Claims

1. An artificial nucleic acid molecule comprising:
   a. at least one 5'-untranslated region element (5'UTR element), which comprises or consists of a nucleic acid sequence, which comprises
      - the 5'UTR of a TOP gene,
      - a fragment of the 5'UTR of a TOP gene, wherein the fragment consists of a continuous stretch of nucleotides corresponding to at least 20% of the full-length 5'UTR of a TOP gene; or
      - a variant of the 5'UTR of a TOP gene, wherein the variant is at least 80% identical to the naturally occuring 5'UTR,
   b. at least one open reading frame (ORF), and
   c. at least one histone stem-loop,

   wherein the at least one 5'UTR element does not comprise a 5'TOP motif and wherein the at least one 5'UTR element increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

2. The artificial nucleic acid molecule according to claim 1, wherein the 5'UTR element and the open reading frame are heterologous.

3. The artificial nucleic acid molecule according to claim 1 or 2, wherein the nucleic acid sequence, which comprises the 5'UTR of a TOP gene or the fragment or variant thereof, preferably the 5'UTR element, terminates at its 3'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 upstream of the start codon of the gene it is derived from.

4. The artificial nucleic acid molecule according to any one of claims 1-3, wherein the 5'UTR element does not comprise a start codon or an open reading frame.

5. The artificial nucleic acid molecule according to any one of claims 1-4, wherein the nucleic acid sequence, which comprises the 5'UTR of a TOP gene or the fragment or the variant thereof, is derived from the 5'UTR of a eukaryotic TOP

gene, preferably from the 5'UTR of a plant or animal TOP gene, more preferably from the 5'UTR of a chordate TOP gene, even more preferably from the 5'UTR of a vertebrate TOP gene, most preferably from the 5'UTR of a mammalian TOP gene, such as a human TOP gene.

6. The artificial nucleic acid molecule according to any one of claims 1-5, wherein the at least one histone stem-loop is selected from following formulae (I) or (II):

formula (I) (stem-loop sequence without stem bordering elements):

$$\underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}$$

formula (II) (stem-loop sequence with stem bordering elements):

$$\underbrace{N_{1-6}}_{\text{stem1}}\ \underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}\ \underbrace{N_{1-6}}_{\text{stem2}}$$

bordering element bordering element

wherein:

stem1 or stem2 bordering elements N_{1-6} is a consecutive sequence of 1 to 6, preferably of 2 to 6, more preferably of 2 to 5, even more preferably of 3 to 5, most preferably of 4 to 5 or 5 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C, or a nucleotide analogue thereof;

stem1 $[N_{0-2}GN_{3-5}]$ is reverse complementary or partially reverse complementary with element stem2, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof, and

wherein G is guanosine or an analogue thereof, and may be optionally replaced by a cytidine or an analogue thereof, provided that its complementary nucleotide cytidine in stem2 is replaced by guanosine;

loop sequence $[N_{0-4}(U/T)N_{0-4}]$ is located between elements stem1 and stem2, and is a consecutive sequence of 3 to 5 nucleotides, more preferably of 4 nucleotides;

wherein each N_{0-4} is independent from another a consecutive sequence of 0 to 4, preferably of 1 to 3, more preferably of 1 to 2 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein U/T represents uridine, or optionally thymidine;

stem2 $[N_{3-5}CN_{0-2}]$ is reverse complementary or partially reverse complementary with element stem1, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide

selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein C is cytidine or an analogue thereof, and may be optionally replaced by a guanosine or an analogue thereof provided that its complementary nucleotide guanosine in stem1 is replaced by cytidine;

