CLAIM

What is claimed is:

1. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:
   a) a 5’ homology arm,
   b) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,
   c) a 5’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 5’ permissive spacer sequence,
   d) an internal ribosome entry site (IRES),
   e) a protein coding region or noncoding region,
   f) a 3’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 3’ permissive spacer sequence,
   g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and
   h) a 3’ homology arm,

   said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

2. The vector of Claim 1, wherein the 3’ Group I intron fragment and the 5’ Group I intron fragment are from a *Cyanobacterium Anabaena sp.* pre-tRNA-Leu gene.

3. The vector of Claim 1, wherein the 3’ Group I intron fragment and the 5’ Group I intron fragment are from a T4 phage Td gene.

4. The vector of Claim 1, wherein the IRES is selected from an IRES sequence of Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus, foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus (EMCV), Drosophila C Virus, Crucifer tobamo virus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPAl, Human AMLl/RUNXl, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAPl , Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Salivirus, Cosavirus, Parechovirus, Human UNR, Mouse UtrA, Human VEGF-A, Human

XIAP, Drosophila hairless, *S. cerevisiae* TFIID, *S. cerevisiae* YAP1, Human c-src, Human FGF-1, Simian picomavirus, Turnip crinkle virus, an aptamer to eIF4G, Coxsackievirus B3 (CVB3) or Coxsackievirus A (CVB1/2).

5. The vector of Claim 1, wherein the protein coding region encodes an eukaryotic protein or a prokaryotic protein.

6. The vector of Claim 1, wherein:
   a) the 5' homology arm is 5-50 nucleotides in length,
   b) the 3' homology arm is 5-50 nucleotides in length,
   or both a) and b).

7. The vector of Claim 1, wherein:
   a) the 5' permissive spacer sequence is a polyA sequence,
   b) the 3' permissive spacer sequence is a polyA sequence,
   or both a) and b).

8. The vector of Claim 1, wherein at least one spacer sequences is a polyA-C sequence.
   a) the 5' permissive spacer sequence is a polyA-C sequence,
   b) the 3' permissive spacer sequence is a polyA-C sequence,
   or both a) and b).

9. The vector of Claim 1, further comprising an RNA polymerase promoter.

10. The vector of Claim 1, wherein the vector is used to transcribe circular RNA with a size range of 500 to 10,000nt.

11. The vector of claim 1, wherein the 3' Group I intron fragment comprises a 3'-proximal Group I intron-derived sequence including the 3' splice site dinucleotide and, optionally, sequence corresponding to the adjacent natural exon, and the 5' Group I intron fragment comprises a 5'-proximal Group I intron-derived sequence including the 5' splice site dinucleotide and, optionally, sequence corresponding to the adjacent exon.

12. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:
   a) a 5' homology arm,
   b) a 3' Group I intron fragment containing a 3' splice site dinucleotide,
   c) a 5' permissive spacer sequence that increases splicing efficiency compared to a vector without the 5' permissive spacer sequence,
   d) an internal ribosome entry site (IRES),
   e) a protein coding region,

f) a 3’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 3’ permissive spacer sequence,

g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and

h) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

13. The vector of Claim 12, wherein the protein coding region encodes a therapeutic protein, a chimeric antigen receptor, an antibody or a bispecific antibody.

14. A composition comprising:

a) the vector for making circular RNA of Claim 12, and

b) a delivery vehicle for delivering the vector to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

15. A circular RNA produced by the vector of Claim 12, wherein the circular RNA comprises in the following order, the 5’ permissive spacer sequence, the Internal Ribosome Entry Site (IRES), the protein coding region, and the 3’ permissive spacer sequence.

16. A composition comprising an effective amount of the circular RNA of Claim 15 and a pharmaceutically acceptable carrier.

17. A composition comprising:

a) an effective amount of the circular RNA produced by the vector of Claim 12, and

b) a delivery vehicle for delivering the circular RNA to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

18. A composition comprising a circular RNA-expressing cell in combination with one or more pharmaceutically or physiologically acceptable carriers, excipients or diluents, wherein the circular RNA is the circular RNA produced by the vector of Claim 12.

