Claims

1. A method for improving the expression and establishment efficiency of a self-replicating non- integrative episomal S/MAR expression vector in a target vertebrate cell comprising the following steps:

a. providing an episomal S/MAR expression vector comprising:

i. a bacterial replication-selection region comprising a bacterial origin of replication and a selectable marker;

ii. a transcription unit for expression of a transgene in a vertebrate cell, comprising a promoter, a 5' UTR, a transgene, and a 3' UTR;

iii. an S/MAR insert located within said 3' UTR; and

b. modifying the episomal S/MAR expression vector such that the S/MAR is flanked by a 5' splice donor site and a 3' splice acceptor site within said 3' UTR, whereby the resultant self-replicating non-integrative episomal S/MAR expression vector has improved expression and establishment efficiency after transfection of a vertebrate cell.

2. The method of claim 1, wherein said S/MAR insert contains internal AATAAA transcription termination motifs.

3. The method of claim 2, wherein said AATAAA transcription termination motifs in said S/MAR are replaced with AATATT motifs.

4. The method of claim 1, wherein said S/MAR is selected from the group consisting of human Interferon beta S/MAR, M18 S/MAR, Apolipoprotein B S/MAR.

5. The method of claim 1, wherein said SMAR flanked by a 5' splice donor site and a 3' splice acceptor site has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23.

6. The method of claim 1, wherein said bacterial origin of replication is an R6K gamma replication origin.

7. The method of claim 1, wherein said bacterial origin of replication is an R6K gamma replication origin with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

8. The method of claim 1, wherein said selectable marker is an RNA-IN regulating RNA-OUT functional variant with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 5, and SEQ ID NO: 7.

9. The method of claim 1, wherein said selectable marker is an RNA-OUT RNA selectable marker that encodes an RNA-IN regulating RNA-OUT RNA with at least 95% sequence identity to SEQ ID NO: 6.

10. The method of claim 1, wherein said bacterial replication-selection region comprising a bacterial origin of replication and a selectable marker is a R6K origin-RNA-OUT RNA selectable marker bacterial replication-selection region with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

11. The method of claim 1, where said 5' UTR further encodes an intron.

12. The method of claim 1, where said transcription unit further encodes an expression enhancer positioned upstream of the promoter.

13. The method of claim 12, wherein said expression enhancer has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 27, and SEQ ID NO: 28.

14. The method of claim 1, wherein said splice donor site has at least 95% sequence identity to SEQ ID NO:25.

15. The method of claim 1, wherein said splice acceptor site has at least 95% sequence identity to SEQ ID NO: 26.

16. The method of claim 1, wherein said self-replicating non-integrative episomal S/MAR expression vector is selected from the group consisting of plasmid vector, Nanoplasmid vector, Integration-Deficient Lentivirus vector, and Non-integrating Lentiviral vectors.

17. A polynucleotide comprising at least one promoter and an S/MAR element, wherein said S/MAR element is located downstream of said promoter and wherein the nucleic acid sequence of said S/MAR element (S/MAR sequence) comprises at least 3 sequence motifs ATTA per 100 nucleotides over a stretch of at most 200 nucleotides, wherein said S/MAR element is flanked by a splice donor and a splice acceptor, and wherein said polynucleotide further comprises an R6K gamma replication origin with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO: 3 and SEQ ID NO: 4.

18. The polynucleotide of claim 17, wherein said promoter is comprised in a transcription unit for expression of a cargo polypeptide and/or a selectable marker in a host cell.

19. The polynucleotide according to claim 18, wherein said transcription unit comprises a promoter, a 5' UTR, a transgene, and a 3' UTR.

20. The polynucleotide of claim 19, wherein said S/MAR is located within said 3' UTR.

21. The polynucleotide of any one of claims 17 to 21, wherein said S/MAR is flanked by a 5' splice donor site and a 3' splice acceptor site.

22. The polynucleotide of any one of claims 17 to 21, wherein said polynucleotide further comprises an RNA-OUT RNA selectable marker comprising an RNA-IN regulating RNA-OUT functional variant with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 5, and SEQ ID NO: 7.

23. A covalently closed circular recombinant DNA molecule comprising:

a. an transcription unit for expression of a transgene in a vertebrate cell, comprising a promoter, a 5' UTR, a transgene, and a 3' UTR;

b. an S/MAR located within said 3' UTR wherein said S/MAR is flanked by a 5' splice donor site and a 3' splice acceptor site;

c. an R6K gamma replication origin with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4; and

d. an RNA-OUT RNA selectable marker comprising an RNA-IN regulating RNA-OUT functional variant with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 5, and SEQ ID NO: 7.

24. The polynucleotide of any one of claims 17 to 22 or the recombinant DNA molecule of claim 23, wherein said R6K gamma replication origin and said RNA-OUT RNA selectable marker comprise a R6K origin-RNA-OUT RNA selectable marker bacterial replication-selection region with at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

25. The polynucleotide of any one of claims 17 to 22 and 24 or the recombinant DNA molecule of claim 23, wherein said S/MAR contains internal AATAAA transcription termination motifs.

26. The polynucleotide of any one of claims 17 to 22 and 24 to 25 or the recombinant DNA molecule of claim 25, wherein said AATAAA transcription termination motifs in said S/MAR are replaced with AATATT motifs.

27. The polynucleotide of any one of claims 17 to 22 and 24 to 26 or the recombinant DNA molecule of claim 23, wherein said S/MAR is selected from the group consisting of human Interferon beta S/MAR, M18 S/MAR, Apolipoprotein B S/MAR.

28. The polynucleotide of any one of claims 17 to 22 and 24 to 27 or the recombinant DNA molecule of claim 23, wherein said SMAR flanked by a 5' splice donor site and a 3' splice acceptor site has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23.

29. The polynucleotide of any one of claims 17 to 22 and 24 to 28 or the recombinant DNA molecule of claim 23, where said 5' UTR further encodes an intron.

30. The polynucleotide of any one of claims 17 to 22 and 24 to 29 or the recombinant DNA molecule of claim 17, where said transcription unit further encodes an expression enhancer positioned upstream of the promoter.

31. The polynucleotide of any one of claims 17 to 22 and 24 to 30 or the recombinant DNA molecule of claim 17, wherein said expression enhancer has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 27, and SEQ ID NO: 28.

32. The polynucleotide of any one of claims 17 to 22 and 24 to 31 or the recombinant DNA molecule of claim 17, wherein said splice donor site has at least 95% sequence identity to SEQ ID NO:25.

33. The polynucleotide of any one of claims 17 to 22 and 24 to 33 or the recombinant DNA molecule of claim 17, wherein said splice acceptor site has at least 95% sequence identity to SEQ ID NO: 26.