at least 1 μ g (e.g., at least 5 μ g, at least 10, at least 25 μ g, at least 50 μ g, at least 100 μ g, at least 250 μ g, at least 500 μ g, at least 750 μ g, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 μ g and 100 μ g, 5 μ g and 500 μ g, 5 μ g and 1 mg, 5 μ g and 10 mg, 500 μ g and 100 mg, 500 μ g and 1 g, 500 μ g and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including the population of polyribonucleotides is at least 500 μ L (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 μ L and 100 mL, 500 μ L and 1 L, 500 μ L and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 ng/ μ L (e.g., at least 500 ng/ μ L, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/ μ L and 100 mg/mL, 200 ng/ μ L and 50 mg/mL, or 1 mg/mL and 50 mg/mL). In some embodiments, the circRNA has a length from

about 100 nucleotides to about 20,000 nucleotides (e.g., about 100 nucleotides to about 500 nucleotides, about 100 nucleotides to about 750 nucleotides, about 100 nucleotides to about 1,000 nucleotides, about 100 nucleotides to about 2,500 nucleotides, about 100 nucleotides to about 5,000 nucleotides, about 100 nucleotides to about 10,000 nucleotides, about 500 nucleotides to about 750 nucleotides, about 750 nucleotides to about 1,000 nucleotides, about 750 nucleotides to about 1,250 nucleotides, about 1,000 nucleotides to about 1,250 nucleotides, about 1,000 nucleotides to about 2,000 nucleotides, about 1,000 nucleotides to about 5,000 nucleotides, about 2,500 nucleotides to about 5,000 nucleotides, about 5,000 nucleotides to about 10,000 nucleotides, about 5,000 nucleotides to about 15,000 nucleotides, about 10,000 nucleotides to about 15,000 nucleotides, about 15,000 nucleotides to about 20,000 nucleotides, about 100 nucleotides, about 200 nucleotides, about 300 nucleotides, about 400 nucleotides, about 500 nucleotides, about 600 nucleotides, about 700 nucleotides, about 800 nucleotides, about 900 nucleotides, about 1,000 nucleotides, about 1,250 nucleotides, about 1,500 nucleotides, about 1,750 nucleotides, about 2,000 nucleotides, about 3,000 nucleotides, about 4,000 nucleotides, about 5,000 nucleotides, about 6,000 nucleotides, about 7,000 nucleotides, about 8,000 nucleotides, about 9,000 nucleotides, about 10,000 nucleotides, about 11,000 nucleotides, about 12,000 nucleotides, about 13,000 nucleotides, about 14,000 nucleotides, about 15,000 nucleotides, about 16,000 nucleotides, about 17,000 nucleotides, about 18,000 nucleotides, about 19,000 nucleotides, or about 20,000 nucleotides). In some embodiments, the circRNA has a length of less than 1,000 nucleotides.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA, wherein: (i) the total weight of polyribonucleotides in the population of polyribonucleotides is at least 1 μ g (e.g., at least 5 μ g, at least 10, at least 25 μ g, at least 50 μ g, at least 100 μ g, at least 250 μ g, at least 500 μ g, at least 750 μ g, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 μ g and 100 μ g, 5 μ g and 500 μ g, 5 μ g and 1 mg, 5 μ g and 10 mg, 500 μ g and 100 mg, 500 μ g and 1 g, 500 μ g and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including the population of polyribonucleotides is at least 500 μ L (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 μ L and 100 mL, 500 μ L and 1 L, 500 μ L and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 ng/ μ L (e.g., at least 500 ng/ μ L, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/ μ L and 100 mg/mL, 200 ng/ μ L and 50 mg/mL, or 1 mg/mL and 50 mg/mL); and (b) separating the circRNA from the linRNA under denaturing conditions, thereby producing an enriched population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In some embodiments, the total weight of polyribonucleotides in the population of polyribonucleotides is between 1 μ g and 1000 mg (e.g., between 5 μ g and 10 mg). In some embodiments, the total volume of the sample including the population of polyribonucleotides is between 500 μ L and 1000 mL. In some embodiments, the concentration of the population of polyribonucleotides in the sample is between 200 ng/ μ L and 50 mg/mL.

In some embodiments, the circRNA has a length from about 100 nucleotides to about 20,000 nucleotides (e.g., about 100 nucleotides to about 500 nucleotides, about 100 nucleotides to about 750 nucleotides, about 100 nucleotides to about 1,000 nucleotides, about 100 nucleotides to about 2,500

mM, 60-225 mM, 70-200 mM, 80-175 mM, 90-150 mM, 100-125 mM, or 110-115 mM) sodium hydroxide, potassium hydroxide, imidazole, histidine, sodium bicarbonate, guanidine, or triethylamine.

