

CLAIMS

1. A composition comprising or encoding: an engineered small nuclear RNA (esnRNA) that promotes trans-splicing of a target RNA molecule and an RNA trans-splicing nucleic acid, wherein the RNA trans-splicing nucleic acid comprises an exonic sequence, an intronic domain, and an antisense domain, wherein the antisense domain in the RNA trans-splicing molecule is complementary to a sequence in the target RNA molecule, wherein the esnRNA comprises an RNA motif at the 5' end of the esnRNA, and wherein the RNA motif binds to an esnRNA-binding site in the intronic domain in the RNA trans-splicing nucleic acid.
2. A composition comprising or encoding: i) an engineered small nuclear RNA (esnRNA) that promotes trans-splicing of a target RNA molecule and an RNA trans-splicing nucleic acid; and ii) the RNA trans-splicing nucleic acid which comprises: (a) an exonic sequence; (b) an intronic domain that promotes RNA splicing of the exonic sequence to an exon of the target RNA molecule; and (c) an antisense domain, wherein the antisense domain in the RNA trans-splicing molecule is complementary to a sequence in the target RNA molecule, wherein the esnRNA comprises an RNA motif at the 5' end of the esnRNA, and wherein the RNA motif binds to an esnRNA-binding site in the intronic domain in the RNA trans-splicing nucleic acid.
3. The composition of claims 1 or 2, wherein the RNA trans-splicing nucleic acid comprises an engineered small nuclear RNA domain.
4. The composition of any one of claims 1-3, wherein the engineered small nuclear RNA is derived or isolated from a human small nuclear RNA gene chosen from a group consisting of: U1, U2, U4, U5, U6, U7, U11, and U12.
5. The composition of any one of claims 1-3, wherein the engineered small nuclear RNA is derived or isolated from a U1 small nuclear RNA gene or variant of a U1 small nuclear RNA gene and contains an RNA motif that begins less than 16 nucleobases from the 5' end that is partially or perfectly complementary to the RNA trans-splicing nucleic acid.
6. The composition of any one of claims 1-5, wherein the engineered small nuclear RNA contains an RNA motif that is partially or perfectly complementary to the RNA trans-splicing nucleic acid.

7. The composition of claim 6, wherein the RNA motif is chosen from a group consisting of: 5'-CGAGCTCTCT-3', 5'-AACGAGCTCT-3', 5'-CGCAACGAGC-3', 5'-TATCGCAACG-3', 5'-AATAATATCG-3', 5'-TAAGAGAGCT-3', 5'-AAGAGAGCTC-3', 5'-AGAGAGCTCGTTGC-3', 5'-GAGAGCTCGT-3', 5'-AGAGCTCGTTGCGA-3', and 5'-GAGCTCGTTG-3'.
8. The composition of any one of claims 1-7, wherein the engineered small nuclear RNA comprises sequences derived or isolated from a U1 small nuclear RNA gene or a variant of U1 small nuclear RNA gene.
9. The composition of any one of claims 1-8, wherein the engineered small nuclear RNA comprises sequences derived or isolated from a U1 small nuclear RNA gene and a variant of the U1 small nuclear RNA gene.
10. The composition of claim 9, wherein the variant of the U1 small nuclear RNA gene is chosen from a group consisting of: vU1.4, vU1.11, vU1.8, vU1.7, vU1.5, and vU1.12.
11. The composition of any one of claims 1-10, wherein the small nuclear RNA comprises RNA, DNA, a DNA/RNA hybrid, a nucleic acid analog, a chemically-modified nucleic acid, or a chimera composed of two or more nucleic acids or nucleic acid analogs.
12. A composition comprising or encoding an RNA trans-splicing nucleic acid, comprising: (a) an exonic sequence; (b) an intronic domain that promotes RNA splicing of the exonic sequence; and (c) an antisense domain that promotes binding to a target RNA molecule; and (d) an engineered small nuclear RNA domain that promotes trans-splicing of the RNA trans-splicing nucleic acid.
13. The composition of claim 12, wherein the engineered small nuclear RNA domain is located less than 200 bases away from a splice donor site in the RNA trans-splicing nucleic acid.
14. The composition of any one of claims 12-13, wherein the engineered small nuclear RNA domain is isolated or derived from a human small nuclear RNA gene.
15. The composition of claim 14, wherein the human small nuclear RNA gene is chosen from a group consisting of: U1, U2, U4, U5, U6, U7, U11, and U12.
16. The composition of any one of claims 12-15, wherein the engineered small nuclear RNA domain is synthetic and binds components of the spliceosome.

