efficiency, of RNA is preferably achieved by genetically modifying expression vectors which preferably serve as template for RNA transcription *in vitro*. These expression vectors allow transcription of RNA with a 3'-untranslated region described according to the invention, and preferably between the sequence coding for a peptide or protein (open reading frame) and the poly(A) sequence.

These vectors may also allow transcription of RNA with a poly(A) sequence which preferably has an open end in said RNA, i.e. no nucleotides other than A nucleotides flank said poly(A) sequence at its 3' end. An open-ended poly(A) sequence in the RNA can be achieved by introducing a type IIS restriction cleavage site into an expression vector which allows RNA to be transcribed under the control of a 5' RNA polymerase promoter and which contains a polyadenyl cassette, wherein the recognition sequence is located 3' of the polyadenyl cassette, while the cleavage site is located upstream and thus within the polyadenyl cassette. Restriction cleavage at the type IIS restriction cleavage site enables a plasmid to be linearized within the polyadenyl cassette. The linearized plasmid can then be used as template for *in vitro* transcription, the resulting transcript ending in an unmasked poly(A) sequence. Furthermore, an optional disruption of the 3' polyadenyl cassette by a random nucleotide sequence, with an equal distribution of the 4 nucleotides (linker), increases the stability of the 3' polyadenyl cassette in E.coli.

Summary

In one aspect, the present disclosure refers to a nucleic acid molecule comprising in the 5' → 3' direction of transcription:(a)a promoter;(b) a transcribable nucleic acid sequence or a nucleic acid sequence for introducing a transcribable nucleic acid sequence; and(c) a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a 3'-untranslated region in the transcript which is not naturally linked to the nucleic acid sequence (b), said 3'-untranslated region comprising (i) (c-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment ; or (ii) (c-8) any combination of (c-4) and one or more nucleic acid sequences selected from the group consisting of:(c-1)the nucleic acid sequence of the 3' untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-2)the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant of said nucleic

acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, (c-4) the nucleic acid sequence of the 3'-untranslated region of AES comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-5)the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, and(c-7) the nucleic sequence of the 3'- untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, wherein the nucleic acid sequences (b) and (c) under the control of the promoter (a) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (c) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (b).

In another aspect, the present disclosure refers to the RNA comprising in the 5' → 3' direction:(a) a 5' untranslated region;(b)a nucleic acid sequence coding for a peptide or protein; and(c) a 3'-untranslated region which is not naturally linked to the nucleic acid sequence (b), said 3'-untranslated region comprising (i) (c-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment; or (ii) (c-8) any combination of (c-4) and one or more nucleic acid sequences selected from the group consisting of:(c-1)the nucleic acid sequence of the 3'-untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-2)the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, (c-4) the nucleic acid sequence of the 3' untranslated region of AES comprising or consisting of

a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-5)the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(c-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, (c-7) the nucleic sequence of the 3'-untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,wherein the nucleic acid sequence (c) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence coding for a peptide or protein.

In yet another aspect, the present disclosure refers to a method of obtaining RNA, comprising:(i)coupling a nucleic acid sequence (b) which, when transcribed, codes for a 3'-untranslated region, at the 3' end of a transcribable nucleic acid sequence (a) comprising a nucleic acid sequence coding for a peptide or protein, wherein nucleic acid sequence (a) is not naturally linked to the nucleic acid sequence (b), and

(ii) transcribing the nucleic acid obtained,said 3'-untranslated region comprising (iia) (b-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment; or(iib) (b-8) any combination of (b-4) and one or more nucleic acid sequences selected from the group consisting of: (b-1) the nucleic acid sequence of the 3'-untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(b-2)the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(b-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(b-4)the nucleic acid sequence of the 3'-untranslated region of AES comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or

fragment,(b-5) the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,(b-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, (b-7) the nucleic sequence of the 3'-untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, wherein the nucleic acid sequences (a) and (b) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (b) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (a).

In a further aspect, the present disclosure refers to an in vitro method of obtaining a peptide or protein, comprising:(i) obtaining RNA by the method as described herein, and(ii) translating the RNA.

In a further aspect, the present disclosure refers to the use of the RNA as described herein in an in vitro method of transfecting a host cell.

In a further aspect, the present disclosure refers to the RNA as described herein for use in vaccination.