In some embodiments, the organic solvent includes at least 0.1% (v/v) (e.g., at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or greater; or between 0.01% and 10%) of dimethyl sulfoxide, triethylammonium acetate, methanol, ethanol, 2-propanol, isopropanol, 1-butanol, 2-butanol, t-butanol, isobutanol, phenol, chloroform, hexane, acetonitrile, formamide, acetone, denatonium, or propylene glycol.

In some embodiments, the chaotropic agent includes between 100 mM and 8 M urea (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between 900 mM and 2 M, between 1 M and 1.5 M, between 1.1 M and 1.4 M, or between 1.2 and 1.3 M), guanidinium chloride, lithium perchlorate, or polyethylene glycol (PEG). In some embodiments, the chaotropic agent includes between 100 mM and 8 M (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between 900 mM and 2 M, between 1 M and 1.5 M, between 1.1 M and 1.4 M, or between 1.2 and 1.3 M) n-dodecyl β -D-maltoside, n-octylglucoside, CHAPS, or deoxycholate.

In some embodiments, the crowding agent includes between 100mM and 8 M (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between 900 mM and 2 M, between 1 M and 1.5 M, between 1.1 M and 1.4 M, or between 1.2 and 1.3 M) PEG, or urea.

In some embodiments, the chelator includes between 1 mM and 10 mM (e.g., 1-2 mM, 2-3 mM, 3-4 mM, 4-5 mM, 5-6 mM, 6-7 mM, 7-8 mM, 8-9 mM, or 9-10 mM) ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetra acetic acid (EGTA) or derivatives thereof, EDTA or derivatives thereof, nitrilotriacetic acid (NTA), imino-disuccinic acid (IDS), polyaspartic acid, *S,S*-ethylenediamine-*N,N'*-disuccinic acid (EDDS), or methylglycinediacetic acid (MGDA).

In some embodiments, the detergent includes between 0.005% and 0.05% (v/v) (e.g., 0.006-0.045%, 0.007-0.04%, 0.008-0.035%, 0.009-0.03%, 0.01-0.025%, 0.011-0.02%, 0.012-0.019%, 0.013-0.018%, 0.014-0.017%, or 0.015-0.016%) Nonidet P-40 (NP40), CHAPS, octyl β -D-glucopyranoside, n-dodecyl β -D-maltoside, Tween-20, or Tween-80. In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) separating the circRNA from the linRNA at a temperature of at least 50°C (e.g., at least 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, or 100°C), thereby producing an enriched population of circRNA, wherein the separating does not include the use of gel electrophoresis. Also contemplated is a method of separating a population of circRNA from a population of linRNA under

the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA, wherein: (i) the total mass of polyribonucleotides in the population of polyribonucleotides is at least 1 µg (e.g., at least 5 µg, at least 10, at least 25 µg, at least 50 µg, at least 100 µg, at least 250 µg, at least 500 µg, at least 750 µg, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 µg and 100 µg, 5 µg and 500 µg, 5 µg and 1 mg, 5 µg and 10 mg, 500 µg and 100 mg, 500 µg and 1 g, 500 µg and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including the population of polyribonucleotides is at least 500 µL (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 µL and 100 mL, 500 µL and 1 L, 500 µL and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 ng/µL (e.g., at least 500 ng/µL, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/µL and 100 mg/mL, 200 ng/µL and 50 mg/mL, or 1 mg/mL and 50 mg/mL); and (b) separating the circRNA from the linRNA at a temperature of at least 50°C (e.g., at least 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, or 100°C), thereby producing an enriched population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) exposing the population of polyribonucleotides to a temperature of at least 50°C (e.g., at least 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, or 100°C), thereby enriching the population of circRNA. In some embodiments, the temperature is between 50°C and 85°C. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) separating the circRNA from the linRNA at a pH of less than 5 (e.g., less than 4, 3, 2, or 1) or greater than 9 (e.g., greater than 10, 11, 12, or 13), thereby producing an enriched population of circRNA, wherein the separating does not include the use of gel electrophoresis. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA, wherein: (i) the total mass of polyribonucleotides in the population of polyribonucleotides is at least 1 µg (e.g., at least 5 µg, at least 10, at least 25 µg, at least 50 µg, at least 100 µg, at least 250 µg, at least 500 µg, at least 750 µg, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 µg and 100 µg, 5 µg and 500 µg, 5 µg and 1 mg, 5 µg and 10 mg, 500 µg and 100 mg, 500 µg and 1 g, 500 µg and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including

