

In one aspect, the present disclosure refers to a linear polyribonucleotide having the formula 5'-(A)-(B)-(C)-(D)-(E)-(F)-(G)-3', wherein:

(A) comprises a 3' half of Group I catalytic intron fragment;

(B) comprises a 3' splice site;

(C) comprises a 3' exon fragment comprising a first annealing region comprising from 8 to 50 ribonucleotides, wherein the first annealing region is flanked on each side by a portion of the 3' exon fragment;

(D) comprises a polyribonucleotide cargo;

(E) comprises a 5' exon fragment comprising a second annealing region comprising from 8 to 50 ribonucleotides that can hybridize to the first annealing region;

(F) comprises a 5' splice site; and

(G) comprises a 5' half of Group I catalytic intron fragment.

In another example, the disclosure features a linear polyribonucleotide having the formula 5'-(A)-(B)-(C)-(D)-(E)-(F)-(G)-3'. The linear polyribonucleotide includes, from 5' to 3', (A) a 3' half of Group I catalytic intron fragment; (B) a 3' splice site; (C) a 3' exon fragment; (D) a polyribonucleotide cargo; (E) a 5' exon fragment; (F) a 5' splice site; and (G) a 5' half of Group I catalytic intron fragment, wherein the 3' half of Group I catalytic intron fragment of (A) and the 5' half of Group I catalytic intron fragment of (G) are from a Cyanobacterium Anabaena pre-tRNA-Leu gene. The polyribonucleotide includes a first annealing region that has from 5 to 50, e.g., 6 to 50 (e.g., from 10 to 30, 10 to 20, or 10 to 15, e.g., at least 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) ribonucleotides and is present within (A) the 3' half of Group I catalytic intron fragment; (B) the 3' splice site; or (C) the 3' exon fragment. The polyribonucleotide also includes a second annealing region that has from 5 to 50, e.g., 6 to 50 (e.g., from 10 to 30, 10 to 20, or 10 to 15, e.g., at least 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) ribonucleotides and is present within (E) the 5' exon fragment; (F) the 5' splice site; or (G) the 5' half of Group I catalytic intron fragment. The first annealing region has from 80% to 100% (e.g., 85% to 100%, e.g., 90% to 100%, e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) complementarity with the

CLAIMS

1. A linear polyribonucleotide having the formula 5'-(A)-(B)-(C)-(D)-(E)-(F)-(G)-3', wherein:
 - (A) comprises a 3' half of Group I catalytic intron fragment;
 - (B) comprises a 3' splice site;
 - (C) comprises a 3' exon fragment;
 - (D) comprises a polyribonucleotide cargo;
 - (E) comprises a 5' exon fragment;
 - (F) comprises a 5' splice site; and
 - (G) comprises a 5' half of Group I catalytic intron fragment;and wherein (A), (B), or (C) comprises a first annealing region comprising from 8 to 50 ribonucleotides and (E), (F), or (G) comprises a second annealing region comprising from 8 to 50 ribonucleotides,
 - wherein the first annealing region and the second annealing region have from 80% to 100% complementarity, or
 - the first annealing region and the second annealing region comprises from zero to 10 mismatched base pairs.
2. The linear polyribonucleotide of claim 1, wherein:
 - i) (A) or (C) comprises the first annealing region and (E) or (G) comprises the second annealing region; and/or
 - ii) the 3' exon fragment of (C) comprises the first annealing region and the 5' exon fragment of (E) comprises the second annealing region; or the 3' half of Group I catalytic intron fragment of (A) comprises the first annealing region and the 5' exon fragment of (E) comprises the second annealing region; and/or
 - iii) the first annealing region comprises from 10 to 30, 10 to 20 or 10 to 15 ribonucleotides, and the second annealing region comprises from 10 to 30, 10 to 20 or 10 to 15 ribonucleotides; and/or
 - iv) the 3' half of Group I catalytic intron fragment of (A) and the 5' half of Group I catalytic intron fragment of (G) are from a cyanobacterium *Anabaena* pre-tRNA-Leu gene, or a *Tetrahymena* pre-rRNA.
3. The linear polyribonucleotide of claim 1 or 2, wherein the first annealing region and the second annealing region:
 - i) has 90% to 100% complementarity;
 - ii) comprise zero or one mismatched base pair; and/or
 - iii) are 100% complementary.
4. The linear polyribonucleotide of claim 1,
 - i) wherein the 3' half of Group I catalytic intron fragment of (A) and the 5' half of Group I catalytic intron fragment of (G) are from a Cyanobacterium *Anabaena* pre-tRNA-Leu gene,
 - and wherein the 3' exon fragment of (C) comprises the first annealing region and the 5' exon fragment of (E) comprises the second annealing region;

optionally wherein the first annealing region comprises from 10 to 15 ribonucleotides and the second annealing region comprises from 10 to 15 ribonucleotides; or

ii) wherein the 3' half of Group I catalytic intron fragment of (A) and the 5' half of Group I catalytic intron fragment of (G) are from a *Tetrahymena* pre-rRNA,

and wherein the 3' half of Group I catalytic intron fragment of (A) comprises the first annealing region and the 5' exon fragment of (E) comprises the second annealing region;

optionally wherein the first annealing region comprises from 10 to 16 ribonucleotides and the second annealing region comprises from 10 to 16 ribonucleotides; or

iii) wherein the 3' half of Group I catalytic intron fragment of (A) and the 5' half of Group I catalytic intron fragment of (G) are from a T4 phage td gene, and wherein the 3' exon fragment of (C) comprises the first annealing region and the 5' half of Group I catalytic intron fragment of (G) comprises the second annealing region;

optionally wherein the first annealing region comprises from 2 to 16 ribonucleotides and the second annealing region comprises from 2 to 16 ribonucleotides.