In a further aspect, the present disclosure refers to the RNA as described herein for use in reprogramming somatic cells to cells having stem cell characteristics.

In one example, the disclosure relates to a nucleic acid molecule comprising in the 5' → 3' direction of transcription:

(a) a promoter;

(b) a transcribable nucleic acid sequence or a nucleic acid sequence for introducing a transcribable nucleic acid sequence; and

(c) a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a 3'-untranslated region in the transcript, said 3'-untranslated region comprising a nucleic acid sequence which is selected from the group consisting of:

(c-1) the nucleic acid sequence of the 3'-untranslated region of FCGRT, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-2) the nucleic acid sequence of the 3'-untranslated region of LSP1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-3) the nucleic acid sequence of the 3'-untranslated region of CCL22, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-5) the nucleic acid sequence of the 3'-untranslated region of PLD3, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-7) the nucleic sequence of the 3'-untranslated region of HLA-DRB4, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

and

(c-8) any combination of two or more of the nucleic acid sequences, fragments and/or variants under (c-1), (c-2), (c-3), (c-4), (c-5), (c-6) and (c-7).

In one example, the nucleic acid sequences (b) and (c) under the control of the promoter (a) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (c) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (b).

In one example, the nucleic acid sequences (b) and (c) are not naturally linked.

In one example, (c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment comprises a nucleic acid sequence selected from the group consisting of a nucleic acid sequence selected from SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment.

In one example, (c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment

comprises a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of SEQ ID NO: 86, a fragment thereof, or a variant of said nucleic acid sequence or fragment.

In one example, (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment comprises a nucleic acid sequence selected from the group consisting of a nucleic acid sequence selected from SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment.

In one example, (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment comprises a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of SEQ ID NO: 115, a fragment thereof, or a variant of said nucleic acid sequence or fragment.

In one example, the nucleic acid sequence (c-8) comprises a combination of two or more identical or different nucleic acid sequences, fragments and/or variants under (c-1), (c-2), (c-3), (c-4), (c-5), (c-6) and (c-7). In various examples, the nucleic acid sequence (c-8) comprises a combination of (c-1) and (c-2), (c-1) and (c-3), (c-1) and (c-4), (c-1) and (c-5), (c-1) and (c-6), (c-1) and (c-7), (c-2) and (c-3), (c-2) and (c-4), (c-2) and (c-5), (c-2) and (c-6), (c-2) and (c-7), (c-3) and (c-4), (c-3) and (c-5), (c-3) and (c-6), (c-3) and (c-7), (c-4) and (c-5), (c-4) and (c-6), (c-4) and (c-7), (c-5) and (c-6), (c-5) and (c-7), or (c-6) and (c-7).

In one example, the nucleic acid sequence (c-8) comprises a combination of (c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment, and (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment. In one example, (c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment is located 5’ to (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment. In one example, the combination of (c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment, and (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment comprises a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of SEQ ID NO: 174, a fragment thereof, or a variant of said nucleic acid sequence or fragment.

In one example, the nucleic acid molecule of the disclosure further comprises (d) a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides. In one example, said

polyadenyl sequence comprises at least 20 A nucleotides, preferably at least 40, at least 80, at least 100 or at least 120 A nucleotides, preferably consecutive A nucleotides. In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is a sequence, preferably an arbitrary sequence, of 2 or more consecutive nucleotides, wherein the first and the last nucleotide of said sequence of 2 or more consecutive nucleotides is a nucleotide other than an A nucleotide. In one example, said nucleic acid sequence (d) is a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a nucleic acid sequence which is a polyadenyl sequence comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides and which exhibits higher stability upon propagation of said nucleic acid molecule in Escherichia coli compared to a nucleic acid molecule which comprises instead of said nucleic acid sequence (d) a nucleic acid sequence (d)' which, when transcribed under the control of the promoter (a), codes for a polyadenyl sequence of the same length as said nucleic acid sequence which is a polyadenyl sequence comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides. In one example, said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides comprises at least 80 nucleotides, preferably at least 90 or 100 nucleotides. In one v, said nucleic acid sequence which is a polyadenyl

sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides comprises at least 90 nucleotides, preferably at least 100 nucleotides, preferably at least 110 nucleotides. In one example, said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides comprises about 120 nucleotides. In particular examples, said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides comprises up to 200, preferably up to 150, and, in particular, up to 130 nucleotides. In one example, at least 90%, preferably at least 92%, preferably at least 95%, 97% or 98% of the nucleotides of said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides are A nucleotides in said polyadenyl sequence (not including A nucleotides in said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides).

