Claims

1. An RNA replicon comprising a modified 5' replication recognition sequence of alphavirus, wherein the modified 5' replication recognition sequence is characterized in that all initiation codons have been removed from the conserved sequence element 2 (CSE 2) of the native alphavirus 5' replication recognition sequence and wherein the modified 5' replication recognition sequence comprises one or more additional nucleotide changes compensating for nucleotide pairing disruptions within one or more stem loops (SL) introduced by the removal of the initiation codons,
wherein said CSE 2 spans from SL2 to SL4 and comprises the native initiation codon that encodes the first amino acid residues of alphavirus non-structural protein nsP1, and
wherein the modified 5’ replication recognition sequence is characterized by a predicted secondary structure that is equivalent to the predicted secondary structure of the 5’ replication recognition sequence of alphaviral genomic RNA.

2. The RNA replicon according to claim 1, which comprises a 3' replication recognition sequence.

3. The RNA replicon according to claim 1 or 2, which comprises a first open reading frame encoding a protein of interest, wherein preferably the protein of interest can be expressed from the RNA replicon as a template.

4. The RNA replicon according to claim 3, which comprises a subgenomic promoter controlling production of subgenomic RNA comprising the first open reading frame encoding a protein of interest, wherein preferably the protein of interest encoded by the first open reading frame can be expressed from the RNA replicon and the subgenomic RNA.

5. The RNA replicon according to claim 3, which comprises a subgenomic promoter controlling production of subgenomic RNA comprising a second open reading frame encoding a protein of interest, which are preferably located downstream from the first open reading frame encoding a protein of interest.

6. The RNA replicon according to any one of claims 3 to 5, wherein the protein of interest encoded by the first and/or second open reading frame is functional alphavirus non-structural protein.

7. The RNA replicon according to claim 6, which can be replicated by the functional alphavirus non-structural protein.

8. The RNA replicon according to any one of claims 1 to 5, which does not comprise an open reading frame encoding functional alphavirus non-structural protein.

9. A system comprising:
an RNA construct for expressing functional alphavirus non-structural protein,
the RNA replicon according to any one of claims 1 to 5 and 8 which can be replicated by the functional alphavirus non-structural protein *in trans*.

10. The RNA replicon according to any one of claims 1 to 8 or the system according to claim 9, wherein the alphavirus is Semliki Forest Virus.

11. A DNA comprising a nucleic acid sequence encoding the RNA replicon of any one of claims 1 to 8 and 10.

12. An *in vitro* method for producing a protein of interest in a cell comprising the steps of:
(a) obtaining the RNA replicon according to any one of claims 1 to 7 and 10 which comprises an open reading frame encoding functional alphavirus non-structural protein, which can be replicated by the functional alphavirus non-structural protein and which comprises an open reading frame encoding the protein of interest, and
(b) inoculating the RNA replicon into the cell.

13. An *in vitro* method for producing a protein of interest in a cell comprising the steps of:
(a) obtaining an RNA construct for expressing functional alphavirus non-structural protein,

(b) obtaining the RNA replicon according to any one of claims 1 to 5, 8 and 10 which can be replicated by the functional alphavirus non-structural protein *in trans* and comprises an open reading frame encoding the protein of interest, and

(c) co-inoculating the RNA construct for expressing functional alphavirus non-structural protein and the RNA replicon into the cell.

14. A cell inoculated according to the method of claim 12 or 13, wherein the cell inoculated according to the method of claim 12 comprises the RNA replicon of any one of claims 1 to 7 or 10 and wherein the cell inoculated according to the method of claim 13 comprises an RNA construct for expressing functional alphavirus non-structural protein and the RNA replicon according to any one of claims 1 to 5, 8 and 10.

15. An RNA replicon according to any one of claims 1 to 7 and 10 for use in therapy, wherein the RNA replicon comprises an open reading frame encoding functional alphavirus non-structural protein, and can be replicated by the functional alphavirus non-structural protein and wherein the RNA replicon comprises an open reading frame encoding a protein of interest, and wherein the therapy comprises the RNA replicon to be administered to a subject.

16. An RNA replicon according to any one of claims 1 to 5, 8 and 10 for use in therapy, wherein the RNA replicon can be replicated by functional alphavirus non-structural protein *in trans* and comprises an open reading frame encoding a protein of interest, and wherein the therapy comprises the RNA replicon and an RNA construct for expressing functional alphavirus non-structural protein to be administered to a subject.