19. The composition of Claim 18, comprising a plurality of circular RNA-expressing cells.

20. The composition of Claim 16, wherein the composition is a nanoparticle formulation.

21. The circular RNA of Claim 15, wherein the protein coding region encodes a chimeric antigen receptor.

22. The composition of Claim 14, which is formulated for intravenous delivery.

23. A method of making circular RNA, said method comprising preparing the vector of Claim 12, and transcribing the vector.

24. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:

a) a 5’ homology arm,

b) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,

c) a 5’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 5’ permissive spacer sequence,

d) an internal ribosome entry site (IRES),

e) a protein coding region encoding a chimeric antigen receptor,

f) a 3’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 3’ permissive spacer sequence,

g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and

h) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

25. The circular RNA produced by the vector of Claim 12, wherein the circular RNA is at least 1 kb.

26. A composition comprising:

a) the vector for making circular RNA of Claim 24, and

b) a delivery vehicle for delivering the vector to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

27. The circular RNA produced by the vector of Claim 24, wherein the circular RNA comprises in the following order, the 5’ permissive spacer sequence, the Internal Ribosome Entry Site (IRES), a protein coding region encoding a chimeric antigen receptor, and the 3’ permissive spacer sequence.

28. A composition comprising:

a) an effective amount of the circular RNA produced by the vector of Claim 24, and

b) a delivery vehicle for delivering the circular RNA to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

29. The composition of Claim 28, wherein the composition is a nanoparticle formulation.

30. An *in vitro* method for making a cell population comprising circular RNA, said method comprising contacting the cells in the population with a composition comprising:

a) circular RNA transcribed from a vector comprising the following elements operably connected to each other and arranged in the following sequence:

i) a 5’ homology arm,

ii) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,

iii) a 5’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 5’ permissive spacer sequence,

iv) an internal ribosome entry site (IRES),

v) a protein coding region,

vi) a 3’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 3’ permissive spacer sequence,

vii) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and

viii) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells, and

b) a delivery vehicle for delivering the circular RNA to the cells, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

31. An *in vitro* method for making a cell population comprising circular RNA, said method comprising contacting the cells in the population with a composition comprising:

a) a vector comprising the following elements operably connected to each other and arranged in the following sequence:

i) a 5’ homology arm,

ii) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,

iii) a 5’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 5’ permissive spacer sequence,

iv) an internal ribosome entry site (IRES),

v) a protein coding region,

vi) a 3’ permissive spacer sequence that increases splicing efficiency compared to a vector without the 5’ permissive spacer sequence,

vii) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and

viii) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells, and

b) a delivery vehicle for delivering the vector to the cells, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

32. The method of Claim 30, wherein the circular RNA comprises in the following order, the 5’ permissive spacer sequence, the Internal Ribosome Entry Site (IRES), the protein coding region, and the 3’ permissive spacer sequence.

33. The method of Claim 32, wherein the protein coding region encodes a chimeric antigen receptor (CAR).

34. The method of Claim 30, wherein the protein coding region encodes a therapeutic protein, a chimeric antigen receptor, an antibody or a bispecific antibody.

35. A circular RNA comprising the following elements arranged in the following sequence:
    a) an adjacent exon sequence of a 3' Group I self-splicing intron-exon,
    b) an internal ribosome entry site (IRES),
    c) a protein coding region or noncoding region, and
    d) an adjacent exon sequence of a 5' Group I self-splicing intron-exon.

36. The circular RNA of claim 35, wherein the adjacent exon sequence of the 3' Group I self-splicing intron-exon or the adjacent exon sequence of the 5' Group I self-splicing intron-exon is at least 1 nucleotide in length.

37. The circular RNA of claim 35, wherein the adjacent exon sequence of the 3' Group I self-splicing intron-exon or the adjacent exon sequence of the 5' Group I self-splicing intron-exon has a length about that of a natural exon.

38. The circular RNA of claim 35, wherein the adjacent exon sequence of the 3' Group I self-splicing intron-exon or the adjacent exon sequence of the 5' Group I self-splicing intron-exon is derived from *Cyanobacterium Anabaena*.

39. The circular RNA of claim 35, wherein the adjacent exon sequence of the 3' Group I self-splicing intron-exon or the adjacent exon sequence of the 5' Group I self-splicing intron-exon is derived from a *Cyanobacterium Anabaena* sp. pre-tRNA-Leu gene.