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is located within a region from position 21 to position 80, preferably from position 21 to position 60, more preferably from position 31 to position 50 of said polyadenyl sequence.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is preceeded by at least 20 A residues, preferably at least 30, 40 or 50 A residues in said polyadenyl sequence. In particular examples, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is preceeded by up to 80 A residues, preferably up to 70 or 60 A residues in said polyadenyl sequence.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is followed by at least 20 A residues, preferably at least 30, 40, 50, 60 or 70 A residues in said polyadenyl sequence. In particular examples, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is followed by up to 100 A residues, preferably up to 80 A residues in said polyadenyl sequence.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is preceeded by 20 to 50, preferably 30 to 40 A residues in said polyadenyl sequence and is followed by 30 to 80, preferably 40 to 70 A residues in said polyadenyl sequence.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides has a length of at least 3, at least 4, at least 5, at least 6, at least 8, preferably at least 10, more preferably at least 15 nucleotides.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides has a length of no more than 50, preferably no more than 30, more preferably no more than 20 nucleotides.

In one example, said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides does not comprise more than 3, preferably no more than 2, preferably no consecutive A residues.

In one example, the nucleic acid sequences (b), (c) and (d) under the control of the promoter (a) can be transcribed to give a common transcript. In one example, the nucleic acid sequences transcribed from the nucleic acid sequences (c) and optionally (d) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (b).

In one example, in the transcript said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is located at the 3' end.

In one example, the nucleic acid molecule of the disclosure is a DNA molecule. In one example, said nucleic acid molecule is an expression vector or plasmid such as an IVT vector.

In one example, the nucleic acid molecule of the disclosure is a closed circular molecule or a linear molecule.

In one example, the transcribable nucleic acid sequence comprises a nucleic acid sequence coding for a peptide or protein and the nucleic acid sequence for introducing a transcribable nucleic acid sequence is a multiple cloning site.

In one example, the nucleic acid molecule of the disclosure further comprises one or more members selected from the group consisting of: (i) a reporter gene; (ii) a selectable marker; and (iii) an origin of replication.

In one example, the nucleic acid molecule of the disclosure is suitable, in particular after linearization, for *in vitro* transcription of RNA, in particular mRNA.

Prior to *in vitro* transcription, circular IVT vectors are generally linearized downstream of the polyadenyl cassette by type II restriction enzymes (recognition sequence corresponds to cleavage site). The polyadenyl cassette thus corresponds to the later poly(A) sequence in the transcript. As a result of this procedure, some nucleotides remain as part of the enzyme cleavage site after linearization and extend or mask the poly(A) sequence at the 3' end. However, it was found that RNA having an open-ended poly(A) sequence is translated more efficiently than RNA having a poly(A) sequence with a masked terminus.

Accordingly, nucleic acid molecules of the disclosure when used as expression vectors preferably allow transcription of RNA with a poly(A) sequence which preferably has an open end in said RNA, i.e. no nucleotides other than A nucleotides flank said poly(A) sequence at its 3' end. An open-ended poly(A) sequence in the RNA can be achieved by introducing a type IIS restriction cleavage site into an expression vector which allows RNA to be transcribed under the control of a 5' RNA polymerase promoter and which contains a polyadenyl cassette, wherein the recognition sequence is located downstream of the polyadenyl cassette, while the cleavage site is located upstream and thus within the polyadenyl cassette. Restriction cleavage at the type IIS restriction cleavage site enables a plasmid to be linearized within the polyadenyl cassette. The linearized plasmid can then be used as template for *in vitro* transcription, the resulting transcript ending in an unmasked poly(A) sequence.

Accordingly, in one example, it is preferred that the nucleic acid molecule of the disclosure can be cleaved, preferably enzymatically or in another biochemical way, within the nucleic acid sequence (d) in such a way that said cleavage results in a nucleic acid molecule which comprises, in the 5' → 3' direction of transcription, the promoter (a), the nucleic acid sequences (b) and (c), and at least a part of the nucleic acid sequence (d), wherein the at least a part of the nucleic acid sequence (d), when transcribed under the control of the promoter (a), codes for said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides

other than A nucleotides and wherein in the transcript the 3'-terminal nucleotide is an A nucleotide of said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

Preferably, after cleavage, the nucleic acid molecule, at the end of the strand that serves as template for the nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides, has a T nucleotide which is part of the nucleic acid sequence which serves as template for the nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

The nucleic acid molecule of the disclosure is preferably a closed circular molecule prior to cleavage and a linear molecule after cleavage.