17. The composition of claim 16, wherein the engineered small nuclear RNA domain comprises sequences isolated or derived from a variant of the human U1 gene.
18. The composition of any one of claims 12-17, wherein the engineered small nuclear RNA domain comprises sequences derived from the human U1 small nuclear RNA gene and a variant of the U1 small nuclear RNA gene.
19. The composition of any one of claims 1-18, wherein the RNA trans-splicing nucleic acid further comprises an untranslated region that enhances translation.
20. The composition of any one of claims 1-19, further comprising an RNA-binding protein that strengthens the interaction among the RNA trans-splicing nucleic acid and the target RNA molecule and increases trans-splicing efficiency.
21. The composition of any one of claims 1-20, wherein the engineered small nuclear RNA contains an RNA motif that is at least 4 nucleotides long and is partially or perfectly complementary to a sequence in the RNA trans-splicing nucleic acid.
22. The composition of claim 21, wherein the RNA motif is chosen from a group consisting of: 5'-CGAGCTCTCT-3', 5'-AACGAGCTCT-3', 5'-CGCAACGAGC-3', 5'-TATCGCAACG-3', 5'-AATAATATCG-3', 5'-TAAGAGAGCT-3', 5'-AAGAGAGCTC-3', 5'-AGAGAGCTCGTTGC-3', 5'-GAGAGCTCGT-3', 5'-AGAGCTCGTTGCGA-3', and 5'-GAGCTCGTTG-3'.
23. The composition of claim 22, wherein the RNA trans-splicing nucleic acid contains an RNA motif that is at least 4 nucleotides long and is partially or perfectly complementary to a sequence in the RNA trans-splicing nucleic acid.
24. The composition of any one of claims 1-23, wherein the RNA trans-splicing nucleic acid further comprises an untranslated region that enhances translation.
25. The composition of claim 24, wherein the translation-enhancing element comprises a sequence derived or isolated from the group consisting of: Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE), triplex from MALAT1, the PRE of Hepatitis B virus (HPRE), and an iron response element.
26. The composition of any one of claims 1-25, further comprising an RNA-binding protein that strengthens the interaction among the RNA trans-splicing nucleic acid and the target RNA molecule and increases trans-splicing efficiency.

27. The composition of any one of claims 1-26, wherein the RNA trans-splicing nucleic acid comprises RNA, DNA, a DNA/RNA hybrid, a nucleic acid analog, a chemically-modified nucleic acid, or a chimera composed of two or more nucleic acids or nucleic acid analogs.
28. The composition of any one of claims 1-27, wherein the RNA trans-splicing nucleic acid further comprises or encodes a heterologous promoter.
29. A vector comprising the composition of claims of any one of claims 1-28.
30. The vector of claim 29, wherein the vector is selected from the group consisting of: adeno-associated virus, retrovirus, lentivirus, adenovirus, nanoparticle, micelle, liposome, lipoplex, polymersome, polyplex, and dendrimer.
31. A cell comprising the vector of claim 29 or 30.
32. The composition of any one of claims 1-28, the vector of claims 29-30, or the cell of claim 31, wherein the RNA motif is between 4 and 16 nucleotides in length.
33. The composition of any one of claims 1-28 or 32, the vector of claims 29-30 or 32, or the cell of claim 31 or 32, wherein the RNA motif binds to the intronic domain at a location between 2 and 200 nucleotides from the exonic sequence in the RNA trans-splicing nucleic acid.
34. The composition of any one of claims 1-28 or 32, the vector of claims 29-30 or 32, or the cell of claim 31 or 32, wherein the RNA motif binds to the intronic domain at a location between 3 and 100 nucleotides from the exonic sequence in the RNA trans-splicing nucleic acid.
35. The composition of any one of claims 1-28 or 32, the vector of any one of claims 29-30 or 32, or the cell of claim 31 or 32, wherein the RNA motif binds to the intronic domain at a location between 4 and 30 nucleotides from the exonic sequence in the RNA trans-splicing nucleic acid.
36. The composition of any one of claims 1-28 or 32-35, the vector of any one of claims 29-30 or 32-35, or the cell of any one of claims 31-35, wherein the RNA trans-splicing molecule comprises, from 5' to 3': the exonic sequence, the intronic domain, and the antisense domain.
37. The composition of any one of claims 1-28 or 32-36, the vector of any one of claims 29-30 or 32-36, or the cell of any one of claims 31-36, wherein the esnRNA and the RNA trans-splicing nucleic acid are in the same nucleic acid molecule.

38. The composition of any one of claims 1-11, 19-28, or 32-36, the vector of claims 29-30 or 32-36, or the cell of any one of claims 31-36, wherein the esnRNA and the RNA trans-splicing nucleic acid are different nucleic acid molecules.

39. Use of the composition of any one of claims 1-28 or 32-38, the vector of any one of claims 29-30 or 32-38, or the cell of any one of claims 31-36 in the manufacture of a medicament for correcting a genetic defect in a subject in need thereof.

40. Use of the composition of any one of claims 1-28 or 32-38, the vector of any one of claims 29-30 or 32-38, or the cell of any one of claims 31-38 in the manufacture of a medicament for treating a disease in a patient in need thereof.

41. An *in vitro* or *ex vivo* trans-splicing method, comprising:

bringing a cell in contact with a composition comprising:

(i) an engineered nucleic acid encoding an RNA trans-splicing nucleic acid comprising: an exonic sequence, an intronic domain, and an antisense domain, and

(ii) a nucleic acid sequence encoding an engineered small nuclear RNA (esnRNA),

wherein upon the composition coming in contact with the cell, the engineered nucleic acid and the nucleic acid sequence encoding the esnRNA enter the cell, and the RNA trans-splicing nucleic acid and the esnRNA are generated in the cell;

wherein the esnRNA comprises an RNA motif at the 5' end of the esnRNA, wherein the antisense domain in the RNA trans-splicing nucleic acid is complementary to a target RNA molecule in the cell, and whereby the exonic sequence is trans-spliced to the target RNA molecule in the cell.

42. A kit for *in vitro* or *ex vivo* trans-splicing, comprising:

(i) an engineered nucleic acid encoding an RNA trans-splicing nucleic acid comprising an exonic sequence, an intronic domain, and an antisense domain, and

(ii) a nucleic acid sequence encoding an engineered small nuclear RNA (esnRNA),

wherein the esnRNA comprises an RNA motif at the 5' end of the esnRNA, wherein the antisense domain in the RNA trans-splicing nucleic acid is complementary to a target RNA molecule in the cell, and whereby the exonic sequence is trans-spliced to the target RNA molecule in the cell.