

CLAIMS

What is claimed is:

1. A linear RNA, comprising from the 5' end to the 3' end:
 - (a) a first portion of a RNA element;
 - (b) an effector RNA sequence; and
 - (c) a second portion of the RNA element;wherein the first portion of the RNA element and the second portion of the RNA element associate with each other to form a double-stranded region of at least 4 basepairs (bp) long; wherein the 5' end of the first portion of the RNA element and the 3' end of the second portion of the RNA element form a nick in the double-stranded region; and wherein the nick can be ligated by a RNA ligase.
2. The linear RNA of claim 1, wherein the double-stranded region is about 6 bp long to about 25 bp long.
3. The linear RNA of any one of claims 1-2, wherein the RNA element is at least 20 nt long.
4. The linear RNA of any one of claims 1-3, wherein the double-stranded region comprises at least 2 bp 3' to the nick, and/or, the double-stranded region comprises at least 2 bp 5' to the nick.
5. The linear RNA of claim 1, wherein the first portion of the RNA element and/or the second portion of the RNA element comprises a sequence that is exogenous to the reference RNA element, particularly, wherein the second portion of the RNA element comprises a first exogenous sequence at the 3' end, wherein the first portion of the RNA element comprises a second exogenous sequence that is complementary of the first exogenous sequence, and wherein the first exogenous sequence and the second exogenous sequence form base pairs flanking the 5' end of the nick.
6. The linear RNA of any one of claims 1-5, wherein the effector RNA sequence is a coding RNA sequence, particularly, wherein the coding RNA sequence encodes a therapeutic polypeptide.

7. The linear RNA of claim 6, wherein the RNA element promotes translation of the coding RNA, particularly, wherein the RNA element is an internal ribosomal entry site (IRES) or a portion thereof, more particularly, wherein the IRES is derived from an IRES selected from the group consisting of Coxsackievirus B3 (CVB3) IRES, Enterovirus 71 (EV71) IRES, encephalomyocarditis virus (EMCV) IRES, picornavirus (PV) IRES, hepatitis C virus (HCV) IRES, adenovirus (AdV) IRES, human papillomavirus type 31 (HPV31) IRES, human herpesvirus (HHV) IRES, Rous sarcoma virus (RSV) IRES, classical swine fever virus (CSFV) IRES, FGF9 IRES, SLC7A1 IRES, and RUNX1 IRES.
8. The linear RNA of claim 7, wherein the IRES is an IRES of a CVB3 virus or a derivative thereof, particularly, wherein the IRES of a CVB3 virus comprises the nucleotide sequence of SEQ ID NO: 1.
9. The linear RNA of claim 8, wherein the first portion of the RNA element comprises nucleotides 386-749 of SEQ ID NO: 1 and the second portion of the RNA element comprises nucleotides 1-385 of SEQ ID NO: 1, or wherein the first portion of the RNA element comprises nucleotides 347-749 of SEQ ID NO: 1 and the second portion of the RNA element comprises nucleotides 1-346 of SEQ ID NO: 1.
10. The linear RNA of any one of claims 6-9, further comprising an in-frame 2A peptide coding sequence that is operably linked to the 3' end of the coding RNA.
11. The linear RNA of any one of claims 1-5, wherein the RNA element is a sgRNA or a H/ACA box small nucleolar RNA (snoRNA).
12. The linear RNA of any one of claims 1-5 and 11, wherein the effector RNA sequence is a sequence of a non-coding RNA selected from the group consisting of a guide RNA (gRNA), a deaminase-recruiting RNA (dRNA), a siRNA, a miRNA, a shRNA, and a long intervening non-coding (linc) RNA.
13. A method of preparing a circRNA, comprising:
 - (a) contacting the linear RNA of any one of claims 1-12 with an RNA ligase under conditions that allow ligation of the nick in the linear RNA to provide a circularized RNA product; and
 - (b) isolating the circularized RNA product, thereby providing the circRNA.

14. A circRNA formed by ligation of the nick in the linear RNA of any one of claims 1-12.
15. A nucleic acid construct comprising a nucleic acid sequence encoding the linear RNA of any one of claims 1-12.