Preferably, cleavage is carried out with the aid of a restriction cleavage site which is preferably a restriction cleavage site for a type IIS restriction endonuclease.

In one example, the recognition sequence for the type IIS restriction endonuclease is located 5-26 base pairs, preferably 24-26 base pairs, downstream of the 3' end of the nucleic acid sequence (d).

In one example, a nucleic acid molecule according to the disclosure is in a closed circular conformation and preferably suitable for *in vitro* transcription of RNA, in particular mRNA, in particular after linearization.

In further examples, the disclosure relates to a nucleic acid molecule obtainable by linearization of an above-described nucleic acid molecule, preferably by cleavage within the nucleic acid sequence (d), and to RNA obtainable by transcription, preferably *in vitro* transcription, with above-described nucleic acid molecules under the control of the promoter (a).

Thus, the disclosure in one example relates to RNA comprising in the 5' → 3' direction:

(a) a 5'-untranslated region;

(b) a nucleic acid sequence coding for a peptide or protein; and

(c) a 3'-untranslated region, said 3'-untranslated region comprising a nucleic acid sequence which is selected from the group consisting of:

(c-1) the nucleic acid sequence of the 3'-untranslated region of FCGRT, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-2) the nucleic acid sequence of the 3'-untranslated region of LSP1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-3) the nucleic acid sequence of the 3'-untranslated region of CCL22, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-5) the nucleic acid sequence of the 3'-

untranslated region of PLD3, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(c-7) the nucleic sequence of the 3'-untranslated region of HLA-DRB4, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

and

(c-8) any combination of two or more of the nucleic acid sequences, fragments and/or variants under (c-1), (c-2), (c-3), (c-4), (c-5), (c-6) and (c-7).

In one example, the nucleic acid sequences (b) and (c) are not naturally linked.

In one example, the RNA further comprises (d) a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides. In one example, said nucleic acid sequence (d) is located at the 3' end of said RNA.

In one example, the nucleic acid sequences (c) and optionally (d) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence coding for a peptide or protein.

In one example, the RNA further comprises (e) a 5’ Cap.

examples of the 3'-untranslated region and the nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of

one or more consecutive nucleotides containing nucleotides other than A nucleotides are as described above for the nucleic acid molecules of the disclosure.

In a further aspect, the disclosure relates to a method of obtaining RNA, comprising:

(i) providing a nucleic acid molecule of the disclosure, and

(ii) transcribing RNA using the nucleic acid molecule as a template.

In a further aspect, the disclosure relates to a method of obtaining a peptide or protein, comprising:

(i) obtaining RNA encoding the peptide or protein according to the method of obtaining RNA of the disclosure, and

(ii) translating the RNA.

In one example, the method of obtaining RNA or the method of obtaining a peptide or protein further comprises, prior to transcription of the nucleic acid molecule, cleavage of the nucleic acid molecule.

In a further example, the disclosure relates to a method of obtaining RNA, comprising:

(i) coupling a nucleic acid sequence (b) which, when transcribed, codes for a 3'-untranslated region, at the 3' end of a transcribable nucleic acid sequence (a) comprising a nucleic acid sequence coding for a peptide or protein, and

(ii) transcribing the nucleic acid obtained, said 3'-untranslated region comprising a nucleic acid sequence which is selected from the group consisting of:

(b-1) the nucleic acid sequence of the 3'

untranslated region of FCGRT, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-2) the nucleic acid sequence of the 3'-untranslated region of LSP1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-3) the nucleic acid sequence of the 3'-untranslated region of CCL22, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-4) the nucleic acid sequence of the 3'-untranslated region of AES, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-5) the nucleic acid sequence of the 3'-untranslated region of PLD3, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

(b-7) the nucleic sequence of the 3'-untranslated region of HLA-DRB4, a fragment thereof, or a variant of said nucleic acid sequence or fragment,

and

(b-8) any combination of two or more of the nucleic acid sequences, fragments and/or variants under (b-1), (b-2), (b-3), (b-4), (b-5), (b-6) and (b-7).