wherein

stem1 and stem2 are capable of base pairing with each other

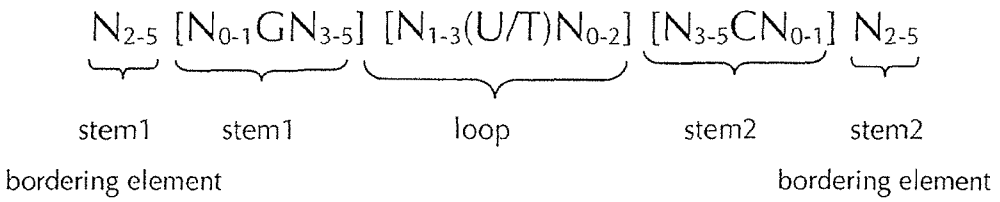
forming a reverse complementary sequence, wherein base pairing may occur between stem1 and stem2, or

forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2.

7. The artificial nucleic acid molecule according to any one of claims 1-6, wherein the at least one histone stem-loop is selected from at least one of following formulae (Ia) or (IIa):

$$\underbrace{[N_{0-1}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{1-3}(U/T)N_{0-2}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-1}]}_{\text{stem2}}$$

formula (Ia) (stem-loop sequence without stem bordering elements)



formula (IIa) (stem-loop sequence with stem bordering elements)

8. The artificial nucleic acid molecule according to any one of claims 1-7, further comprising

   d. a poly(A) sequence and/or a polyadenylation signal.

9. The artificial nucleic acid molecule according to any of claims 1-8, further comprising:

   e. a poly(C) sequence.

10. The artificial nucleic acid molecule according to any one of claims 1-9, further comprising:

    f. at least one 3'UTR element.

11. The artificial nucleic acid molecule according to claim 10, wherein the at least one 3'UTR element comprises or consists of a nucleic acid sequence, which is at least 80% identical to a 3'UTR of a gene providing a stable mRNA.

12. The artificial nucleic acid molecule according to any one of claims 1-11, wherein the 5'UTR element comprises or consists of a nucleic acid sequence, which has an identity of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to a nucleic acid sequence selected from SEQ ID NOs. 1-1363, SEQ ID NO. 1435, SEQ ID NO. 1461 or SEQ ID NO. 1462, or to a corresponding RNA sequence, or
wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence, which is 100% identical over at least 20% of the full length 5'UTR element and which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%,

more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to a nucleic acid sequence selected from SEQ ID NOs. 1-1363, SEQ ID NO. 1435, SEQ ID NO. 1461 or SEQ ID NO. 1462, or to a corresponding RNA sequence,
wherein the fragment of the at least one 5'UTR element of a TOP gene increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

13. The artificial nucleic acid molecule according to any one of claims 1-12, wherein the 5'UTR element comprises or consists of a nucleic acid sequence, which has an identity of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the 5'UTR of a nucleic acid sequence according to any of SEQ ID NOs: 170, 232, 244, 259, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, or 1360; or to a corresponding RNA sequence, or
wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence, which is 100% identical over at least 20% of the full length 5'UTR element and which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the 5'UTR of a nucleic acid sequence according to SEQ ID NOs: 170, 232, 244, 259, 1284, 1285, 1286, 1287, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, or 1360; or to a corresponding RNA sequence,

wherein the fragment of the at least one 5'UTR element of a TOP gene increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

14. The artificial nucleic acid molecule according to any one of claims 1-13, wherein the 5'UTR element is at least 80% identical to a 5'UTR of a TOP gene encoding a ribosomal Large protein (RPL), preferably to a 5'UTR of a nucleic acid sequence according to any of SEQ ID NOs: 67, 259, 1284-1318, 1344, 1346, 1348-1354, 1357, 1461 and 1462, a corresponding RNA sequence or a homolog thereof.

