

CLAIMS

1. A method of separating a linear polyribonucleotide from a plurality of polyribonucleotides comprising a mixture of linear polyribonucleotides and circular polyribonucleotides comprising an open reading frame (ORF) encoding a polypeptide, 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 a target region to the linear polyribonucleotide;
- (c) contacting the sample with an oligonucleotide that hybridizes to the target region; and
- (d) separating the linear polyribonucleotide comprising the target region that is hybridized to the oligonucleotide from the plurality of polyribonucleotides in the sample.

2. The method of claim 1, wherein the oligonucleotide is conjugated to a particle.

3. The method of claim 2, wherein the particle comprises a magnetic bead or wherein the oligonucleotide is conjugated to a resin comprising a plurality of the particles.

4. The method of any one of claims 1-3, wherein separating the linear polyribonucleotide comprises immobilizing the oligonucleotide and/or collecting a portion of the sample that is not hybridized to the oligonucleotide.

5. The method of claim 4, wherein the portion of the sample that is not hybridized to the oligonucleotide comprises the circular polyribonucleotide.

6. 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 a target region to the linear polyribonucleotide;
- (c) contacting the sample with a column comprising a resin comprising a plurality of particles conjugated to an oligonucleotide that hybridizes to the target region; and
- (d) collecting an eluate comprising a portion of the sample that is not hybridized to the oligonucleotide from the plurality of polyribonucleotides in the sample.

7. The method of claim 6, wherein the portion of the sample that is not hybridized to the oligonucleotide comprises the circular polyribonucleotide.

8. The method of any one of claims 1-7, wherein the method comprises a step of circularizing the circular polyribonucleotide from a linear precursor prior to step (a).

9. The method of claim 8, wherein the linear precursor comprises a 5' self-splicing intron fragment and a 3' self-splicing intron fragment, and wherein the circular polyribonucleotide is produced by self-splicing of the linear precursor.

10. 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) circularizing a linear precursor to form the circular polyribonucleotide;
 - (b) providing a sample comprising the plurality of polyribonucleotides, wherein a subset of the plurality of polyribonucleotides comprise the linear polyribonucleotide;
 - (c) attaching a target region to the linear polyribonucleotide;
 - (d) contacting the sample with an oligonucleotide that hybridizes to the target region; and
 - (e) separating the linear polyribonucleotide comprising the target region that is hybridized to the oligonucleotide from the plurality of polyribonucleotides in the sample.
11. The method of any one of claims 6-10, wherein the circular polyribonucleotide comprises an ORF and/or an internal ribosome entry site (IRES), wherein optionally, the ORF encodes a polypeptide.
12. The method of any one of claims 1-11, wherein the method comprises attaching the target region to a 3' or 5' terminus of the linear polyribonucleotide.
13. The method of claim 12, wherein the attaching step comprises polyadenylating the 3' terminus of the linear polyribonucleotide and/or ligating the target region to the 3' terminus of the linear polyribonucleotide.
14. The method of any one of claims 1-13, wherein the circular polyribonucleotide does not comprise a polyA sequence, wherein, optionally, the circular polyribonucleotide does not comprise a polyA sequence comprising at least 10 contiguous adenosine nucleotides.
15. The method of any one of claims 1-14, wherein the linear polyribonucleotide of step (a) comprises the target region.
16. The method of any one of claims 1-15, wherein the target region comprises a polyA sequence and/or the oligonucleotide comprises a polyU or polydT sequence, wherein, optionally, the polyA sequence comprises at least 10 contiguous adenosine nucleotides.
17. The method of any one of claims 10-16, wherein the oligonucleotide is conjugated to a particle, wherein, optionally, the oligonucleotide is conjugated to a resin comprising a plurality of the particles, wherein, optionally, a column comprises the resin.
18. The method of any one of claims 1-17, wherein the method comprises providing a plurality of oligonucleotides, wherein each oligonucleotide hybridizes to a distinct target region.
19. The method of any one of claims 1-18, wherein the oligonucleotide has at least 80%, 85%, 90%, 95%, 97%, 99%, or 100% complementarity an equal length portion of the target region.

20. A population of polyribonucleotides produced by the method of any one of claims 1-19, wherein, optionally the population comprises a circular polyribonucleotide lacking the target region and the circular polyribonucleotide comprises at least 40% (mol/mol) of the total polyribonucleotides in the composition and/or wherein the population comprises less than 40% (mol/mol) linear polyribonucleotides of the total polyribonucleotides in the composition.