In one example, the nucleic acid sequences (a) and (b) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (b) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the

transcribable nucleic acid sequence (a).

In one example, the nucleic acid sequences (a) and (b) are not naturally linked.

In one example, the method further comprises coupling a nucleic acid sequence (c) which, when transcribed, codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides, at the 3' end of the nucleic acid sequence (b).

In one example, the nucleic acid sequences (a), (b), and (c) can be transcribed to give a common transcript in which the nucleic acid sequences transcribed from the nucleic acid sequences (b) and, optionally, (c) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (a).

Examples of the 3'-untranslated region and the nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides are as described above for the nucleic acid molecules of the invention.

In a further aspect, the disclosure relates to a method of obtaining a peptide or protein, comprising:
(i) obtaining RNA by the method of obtaining RNA of the disclosure, and
(ii) translating the RNA.

The methods of the disclosure may be performed *in vitro* or *in vivo*. In one example of any of the methods of the disclosure, transcription is carried out *in vitro*.

In one example, the method of obtaining RNA or the method of obtaining a peptide or protein further comprises, prior to transcription of the nucleic acid molecule, cleavage of the nucleic acid molecule.

In one example, cleavage is within the nucleic acid sequence which, when transcribed, codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides in such a way that transcription of the nucleic acid obtained in this way generates a transcript which has at its 3'-terminal end said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides, wherein the 3'-terminal nucleotide of said transcript is an A nucleotide of the nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

In all examples of the methods according to the disclosure, cleavage is preferably carried out with the aid of a restriction cleavage site which is preferably a restriction cleavage site for a type IIS restriction endonuclease.

In one example, the recognition sequence for the type

IIS restriction endonuclease is 5-26 base pairs, preferably 24-26 base pairs, downstream of the 3' end of the nucleic acid sequence which, when transcribed, codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

The disclosure also relates to RNA obtainable by the methods according to the disclosure of obtaining RNA.

The disclosure may be utilized, for example, for increasing expression of recombinant proteins in cellular transcription and expression. More specifically, it is possible, when producing recombinant proteins, to use expression vectors of the disclosure for transcription of recombinant nucleic acids and expression of recombinant proteins in cell-based systems. This includes, for example, the preparation of recombinant antibodies, hormones, cytokines, enzymes, and the like. This allows inter alia production costs to be reduced.

It is also possible to use the nucleic acid molecules of the disclosure for gene therapy applications. Accordingly, a nucleic acid molecule of the disclosure may be a gene therapy vector and used for expression of a transgene. To this end, any nucleic acid (DNA/RNA)-based vector systems (for example plasmids, adenoviruses, poxvirus vectors, influenza virus vectors, alphavirus vectors, and the like) may be used. Cells can be transfected with these vectors *in vitro*, for example in lymphocytes or dendritic cells, or else

in vivo by direct administration.

RNA of the disclosure (e.g. obtained using a nucleic acid molecule described herein as a transcription template) may be employed, for example, for transient expression of genes, with possible fields of application being RNA-based vaccines which are transfected into cells *in vitro* or administered directly *in vivo*, transient expression of functional recombinant proteins *in vitro*, for example in order to initiate differentiation processes in cells or to study functions of proteins, and transient expression of functional recombinant proteins such as erythropoietin, hormones, coagulation inhibitors, etc., *in vivo*, in particular as pharmaceuticals.

RNA of the disclosure may be used in particular for transfecting antigen-presenting cells and thus as a tool for delivering the antigen to be presented and for loading antigen-presenting cells, with said antigen to be presented corresponding to the peptide or protein expressed from said RNA or being derived therefrom, in particular by way of intracellular processing such as cleavage, i.e. the antigen to be presented is, for example, a fragment of the peptide or protein expressed from the RNA. Such antigen-presenting cells may be used for stimulating T cells, in particular $CD4^+$ and/or $CD8^+$ T cells.

Accordingly, in a further example, the disclosure relates to a use of the RNA of the disclosure for transfecting a host cell. In one example, the host cell is an antigen-presenting cell, in particular a dendritic cell, a monocyte or a macrophage.

In a further example, the disclosure relates to a use of the RNA of the disclosure for therapy, in particular for vaccination.

