COMPOSITIONS AND METHODS FOR PURIFYING POLYRIBONUCLEOTIDES

Sequence Listing

This application contains a Sequence Listing which has been filed electronically in Extensible Markup Language (XML) format and is hereby incorporated by reference in its entirety. Said XML copy, created on October 30, 2023, is named 51509-067WO2_Sequence_Listing_10_27_23.XML and is 143,826 bytes in size.

Background

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

Summary of the Invention

In one aspect, the disclosure features a method of separating a linear polyribonucleotide comprising an aptamer from a plurality of polyribonucleotides comprising a mixture of linear polyribonucleotides and circular polyribonucleotides, the method comprising: (a) providing a sample comprising the plurality of polyribonucleotides, wherein a subset of the plurality of polyribonucleotides comprise the linear polyribonucleotide comprising the aptamer; (b) contacting the sample with a reagent that binds to the aptamer; and (c) separating the linear polyribonucleotide comprising the aptamer bound to the reagent from the plurality of polyribonucleotides.

In some embodiments, the linear polyribonucleotide with the aptamer is transcribed from a deoxyribonucleotide encoding the linear polyribonucleotide including the aptamer.

In some embodiments, the method further includes producing the linear polyribonucleotide with the aptamer by attaching the aptamer to the linear polyribonucleotide.

In another aspect, the disclosure features a method of separating a linear polyribonucleotide from a plurality of polyribonucleotides comprising a mixture of linear polyribonucleotides and circular polyribonucleotides, the method comprising: (a) providing a sample comprising the plurality of polyribonucleotides, wherein a subset of the plurality of polyribonucleotides comprise the linear polyribonucleotide; (b) attaching an aptamer to the linear polyribonucleotide; (c) contacting the sample with a reagent that binds to the aptamer; and (d) separating the linear polyribonucleotide comprising the aptamer bound to the reagent from the plurality of polyribonucleotides.

In some embodiments, the step of attaching the aptamer to the linear polyribonucleotide includes covalently attaching the aptamer to a 3’ or 5’ terminus of the linear polyribonucleotide.

In some embodiments, the step of attaching the aptamer to the linear polyribonucleotide includes hybridizing the aptamer to a region of the linear polyribonucleotide.

In some embodiments of either of the foregoing aspects, the circular polyribonucleotides lack the aptamer.

In some embodiments, the step of separating includes collecting a portion of the sample that is not bound by the reagent. For example, the portion of the sample that is not bound by the reagent may include the circular polyribonucleotide.

In an example, the disclosure features a composition that includes a population of polyribonucleotides as described herein (e.g., produced by a method as described herein) and a diluent, carrier, or excipient.

Definitions

To facilitate the understanding of this disclosure, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the disclosure. Terms such as "a", "an," and "the" are not intended to refer to only a singular entity but include the general class of which a specific example may be used for illustration. The term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." The terminology herein is used to describe specific embodiments, but their usage is not to be taken as limiting, except as outlined in the claims.

As used herein, any values provided in a range of values include both the upper and lower bounds, and any values contained within the upper and lower bounds.

As used herein, the term “about” refers to a value that is within ± 10% of a recited value.

As used herein, the term “aptamer” is a polynucleotide that specifically binds to a molecule (e.g., a reagent). An aptamer may be a portion of a polyribonucleotide molecule. Typically, an aptamer is from 5 to 500 nucleotides (e.g., between 5 and 200, 5 and 150, 5 and 100, 5 and 50, 10 and 200, 10 and 150, 10 and 100, 10 and 50, 20 and 200, 20 and 150, 20 and 100, or 20 and 50 nucleotides). An aptamer binds to its target through secondary structure rather than sequence homology.

As used herein, the term “carrier” is a compound, composition, reagent, or molecule that facilitates the transport or delivery of a composition (e.g., a circular polyribonucleotide) into a cell by a covalent modification of the circular polyribonucleotide, *via* a partially or completely encapsulating agent, or a combination thereof. Non-limiting examples of carriers include carbohydrate carriers (e.g., an anhydride-modified phytoglycogen or glycogen-type material), nanoparticles (e.g., a nanoparticle that encapsulates or is covalently linked binds to the circular polyribonucleotide), liposomes, fusosomes, ex vivo differentiated reticulocytes, exosomes, protein carriers (e.g., a protein covalently linked to the circular polyribonucleotide), or cationic carriers (e.g., a cationic lipopolymer or transfection reagent).

As used herein, the terms “circular polyribonucleotide,” “circular RNA,” and “circRNA” are used interchangeably and mean a polyribonucleotide molecule that has a structure having no free ends (i.e., no free 3’ or 5’ ends), for example a polyribonucleotide molecule that forms a circular or end-less structure through covalent or non-covalent bonds. The circular polyribonucleotide may be, e.g., a covalently closed polyribonucleotide.

As used herein, the terms “disease,” “disorder,” and “condition” each refer to a state of sub-optimal health, for example, a state that is or would typically be diagnosed or treated by a medical professional.

As used herein, the term “expression sequence” is a nucleic acid sequence that encodes a product, e.g., a peptide or polypeptide. An exemplary expression sequence that codes for a peptide or polypeptide can include a plurality of nucleotide triads, each of which can code for an amino acid and is termed as a “codon.”