the population of polyribonucleotides is at least 500 μ L (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 μ L and 100 mL, 500 μ L and 1 L, 500 μ L and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 ng/ μ L (e.g., at least 500 ng/ μ L, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/ μ L and 100 mg/mL, 200 ng/ μ L and 50 mg/mL, or 1 mg/mL and 50 mg/mL); and (b) separating the circRNA from the linRNA at a pH of less than 5 (e.g., less than 4, 3, 2, or 1) or greater than 9 (e.g., greater than 10, 11, 12, or 13), thereby producing an enriched population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) exposing the population of polyribonucleotides to a pH of less than 5 (e.g., less than 4, 3, 2, or 1) or greater than 9 (e.g., greater than 10, 11, 12, or 13), thereby enriching the population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In some embodiments, the pH is less than 5 (e.g., less than 4, 3, 2, or 1). In some embodiments, the pH is greater than 9 (e.g., greater than 10, 11, 12, or 13).

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) separating the circRNA from the linRNA under conditions including an acid, base, organic solvent, chaotropic agent, crowding agent, chelator, detergent, or salt solution, thereby producing an enriched population of circRNA, wherein the separating does not include the use of gel electrophoresis. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA, wherein: (i) the total mass of polyribonucleotides in the population of polyribonucleotides is at least 1 μ g (e.g., at least 5 μ g, at least 10, at least 25 μ g, at least 50 μ g, at least 100 μ g, at least 250 μ g, at least 500 μ g, at least 750 μ g, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 μ g and 100 μ g, 5 μ g and 500 μ g, 5 μ g and 1 mg, 5 μ g and 10 mg, 500 μ g and 100 mg, 500 μ g and 1 g, 500 μ g and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including the population of polyribonucleotides is at least 500 μ L (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 μ L and 100 mL, 500 μ L and 1 L, 500 μ L and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 200 ng/ μ L (e.g., at least 500 ng/ μ L, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/ μ L and 100 mg/mL, 200 ng/ μ L and 50 mg/mL, or 1 mg/mL and 50 mg/mL); and (b) separating the circRNA from the linRNA under conditions including an acid, base, organic solvent, chaotropic agent, crowding agent, chelator, detergent, or salt solution, thereby producing an enriched population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above,

optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In an example, the disclosure provides a method for producing an enriched population of circRNA, the method including: (a) providing a sample including a population of polyribonucleotides including circRNA and linRNA; and (b) exposing the population of polyribonucleotides to an acid, base, organic solvent, chaotropic agent, crowding agent, chelator, detergent, or salt solution, thereby enriching the population of circRNA. Also contemplated is a method of separating a population of circRNA from a population of linRNA under the conditions described above, optionally further including a step of quantifying the population of circRNA and/or quantifying the population of linRNA.

In some embodiments, the acid includes at least 0.5% (v/v) (e.g., at least 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more) acetic acid, hydrochloric acid, salicylic acid, phosphoric acid, boric acid, formic acid, oxalic acid, citric acid, benzoic acid, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, ascorbic acid, or nitric acid.

In some embodiments, the base includes between 1 mM and 500 mM (e.g., between 2-475 mM, 3-450 mM, 4-425 mM, 5-400 mM, 10-375 mM, 15-350 mM, 20-325 mM, 30-300 mM, 40-275 mM, 50-250 mM, 60-225 mM, 70-200 mM, 80-175 mM, 90-150 mM, 100-125 mM, or 110-115 mM) sodium hydroxide, potassium hydroxide, imidazole, histidine, sodium bicarbonate, guanidine, or triethylamine.

In some embodiments, the organic solvent includes at least 0.1% (v/v) (e.g., at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or greater; or between 0.01% and 10%) of dimethyl sulfoxide, triethylammonium acetate, methanol, ethanol, 2-propanol, isopropanol, 1-butanol, 2-butanol, t-butanol, isobutanol, phenol, chloroform, hexane, acetonitrile, formamide, acetone, denatonium, or propylene glycol.

In some embodiments, the chaotropic agent includes between 100 mM and 8 M (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between 900 mM and 2 M, between 1 M and 1.5 M, between 1.1 M and 1.4 M, or between 1.2 and 1.3 M) urea, guanidinium chloride, lithium perchlorate, or PEG. In some embodiments, the chaotropic agent includes between 100 mM and 8 M (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between 900 mM and 2 M, between 1 M and 1.5 M, between 1.1 M and 1.4 M, or between 1.2 and 1.3 M) n-dodecyl β -d-maltoside, n-octylglucoside, CHAPS, or deoxycholate.