In a further example, the disclosure relates to a pharmaceutical composition such as a vaccine composition comprising the RNA of the disclosure.

In a further example, the disclosure relates to the RNA of the disclosure for the uses described herein.

Detailed description of the invention

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously

Claims

1. A nucleic acid molecule comprising in the 5' → 3' direction of transcription:

(a) a promoter;

(b) a transcribable nucleic acid sequence or a nucleic acid sequence for introducing a transcribable nucleic acid sequence; and

(c) a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a 3'-untranslated region in the transcript which is not naturally linked to the nucleic acid sequence (b), said 3'-untranslated region comprising (i) (c-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment ; or

(ii) (c-8) any combination of (c-4) and one or more nucleic acid sequences selected from the group consisting of:

(c-1) the nucleic acid sequence of the 3'-untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-2) the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting

of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-4) the nucleic acid sequence of the 3'-untranslated region of AES comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-5) the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, and

(c-7) the nucleic sequence of the 3'-

untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, wherein the nucleic acid sequences (b) and (c) under the control of the promoter (a) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (c) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (b).

2. The nucleic acid molecule as claimed in claim 1, wherein (c-4) the nucleic acid sequence of the 3'-untranslated region of AES comprises or consists of the nucleic acid sequence of SEQ ID NO: 86, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

3. The nucleic acid molecule as claimed in claim 1 or 2, wherein (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1 comprises or consists of the nucleic acid sequence of SEQ ID NO: 115, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

4. The nucleic acid molecule as claimed in any one of claims 1 to 3, wherein the nucleic acid sequence (c-8) comprises a combination of (c-4) and (c-6).

5. The nucleic acid molecule as claimed in claim 4, wherein (c-4) is located 5’ to (c-6).

6. The nucleic acid molecule as claimed in claim 4 or 5, wherein the combination of (c-4) and (c-6) comprises the nucleic acid sequence of SEQ ID NO: 174, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

7. The nucleic acid molecule as claimed in any one of claims 1 to 6, further comprising (d) a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

8. The nucleic acid molecule as claimed in claim 7, wherein said polyadenyl sequence comprises at least 20 A, at least 40, at least 80 or at least 100 A nucleotides or consecutive A nucleotides.

9. The nucleic acid molecule as claimed in claim 7 or 8, wherein said polyadenyl sequence comprises at least 120 A nucleotides or consecutive A nucleotides.

10. The nucleic acid molecule as claimed in any one of claims 7 to 9, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is a sequence of 2 or more consecutive nucleotides, wherein the first and the last nucleotide of said sequence of 2 or more consecutive nucleotides is a nucleotide

other than an A nucleotide.

11. The nucleic acid molecule as claimed in claim 10, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is an arbitrary sequence.

12. The nucleic acid molecule as claimed in any one of claims 7 to 11, wherein said nucleic acid sequence (d) is a nucleic acid sequence which, when transcribed under the control of the promoter (a), codes for a nucleic acid sequence which is a polyadenyl sequence comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides and which exhibits higher stability upon propagation of said nucleic acid molecule in Escherichia coli compared to a nucleic acid molecule which comprises instead of said nucleic acid sequence (d) a nucleic acid sequence (d)' which, when transcribed under the control of the promoter (a), codes for a polyadenyl sequence of the same length as said nucleic acid sequence which is a polyadenyl sequence comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

13. The nucleic acid molecule as claimed in any one of claims 7 to 12, wherein said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides comprises at least 80, 90 or 100 nucleotides.

14. The nucleic acid molecule as claimed in any one of claims 7 to 13, wherein said sequence of one or

more consecutive nucleotides containing nucleotides other than A nucleotides is located within a region from position 21 to position 80, from position 21 to position 60, or from position 31 to position 50 of said polyadenyl sequence.

15. The nucleic acid molecule as claimed in any one of claims 7 to 14, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is preceeded by at least 20 A residues in said polyadenyl sequence and/or is followed by at least 20 A residues in said polyadenyl sequence.

16. The nucleic acid molecule as claimed in any one of claims 7 to 15, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides has a length of at least 3, at least 4, at least 5, at least 6, at least 8, at least 10, or at least 15 nucleotides.

17. The nucleic acid molecule as claimed in any one of claims 7 to 16, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides has a length of no more than 50, no more than 30, or no more than 20 nucleotides.

