There is, nonetheless, a need in the art for improved means and methods for stable transfection of cells, in particular using S/MAR elements and avoiding the risks involved with integration of the transgene into the genome of the host cell. This problem is solved by the means and methods disclosed herein.

Summary

In one aspect, the present disclosure relates to 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 (SEQ ID NO:1) 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.

In another aspect, the present disclosure relates to a composition comprising a polynucleotide according to the disclosure, preferably wherein said composition is a pharmaceutical composition.

In another aspect, the present disclosure relates to a host cell comprising the polynucleotide according to the disclosure, preferably wherein said host cell is a CD34+ Progenitor Cell; a CD61+ Thrombocyte; a CD19+ B-Lymphocyte; a CD14+ Monocyte; a CD15+ Granulocyte; a CD3+ Cytotoxic T-Lymphocyte, preferably also positive for CD8 and CD45; a CD3+ Helper T-Lymphocyte, preferably also positive for CD4 and CD45; a CD3+ activated T-Lymphocyte, preferably also positive for CD25 and CD45, a Tumor infiltrating Lymphocyte, or a Natural Killer (NK) cell.

In another aspect, the present disclosure relates to a polynucleotide according to the disclosure, a composition according to the disclosure, and/or a host cell according to the disclosure, for use in medicine, preferably, for use in treating genetic disease.

In another aspect, the present disclosure relates to a kit comprising a polynucleotide according to the disclosure and a compound mediating cell entry.

In another aspect, the present disclosure relates to a device comprising a polynucleotide according to the disclosure, a composition according to the disclosure, and/or a host cell according to the disclosure.

In another aspect, the present disclosure relates to a method for stably transfecting a host cell, comprising a) contacting said host cell with a polynucleotide according to the disclosure, and/or a composition according to the disclosure, and/or a host cell according to the disclosure, and, b) thereby, stably transfecting a host cell.

Detailed Description of the Invention

Accordingly, the present invention relates to 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.

As used in the following, the terms “have”, “comprise” or “include” or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms may both refer to a situation in which, besides the feature introduced by these terms, no further features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions “A has B”, “A comprises B” and “A includes B” may both refer to a situation in which, besides B, no other element is present in A (i.e. a situation in which A solely and exclusively consists of B) and to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C and D or even further elements.

Further, as used in the following, the terms "preferably", "more preferably", "most preferably", "particularly", "more particularly", "specifically", "more specifically" or similar terms are used in conjunction with optional features, without restricting further possibilities. Thus, features introduced by these terms are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by using alternative features. Similarly, features introduced by "in an embodiment of the invention" or similar expressions are intended to be optional features,

without any restriction regarding further embodiments of the invention, without any restrictions regarding the scope of the invention and without any restriction regarding the possibility of combining the features introduced in such way with other optional or non-optional features of the invention.

Claims

1. 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 (SEQ ID NO:1) 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.

2. The polynucleotide of claim 1, wherein said polynucleotide further comprises a coding sequence encoding a polypeptide, preferably a selectable marker, wherein said coding sequence encoding a polypeptide intervenes said promoter and said S/MAR element.

3. The polynucleotide of any one of claims 1 to 2, wherein said polynucleotide further comprises a coding sequence encoding a selectable marker (selectable marker sequence), said selectable marker sequence intervening said promoter and said S/MAR element, wherein said promoter and said selectable marker sequence together constitute a selectable marker gene, and wherein said selectable marker is a selectable marker of a eukaryotic cell.

4. The polynucleotide of claim 3, wherein said selectable marker gene is a puromycin resistance gene, a blasticidin resistance gene, a neomycin resistance gene, or a zeocin resistance gene, preferably is a puromycin resistance gene.

5. The polynucleotide of any one of claims 1 to 4, wherein a transcript is transcribed from said promoter, from which transcript the sequence of the S/MAR element is spliced out..

6. The polynucleotide of any one of claims 1 to 5, wherein said polynucleotide is devoid of a simian virus 40 (SV40) origin of replication, a bovine papillomavirus (BPV) origin of replication, and an Epstein-Barr virus (EBV) origin of replication.

7. The polynucleotide of any one of claims 1 to 6, wherein said polynucleotide replicates episomally in a host cell, preferably wherein episomal replication is stable episomal replication, preferably in a mammalian cell

8. A composition comprising a polynucleotide according to any one of claims 1 to 7, preferably wherein said composition is a pharmaceutical composition.

9. A host cell comprising the polynucleotide according to any one of claims 1 to 7, preferably wherein said host cell is a CD34+ Progenitor Cell; a CD61+ Thrombocyte; a CD19+ B-Lymphocyte; a CD14+ Monocyte; a CD15+ Granulocyte; a CD3+ Cytotoxic T-Lymphocyte, preferably also positive for CD8 and CD45; a CD3+ Helper T-Lymphocyte, preferably also positive for CD4 and CD45; a CD3+ activated T-Lymphocyte, preferably also positive for CD25 and CD45, a Tumor infiltrating Lymphocyte, or a Natural Killer (NK) cell.

10. A polynucleotide according to any one of claims 1 to 7, a composition according to claim 8, and/or a host cell according to claim 9, for use in medicine, preferably, for use in treating genetic disease.

11. A kit comprising a polynucleotide according to any one of claims 1 to 7 and a compound mediating cell entry.

12. A device comprising a polynucleotide according to any one of claims 1 to 7, a composition according to claim 8, and/or a host cell according to claim 9.

13. A method for stably transfecting a host cell, comprising

a) contacting said host cell with a polynucleotide according to any one of claims 1 to 7, and/or a composition according to claim 8, and/or a host cell according to claim 9, and,

b) thereby, stably transfecting a host cell.