15. The artificial nucleic acid molecule according to any one of claims 1-14, wherein the 5'UTR element comprises or consists of a nucleic acid sequence having an identity of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the 5'UTR of a nucleic acid sequence according to any of SEQ ID NOs: 67, 259, 1284-1318, 1344, 1346, 1348-1354, 1357, 1461 and 1462, or to a corresponding RNA sequence, or

wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence, which is 100% identical over at least 20% of the full length 5'UTR element and which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the 5'UTR of a nucleic acid sequence according to SEQ ID No. SEQ ID NOs: 67, 259, 1284-1318, 1344, 1346, 1348-1354, 1357, 1461 and 1462 or to a corresponding RNA sequence,

wherein the fragment of the at least one 5'UTR element of a TOP gene increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

16. The artificial nucleic acid molecule according to any one of claims 1-15, wherein the 5'UTR element comprises or consists of a nucleic acid sequence, which is at least 80% identical to the 5'UTR of a ribosomal protein Large 32 gene, preferably to the 5'UTR of a vertebrate ribosomal protein Large 32 (L32) gene, more preferably to

the 5'UTR of a mammalian ribosomal protein Large 32 (L32) gene, most preferably to the 5'UTR of a human ribosomal protein Large 32 (L32) gene.

17. The artificial nucleic acid molecule according to any one of claims 1-16, wherein the 5'UTR element comprises or consists of a nucleic acid sequence, which is 100% identical over at least 20% of the full length 5'UTR element and which has an identity of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NOs. 1368 or 1452-1460, or a corresponding RNA sequence, or

wherein the at least one 5'UTR element comprises or consists of a fragment of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NOs. 1368 or 1452-1460, or to a corresponding RNA sequence,

wherein the fragment of the at least one 5'UTR element of a TOP gene increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

18. The artificial nucleic acid molecule according to any one of claims 1-17, wherein the 5'UTR element comprises or consists of a nucleic acid sequence, which has an identity of at least 80% to the 5'UTR of a TOP gene selected from RPSA, RPS2, RPS3, RPS3A, RPS4, RPS5, RPS6, RPS7, RPS8, RPS9, RPS10, RPS11, RPS12, RPS13, RPS14, RPS15, RPS15A, RPS16, RPS17, RPS18, RPS19, RPS20, RPS21, RPS23, RPS24, RPS25, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS30, RPL3, RPL4, RPL5, RPL6, RPL7, RPL7A, RPL8, RPL9, RPL10, RPL10A, RPL11, RPL12, RPL13, RPL13A, RPL14, RPL15, RPL17, RPL18, RPL18A, RPL19, RPL21, RPL22, RPL23, RPL23A, RPL24, RPL26, RPL27, RPL27A, RPL28, RPL29, RPL30, RPL31, RPL32, RPL34, RPL35, RPL35A, RPL36, RPL36A, RPL37, RPL37A, RPL38, RPL39, RPL40, RPL41, RPLP0, RPLP1, RPLP2, RPLP3, UBA52.

19. The artificial nucleic acid molecule according to any one of claims 1-18, wherein the at least one histone stem-loop comprises or consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs. 1391-1433, preferably from the group consisting of SEQ ID NOs. 1403-1433.

20. The artificial nucleic acid molecule according to any one of claims 1-19, wherein the histone stem-loop comprises or consists of a nucleic acid sequence having a sequence identity of at least about 80%, preferably of at least about 85%, more preferably of at least about 90%, even more preferably of at least about 95% to the sequence according to SEQ ID NO. 1433 or to the corresponding RNA sequence,
wherein the histone stem-loop increases protein production from the artificial nucleic acid molecule or increases stability of the artificial nucleic acid molecule, and

wherein preferably positions 6, 13 and 20 of the sequence having a sequence identity of at least about 80%, preferably at least about 85%, more preferably at least about 90%, even more preferably at least about 95% to the sequence according to SEQ ID NO. 1433 or to the corresponding RNA sequence are conserved, i.e. are identical to the nucleotides at positions 6, 13 and 20 of SEQ ID NO. 1433 or to the corresponding RNA nucleotides.