18. The nucleic acid molecule as claimed in any one of claims 7 to 17, wherein said sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides does not comprise more than 3 or more than 2 consecutive A residues.

19. The nucleic acid molecule as claimed in any one of claims 7 to 18, wherein the nucleic acid sequences

(b), (c) and (d) under the control of the promoter (a) can be transcribed to give a common transcript.

20. The nucleic acid molecule as claimed in claim 19, wherein the nucleic acid sequences transcribed from the nucleic acid sequences (c) and (d) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (b).

21. The nucleic acid molecule as claimed in any one of claims 7 to 20, wherein in the transcript said nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides is located at the 3' end.

22. The nucleic acid molecule as claimed in any one of claims 1 to 21, which is a closed circular molecule or a linear molecule.

23. The nucleic acid molecule as claimed in any one of claims 1 to 22, wherein the transcribable nucleic acid sequence comprises a nucleic acid sequence coding for a peptide or protein and the nucleic acid sequence for introducing a transcribable nucleic acid sequence is a multiple cloning site.

24. The nucleic acid molecule as claimed in any one of claims 1 to 23, further comprising one or more members selected from the group consisting of: (i) a reporter gene; (ii) a selectable marker; and (iii) an origin of replication.

25. RNA comprising in the 5' → 3' direction:

(a) a 5'-untranslated region;

(b) a nucleic acid sequence coding for a peptide or protein; and

(c) a 3'-untranslated region which is not naturally linked to the nucleic acid sequence (b), said 3'-untranslated region comprising (i) (c-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment; or (ii) (c-8) any combination of (c-4) and one or more nucleic acid sequences selected from the group consisting of:

(c-1) the nucleic acid sequence of the 3'-untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-2) the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant

of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-4) the nucleic acid sequence of the 3'-untranslated region of AES comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-5) the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(c-7) the nucleic sequence of the 3'-untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

wherein the nucleic acid sequence (c) is active so

as to increase the translation efficiency and/or the stability of the nucleic acid sequence coding for a peptide or protein.

26. The RNA as claimed in claim 25, wherein (c-4) the nucleic acid sequence of the 3'-untranslated region of AES comprises or consists of the nucleic acid sequence of SEQ ID NO: 86, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

27. The RNA as claimed in claim 25 or 26, wherein (c-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1 comprises or consists of the nucleic acid sequence of SEQ ID NO: 115, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

28. The RNA as claimed in any one of claims 25 to 27, wherein the nucleic acid sequence (c-8) comprises a combination of (c-4) and (c-6).

29. The RNA as claimed in claim 28, wherein (c-4) is located 5’ to (c-6).

30. The RNA as claimed in any one of claims 25 to 29, further comprising (d) a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides.

31. The RNA as claimed in claim 30, wherein said nucleic acid sequence (d) is located at the 3' end

of said RNA.

32. The RNA as claimed in claim 30 or 31, wherein the polyadenyl sequence comprises at least 120 A nucleotides.

33. The RNA as claimed in any one of claims 30 to 32, wherein the nucleic acid sequences (c) and (d) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence coding for a peptide or protein.

34. The RNA as claimed in any one of claims 30 to 33, further comprising (e) a 5′ Cap or 5′ Cap analog.

35. The RNA as claimed in any one of claims 25 to 34, wherein the peptide or protein encoded by the nucleic acid sequence (b) is a tumor-associated antigen, which antigen is not expressed in normal tissues or is mutated in tumor cells.

36. A method of obtaining RNA, comprising:
(i) providing a nucleic acid molecule as claimed in any one of claims 1 to 24, and
(ii) transcribing RNA using the nucleic acid molecule as a template.

37. A method of obtaining a peptide or protein, comprising:
(i) obtaining RNA encoding the peptide or protein according to the method of claim 36, and
(ii) translating the RNA.

38. The method as claimed in claim 36 or 37, characterized in that it further comprises, prior to transcription of the nucleic acid molecule, cleavage of the nucleic acid molecule.