In some embodiments, the crowding agent includes between 100mM and 8 M (e.g., between 150 mM and 7.5 M, between 200 mM and 7 M, between 250 mM and 6.5 M, between 300 mM and 6 M, between 350 mM and 5.5 M, between 400 mM and 5 M, between 450 mM and 4.5 M, between 500 mM and 4 M, between 600 mM and 3.5 M, between 700 mM and 3 M, between 800 mM and 2.5 M, between

In some embodiments, the circRNA has a length from about 100 nucleotides to about 20,000 nucleotides (e.g., about 100 nucleotides to about 500 nucleotides, about 100 nucleotides to about 750 nucleotides, about 100 nucleotides to about 1,000 nucleotides, about 100 nucleotides to about 2,500 nucleotides, about 100 nucleotides to about 5,000 nucleotides, about 100 nucleotides to about 10,000 nucleotides, about 500 nucleotides to about 750 nucleotides, about 750 nucleotides to about 1,000 nucleotides, about 750 nucleotides to about 1,250 nucleotides, about 1,000 nucleotides to about 1,250 nucleotides, about 1,000 nucleotides to about 2,000 nucleotides, about 1,000 nucleotides to about 5,000 nucleotides, about 2,500 nucleotides to about 5,000 nucleotides, about 5,000 nucleotides to about 10,000 nucleotides, about 5,000 nucleotides to about 15,000 nucleotides, about 10,000 nucleotides to about 15,000 nucleotides, about 15,000 nucleotides to about 20,000 nucleotides, about 100 nucleotides, about 200 nucleotides, about 300 nucleotides, about 400 nucleotides, about 500 nucleotides, about 600 nucleotides, about 700 nucleotides, about 800 nucleotides, about 900 nucleotides, about 1,000 nucleotides, about 1,250 nucleotides, about 1,500 nucleotides, about 1,750 nucleotides, about 2,000 nucleotides, about 3,000 nucleotides, about 4,000 nucleotides, about 5,000 nucleotides, about 6,000 nucleotides, about 7,000 nucleotides, about 8,000 nucleotides, about 9,000 nucleotides, about 10,000 nucleotides, about 11,000 nucleotides, about 12,000 nucleotides, about 13,000 nucleotides, about 14,000 nucleotides, about 15,000 nucleotides, about 16,000 nucleotides, about 17,000 nucleotides, about 18,000 nucleotides, about 19,000 nucleotides, or about 20,000 nucleotides). In some embodiments, the circRNA has a length of less than 1,000 nucleotides.

In an aspect, the disclosure provides a composition including a population of polyribonucleotides including 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 (e.g., at least 5 µg, at least 10, at least 25 µg, at least 50 µg, at least 100 µg, at least 250 µg, at least 500 µg, at least 750 µg, at least 1 mg, 5 mg, 10 mg, 100 mg, or 1 g; or between 5 µg and 100 µg, 5 µg and 500 µg, 5 µg and 1 mg, 5 µg and 10 mg, 500 µg and 100 mg, 500 µg and 1 g, 500 µg and 10 g, 100 mg and 1g, or 1 g and 10 g); (ii) the total volume of the sample including the population of polyribonucleotides is at least 500 µL (e.g., at least 1 mL, 5 mL, 10 mL, 100 mL, or 1 L; or between 500 µL and 100 mL, 500 µL and 1 L, 500 µL and 10 L or 1L and 10 L); or (iii) the concentration of the population of polyribonucleotides in the sample is at least 500 ng/µL (e.g., at least 500 ng/µL, 1 mg/mL, 5 mg/mL, 10 mg/mL, or 50 mg/mL; or between 200 ng/µL and 100 mg/mL, 200 ng/µL and 50 mg/mL, or 1 mg/mL and 50 mg/mL).

In an example, the disclosure provides a composition including a population of polyribonucleotides including circRNA and linRNA, wherein the population of polyribonucleotides is in solution under denaturing conditions, and wherein, the solution is substantially free of the one or more impurities or by-products.

In an example, the disclosure provides a composition including a population of polyribonucleotides including circRNA and linRNA, wherein: (a) the composition is obtained from a sample including a population of nucleic acids; (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.

In an aspect, the disclosure provides a composition including an enriched population of circRNA, wherein: (a) the composition is obtained from a sample including a population of polyribonucleotides