5. The linear polyribonucleotide of any one of claims 1-4, wherein:

i) the 3' half of Group I catalytic intron fragment of (A) is the 5' terminus of the linear polynucleotide; and/or

ii) the 5' half of Group I catalytic intron fragment of (G) is the 3' terminus of the linear polyribonucleotide; and/or

iii) the linear polyribonucleotide does not comprise an annealing region 3' to (A) that comprises partial or complete nucleic acid complementarity with an annealing region 5' to (G); and/or

iv) the polyribonucleotide cargo of (D) comprises an expression sequence, a non-coding sequence, or an expression sequence and a non-coding sequence; and/or

v) the polyribonucleotide cargo of (D) comprises an expression sequence encoding a polypeptide; and/or

vi) the polyribonucleotide cargo of (D) comprises an IRES operably linked to an expression sequence encoding a polypeptide; and/or

vii) the polyribonucleotide cargo of (D) comprises an expression sequence that encodes a polypeptide that has a biological effect on a subject.

6. The linear polyribonucleotide of any one of claims 1-5, wherein the linear polyribonucleotide does not comprise a further annealing region.

7. The linear polyribonucleotide of claim 6, wherein the linear polyribonucleotide does not comprise a further annealing region comprising at least 6 ribonucleotides.

8. The linear polyribonucleotide of any one of claims 1-7, wherein the linear polyribonucleotide further comprises:
- i) a first spacer region between the 3' exon fragment of (C) and the polyribonucleotide cargo of (D); and/or
 - ii) a second spacer region between the polyribonucleotide cargo of (D) and the 5' exon fragment of (E); preferably wherein each spacer region is at least 5 ribonucleotides in length; and more preferably wherein each spacer region is from 5 to 500 ribonucleotides in length.
9. The linear polyribonucleotide of claim 8, wherein the first spacer region, the second spacer region, or the first spacer region and the second spacer region comprises a polyA sequence; or a polyA-C sequence.
10. The linear polyribonucleotide of any one of claims 1-9, wherein:
- i) the linear polyribonucleotide is from 300 to 20,000 ribonucleotides in length; and/or
 - ii) the linear polyribonucleotide is at least 1,000 ribonucleotides in length, optionally wherein the linear polyribonucleotide is at least 3,000 ribonucleotides in length.
11. A linear polyribonucleotide having the formula 5'-(A)-(B)-(C)-(D)-(E)-(F)-(G)-3', wherein:
- (A) comprises a 3' half of Group I catalytic intron fragment;
 - (B) comprises a 3' splice site;
 - (C) comprises a 3' exon fragment comprising a first annealing region comprising from 8 to 50 ribonucleotides, wherein the first annealing region is flanked on each side by a portion of the 3' exon fragment;
 - (D) comprises a polyribonucleotide cargo;
 - (E) comprises a 5' exon fragment comprising a second annealing region comprising from 8 to 50 ribonucleotides that can hybridize to the first annealing region;
 - (F) comprises a 5' splice site; and
 - (G) comprises a 5' half of Group I catalytic intron fragment.
12. The linear polyribonucleotide of claim 11, wherein the linear polyribonucleotide does not comprise a further annealing region comprising at least 6 ribonucleotides.
13. A DNA vector comprising an RNA polymerase promoter operably linked to a DNA sequence that encodes the linear polyribonucleotide of any one of claims 1-12.

14. A circular polyribonucleotide produced from the linear polyribonucleotide of any one of claims 1-12 or from the DNA vector of claim 13.

15. A circular polyribonucleotide comprising a splice junction joining a 5' exon fragment and a 3' exon fragment,

wherein the 3' exon fragment comprises a first annealing region comprising 8 to 50 ribonucleotides, and the 5' exon fragment comprises a second annealing region comprising 8 to 50 ribonucleotides,

and wherein the first annealing region and the second annealing region comprises from 80% to 100% complementarity, or the first annealing region and the second annealing region comprises from zero to 10 mismatched base pairs.

16. The circular polyribonucleotide of claim 15, wherein the circular polyribonucleotide further comprises a polyribonucleotide cargo;

i) preferably wherein the polyribonucleotide cargo comprises an expression sequence, a non-coding sequence, or a combination of an expression sequence and a non-coding sequence; and

ii) more preferably wherein the polyribonucleotide cargo comprises an expression sequence encoding a polypeptide; and

iii) more preferably wherein the polyribonucleotide comprises an IRES operably linked to an expression sequence encoding a polypeptide; and

iv) more preferably wherein the circular polyribonucleotide further comprises a spacer region between the IRES and the 3' exon fragment or the 5' exon fragment; and

v) more preferably wherein the spacer region is at least 5 ribonucleotides in length; and

vi) more preferably wherein the spacer region is from 5 to 500 ribonucleotides in length.

17. The circular polyribonucleotide of claim 16, wherein the spacer region comprises a polyA sequence; or a polyA-C sequence.

18. The circular polyribonucleotide of any one of claims 15-17, wherein:

i) the circular polyribonucleotide is at least 500 ribonucleotides in length; optionally wherein the circular polyribonucleotide is from 500 to 20,000 ribonucleotides in length; and/or

ii) produced from the linear polyribonucleotide of any one of claims 1-12 or from the vector of claim 13.

19. A method of expressing a polypeptide in a cell, the method comprising providing the linear polyribonucleotide of any one of claims 1-12, the DNA vector of claim 13, or the circular polyribonucleotide of any one of claims 14-18 to the cell.

20. A method of producing a circular polyribonucleotide from the linear polyribonucleotide of any one of claims 1-12, the method comprising providing the linear polyribonucleotide under conditions suitable for self-splicing of the linear polyribonucleotide to produce the circular polyribonucleotide.