40. The circular RNA of claim 35, further comprising one or more of a 5' spacer sequence and a 3' spacer sequence.

41. The circular RNA of claim 40, wherein:
    a) the 5' spacer sequence comprises a 5' internal homology region, and
    b) the 3' spacer sequence comprises a 3' internal homology region.

42. The circular RNA of claim 40, wherein:
    a) the 5' spacer sequence is at least 7 nucleotides in length,
    b) the 3' spacer sequence is at least 7 nucleotides in length, or
    both a) and b).

43. The circular RNA of claim 40, wherein:
    a) the 5' spacer sequence is no more than 100 nucleotides in length,
    b) the 3' spacer sequence is no more than 100 nucleotides in length, or
    both a) and b).

44. The circular RNA of claim 40, wherein:

a) the 5’ spacer sequence is 10-60 nucleotides in length,

b) the 3’ spacer sequence is 10-60 nucleotides in length, or

both a) and b).

45. The circular RNA of claim 40, wherein:

a) the 5’ spacer sequence comprises a polyA sequence, a polyC sequence, a polyA-C sequence, or a poly U sequence,

b) the 3’ spacer sequence comprises a polyA sequence, a polyC sequence, a polyA-C sequence, or a poly U sequence, or

both a) and b).

46. The circular RNA of claim 35, comprising the following elements arranged in the following sequence:

a) the adjacent exon sequence of the 3’ Group I self-splicing intron-exon,

b) a 5’ spacer sequence,

c) the internal ribosome entry site (IRES),

d) the protein coding region or noncoding region,

e) a 3’ spacer sequence, and

f) the adjacent exon sequence of the 5’ Group I self-splicing intron-exon.

47. The circular RNA of claim 35, wherein the IRES is selected from an IRES sequence of Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus-1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus-1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus, foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus (EMCV), Drosophila C Virus, Crucifer tobamo virus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPAl, Human AMLl/RUNXl, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAPl , Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Salivirus, Cosavirus, Parechovirus, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, *S. cerevisiae* TFIID, *S. cerevisiae* YAP1, Human c-src, Human FGF-1, Simian picornavirus, Turnip crinkle virus, an aptamer to eIF4G, Coxsackievirus B3 (CVB3) or Coxsackievirus A (CVB1/2).

48. The circular RNA of claim 35, wherein the IRES is an IRES sequence from Coxscakievirus B3 (CBV3), Encephalomyocarditis virus (EMCV), or Salivirus.

49. The circular RNA of claim 35, wherein the protein coding region or noncoding region is a protein coding region that encodes a eukaryotic protein or a prokaryotic protein.

50. The circular RNA of claim 35, wherein the protein coding region or noncoding region is a protein coding region that encodes a human protein, a non-human protein, or a synthetic protein.

51. The circular RNA of claim 35, wherein the protein coding region or noncoding region is a protein coding region that encodes a chimeric antigen receptor, a therapeutic protein, or a binding protein.

52. The circular RNA of claim 35, wherein the circular RNA has a size range of 500 to 10,000 nucleotides.

53. The circular RNA of claim 35, wherein the circular RNA has a size range of at least 1,000 nucleotides.

54. A composition comprising:

a) an effective amount of circular RNA, wherein the circular RNA comprises the following elements arranged in the following sequence:

i) an adjacent exon sequence of a 3’ Group I self-splicing intron-exon,

ii) an internal ribosome entry site (IRES),

iii) a protein coding region or noncoding region, and

iv) an adjacent exon sequence of a 5’ Group I self-splicing intron-exon; and

b) a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

55. A composition comprising:

a) an effective amount of the circular RNA, wherein the circular RNA comprises the following elements arranged in the following sequence:

i) an adjacent exon sequence of a 3’ Group I self-splicing intron-exon,

ii) an internal ribosome entry site (IRES),

iii) a protein coding region or noncoding region, and

iv) an adjacent exon sequence of a 5’ Group I self-splicing intron-exon; and

b) a pharmaceutically acceptable carrier, excipient or diluent.

56. The circular RNA of any one of claims 35-53, or the composition of claim 54 or 55, for use in therapy.