

DEVICES, SYSTEMS AND METHODS FOR PROCESSING

Background

Methods are needed for the production and processing of circular polyribonucleotides.

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Summary

This disclosure relates generally to devices, systems, and methods for the preparation and processing of circular polyribonucleotides.

10 In one example, the disclosure features a method of processing circular polyribonucleotides. This method includes the steps of: (a) providing a device that includes a reservoir that has (i) a filter disposed between (ii) a filtrate chamber and a (iii) waste collection chamber the reservoir further including a removable cap having a pressure inlet port; wherein the device is in fluidic communication with a positive pressure source through the pressure inlet port; (b) providing circular polyribonucleotides in a first fluid in the filtrate chamber; (c) pressurizing the reservoir with the positive pressure source such that the first fluid passes through the filter and the circular polyribonucleotides do not pass through the filter; (d) applying a second fluid to the reservoir; and (e) pressurizing the reservoir with the positive pressure source such that the second buffer passes through the filter and the circular polyribonucleotides do not pass through the filter.

20 In one aspect, the present disclosure refers to a method of processing circular polyribonucleotides, the method comprising the steps of:

(a) providing a device comprising: a reservoir comprising a filter disposed between a filtrate chamber and a waste collection chamber; the reservoir further comprising a removable cap having a pressure inlet port; wherein the device is in fluidic communication with a positive pressure source through the pressure inlet port;

25 (b) providing circular polyribonucleotides in a first fluid in the filtrate chamber; (c) pressurizing the reservoir with the positive pressure source, wherein the first fluid passes through the filter and the circular polyribonucleotides do not pass through the filter;

(d) applying a second fluid to the reservoir; and (e) pressurizing the reservoir with the positive pressure source, wherein the second buffer passes through the filter and the circular polyribonucleotides do not pass through the filter.

30 In some examples, the method further includes the step of repeating steps (d) and (e) from 1 to 5 times. In some examples, the method further includes the step of (f) aspirating the circular polynucleotides from the filter following step (e). In some examples, from 1 mg to 1000 mg of circular polyribonucleotides are aspirated from the filter. In some examples, from 50 mg to 150 mg of circular polyribonucleotides are aspirated from the filter. In some examples, about 100 mg of circular polyribonucleotides are aspirated from the filter.

35 In some examples, the second fluid is applied through a fluid inlet port in the cap. In some examples, the fluid inlet port is in fluidic communication with a fluid source. In some examples, the first and/or second fluid is a liquid. In some examples, the liquid is a solution. In some examples, the solution is a buffer. In some examples, the buffer is a formulation buffer, storage buffer, or purification buffer.

40 In some examples, pressurizing step (c) and step (d) include pressurizing the reservoir with from 10 PSI to 100 PSI. In some examples, applying step (d) includes applying from 5 mL to 15 mL of fluid to the reservoir.

45 In another example, the disclosure features a device includes: a reservoir that has a filtrate chamber and a waste collection chamber separated by a filter, and a removable cap in contact with a pressure inlet port; wherein the device is in fluidic communication with a positive pressure source. In one aspect, the present disclosure refers to a device comprising: a reservoir comprising a filtrate chamber and a waste collection chamber separated by a filter; the reservoir further comprising a removable cap in contact with a pressure inlet port; wherein the device is in fluidic communication with a positive pressure source.

50 In some examples, the reservoir is a tube. In some examples, the tube is a centrifugal tube. In some examples, the filtrate chamber is funneled. In some examples, the cap further includes a fluid inlet port. In some examples, the volume of the reservoir is from 5 mL to 100 mL. In some examples, the volume of the filtrate chamber is from 5 mL to 15 mL. In some examples, the filter is from 2 kDa to 200 kDa. In some examples, the filtrate chamber and filter are stacked on, screwed into, or nested in the reservoir. In some examples, the pressure inlet port is sealed.

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In some examples, the device further includes a sensor (e.g., a liquid detection sensor). In some examples, the liquid detection sensor is selected from the list consisting of an optical sensor, a vibrating sensor, an ultrasonic sensor, a float sensor, a capacitance sensor, a radar sensor, a conductivity sensor, or a resistance sensor. In some examples, the sensor is an optical sensor.

In another example, the disclosure features a system that includes (i) a filter tray having a sample reservoir including filtrate chamber having a filter, the filter tray disposed between (ii) a lid having a pressure inlet port, and (iii) a waste reservoir; wherein the pressure inlet port is in fluidic communication with a (iv) a positive pressure source. In one aspect, the present disclosure refers to a system comprising a filter tray having a sample reservoir comprising filtrate chamber having a filter, the filter tray disposed between a lid having a pressure inlet port, and a waste reservoir; wherein the pressure inlet port is in fluidic communication with a positive pressure source.

In some examples, the filtrate chamber is funneled. In some examples, the volume of the filtrate chamber is from 5 mL to 15 mL. In some examples, the waste reservoir includes a gravity or vacuum drain. In some examples, the system further includes a liquid detection sensor. In some examples, the system further includes a vent. In some examples, the filter tray, lid, and waste reservoir are sealably connected together. In some examples, the filtrate chamber is stacked on, screwed into, or nested in the sample reservoir. In some examples, the lid further includes a fluid inlet port. In some examples, the filter tray includes a plurality of sample reservoirs.

Definitions

The present invention will be described with respect to particular embodiments and with reference to certain figures, but the invention is not limited thereto but only by the claims. Terms as set forth hereinafter are generally to be understood in their common sense unless indicated otherwise.

As used herein, the term “circular polyribonucleotide” or “circRNA” or “circular RNA” are used interchangeably and mean a polyribonucleotide molecule that has a structure having no free ends (i.e., no free 3' and/or 5' ends), for example a polyribonucleotide molecule that forms a circular or end-less structure through covalent or non-covalent bonds. In some embodiments, a circular polyribonucleotide is a “covalently closed polyribonucleotide” that formed a circular or end-less structure through covalent bonds.

As used herein, the terms “circular polyribonucleotide sample”, “circRNA sample”, and “circular RNA sample” are used interchangeably and mean a composition including circRNA molecules and a solution.

As used herein, the term “expression sequence” refers to a nucleic acid sequence that encodes a product, e.g., a peptide or polypeptide, or a regulatory nucleic acid.

“Polypeptide” and “protein” are used interchangeably and refer to a polymer of two or more amino acids joined by a covalent bond (e.g., an amide bond). Polypeptides as described herein can include full length proteins (e.g., fully processed proteins) as well as shorter amino acid sequences (e.g., fragments of naturally-occurring proteins or synthetic polypeptide fragments). Polypeptides can include naturally occurring amino acids (e.g., one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V) and non-naturally occurring amino acids (e.g., amino acids which is not one of the twenty amino acids commonly found in peptides synthesized in nature, including synthetic amino acids, amino acid analogs, and amino acid mimetics).

As used herein, the term “aptamer sequence” or “aptamer” refers to a non-naturally occurring or synthetic oligonucleotide that specifically binds to a target molecule. Typically, an aptamer is from 20 to