21. The artificial nucleic acid molecule according to any one of claims 10-20, wherein the 3'UTR element comprises or consists of a nucleic acid sequence at least 80% identical to a 3'UTR of a gene selected from the group consisting of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, and a collagen alpha gene,
wherein the 3'UTR element increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule,
wherein the at least one 3'UTR element preferably comprises or consists of a nucleic acid sequence, which is at least 80% identical to the 3'UTR of a vertebrate α-globin gene, preferably to the 3'UTR of a mammalian α-globin gene, more preferably to the 3'UTR of a human α-globin gene.

22. The artificial nucleic acid molecule according to any one of claims 10-21, wherein the at least one 3'UTR element comprises or consists of a nucleic acid sequence, which is at least 80% identical to the 3'UTR of a vertebrate albumin gene, preferably to the 3'UTR of a mammalian albumin gene or from a variant thereof, more preferably to the 3'UTR of a human albumin gene or from a variant thereof, even more preferably to the 3'UTR of the human albumin gene according to GenBank Accession number NM_000477.5,
wherein the 3'UTR element increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

23. The artificial nucleic acid molecule according to any one of claims 10-22, wherein the at least one 3'UTR element comprises or consists of a nucleic acid sequence, which has an identity of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to a nucleic acid sequence selected from SEQ ID NOs. 1369-1377 and 1434 or to a corresponding RNA sequence, or
wherein the at least one 3'UTR element comprises or consists of a fragment of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to a nucleic acid sequence selected from SEQ ID NOs. 1369-1377 and 1434 or to a corresponding RNA sequence,
wherein the 3'UTR element increases and/or prolongs production of the protein encoded by the at least one open reading frame from the artificial nucleic acid molecule.

24. The artificial nucleic acid molecule according to any one of claims 1-23, wherein the artificial nucleic acid molecule, preferably the open reading frame, is at least partially G/C modified, preferably wherein the G/C content of the open reading frame is increased compared to the wild type open reading frame.

25. The artificial nucleic acid molecule according to any one of claims 1-24, which is an RNA, preferably an mRNA molecule.

26. A vector comprising:

a. at least one 5'-untranslated region element (5'UTR element), which comprises or consists of a nucleic acid sequence, which comprises

- the 5'UTR of a TOP gene,
- a fragment of the 5'UTR of a TOP gene, wherein the fragment consists of a continuous stretch of nucleotides corresponding to at least 20% of the full-length 5'-UTR of a TOP gene; or
- a variant of the 5'UTR of a TOP gene, wherein the variant is at least 80% identical to the naturally occuring 5'UTR;

b. at least one open reading frame (ORF) and at least one cloning site; and

c. at least one histone-stem loop,

wherein the at least one 5'UTR element does not comprise a 5'TOP motif and wherein the at least one 5'UTR element increases and/or prolongs production of the protein encoded by the at least one open reading frame.

27. The vector according to claim 26, which is a DNA vector.

28. The vector according to any one of claims 26 or 27, which is a plasmid vector or a viral vector, preferably a plasmid vector.

29. The vector according to any one of claims 26-28, which comprises or codes for an artificial nucleic acid molecule according to any one of claims 1-25.

30. The vector according to any one of claims 26-29, which is a circular molecule.

31. A cell comprising the artificial nucleic acid molecule according to any one of claims 1-25 or the vector according to any one of claims 26-30.

32. The cell according to claim 31, which is a mammalian cell, preferably an isolated cell of a mammalian subject, preferably of a human subject.

33. A pharmaceutical composition comprising the artificial nucleic acid molecule according to any one of claims 1-25, the vector according to any one of claims 26-30, or the cell according to claim 31 or 32, optionally further comprising one or more pharmaceutically acceptable diluents and/or excipients and/or one or more adjuvants.

34. The artificial nucleic acid molecule according to any one of claims 1-25, the vector according to any one of claims 26-30, the cell according to claim 31 or 32, or the pharmaceutical composition according to claim 33 for use as a medicament, preferably for use as a vaccine or for use in gene therapy.