39. A method of obtaining RNA, comprising:

(i) coupling a nucleic acid sequence (b) which, when transcribed, codes for a 3'-untranslated region, at the 3' end of a transcribable nucleic acid sequence (a) comprising a nucleic acid sequence coding for a peptide or protein, wherein nucleic acid sequence (a) is not naturally linked to the nucleic acid sequence (b), and

(ii) transcribing the nucleic acid obtained,

said 3'-untranslated region comprising (iia) (b-4) the nucleic acid sequence of the 3'-untranslated region of Amino-Terminal Enhancer Of Split (AES) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment; or

(iib) (b-8) any combination of (b-4) and one or more nucleic acid sequences selected from the group consisting of:

(b-1) the nucleic acid sequence of the 3'-untranslated region of Fc Fragment Of IgG, Receptor, Transporter, Alpha (FCGRT) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 to 50, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(b-2) the nucleic acid sequence of the 3'-untranslated region of Lymphocyte Specific Protein 1 (LSP1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51 to 72, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic

acid sequence or fragment,

(b-3) the nucleic acid sequence of the 3'-untranslated region of Chemokine Ligand 22 (CCL22) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 73 to 85, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(b-4) the nucleic acid sequence of the 3'-untranslated region of AES comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 86 to 89, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(b-5) the nucleic acid sequence of the 3'-untranslated region of Phospholipase D Family Member 3 (PLD3) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 90 to 104, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(b-6) the nucleic acid sequence of the non-coding RNA of Mitochondrially Encoded 12S RNA (MT-RNR1) comprising or consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105 to 121, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment,

(b-7) the nucleic sequence of the 3'-untranslated region of Major Histocompatibility Complex Class II DR Beta 4 (HLA-DRB4) comprising or consisting of a nucleic acid sequence selected

from the group consisting of SEQ ID NOs: 122 to 143, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment, wherein the nucleic acid sequences (a) and (b) can be transcribed to give a common transcript in which the nucleic acid sequence transcribed from the nucleic acid sequence (b) is active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (a).

40. The RNA as claimed in claim 39, wherein (b-4) the nucleic acid sequence of the 3'-untranslated region of AES comprises or consists of the nucleic acid sequence of SEQ ID NO: 86, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

41. The RNA as claimed in claim 39 or 40, wherein (b-6) the nucleic acid sequence of the non-coding RNA of MT-RNR1 comprises or consists of the nucleic acid sequence of SEQ ID NO: 115, a fragment thereof, or a variant of said nucleic acid sequence or fragment which is at least 90% identical to said nucleic acid sequence or fragment.

42. The RNA as claimed in any one of claims 39 to 41, wherein the nucleic acid sequence (b-8) comprises a combination of (b-4) and (b-6).

43. The RNA as claimed in claim 42, wherein (b-4) is located 5’ to (b-6).

44. The method as claimed in any one of claims 39 to 43, which further comprises coupling a nucleic acid sequence (c) which, when transcribed, codes for a nucleic acid sequence which is a polyadenyl sequence optionally comprising within the polyadenyl sequence a sequence of one or more consecutive nucleotides containing nucleotides other than A nucleotides, at the 3' end of the nucleic acid sequence (b).

45. The method as claimed in claim 44, wherein the nucleic acid sequences (a), (b), and (c) can be transcribed to give a common transcript in which the nucleic acid sequences transcribed from the nucleic acid sequences (b) and (c) are active so as to increase the translation efficiency and/or the stability of the nucleic acid sequence transcribed from the transcribable nucleic acid sequence (a).

46. The method as claimed in any one of claims 39 to 45, wherein the peptide or protein encoded by the nucleic acid sequence (a) is a tumor-associated antigen, which antigen is not expressed in normal tissues or is mutated in tumor cells.

47. An in vitro method of obtaining a peptide or protein, comprising:
(i) obtaining RNA by the method of any one of claims 39 to 46, and
(ii) translating the RNA.

48. The method as claimed in any one of claims 36 to 46, wherein transcription is carried out in vitro.

49. A method of obtaining a peptide or protein, comprising translating the RNA as claimed in any

one of claims 25 to 35.

50. Use of the RNA as claimed in any one of claims 25 to 35 in an in vitro method of transfecting a host cell.

51. The use as claimed in claim 50, wherein the host cell is an antigen-presenting cell.

52. The use as claimed in claim 51, wherein the antigen-presenting cells is a dendritic cell, a monocyte or a macrophage.

53. The RNA as claimed in any one of claims 25 to 35 for use in vaccination.

54. The RNA as claimed in any one of claims 25 to 35 for use in reprogramming somatic cells to cells having stem cell characteristics.