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(54) **Title**: NUCLEIC ACID COMPRISING OR CODING FOR A HISTONE STEM-LOOP AND A POLY(A) SEQUENCE OR A POLYADENYLATION SIGNAL FOR INCREASING THE EXPRESSION OF AN ENCODED PROTEIN

(57) **Abstract**: The present application describes a coding nucleic acid sequence, particularly a messenger RNA (mRNA), comprising or coding for a histone stem-loop and a poly(A) sequence or a polyadenylation signal and the use thereof for increasing the expression of an encoded protein. It also discloses its use for the preparation of a pharmaceutical composition, especially a vaccine e.g. for the use in the treatment of tumours and cancer diseases, cardiovascular diseases, infectious diseases, autoimmune diseases or genetic diseases, or in gene therapy. The present invention further describes an *in vitro* transcription method, *in vitro* methods for increasing the expression of a protein using the nucleic acid comprising or coding for a histone stem-loop and a poly(A) sequence or a polyadenylation signal and an ex vivo and *in vivo* method.

Nucleic acid comprising or coding for a histone stem-loop and a poly(A) sequence or a polyadenylation signal for increasing the expression of an encoded protein

The present application describes a coding nucleic acid sequence, particularly a messenger RNA (mRNA), comprising or coding for a histone stem-loop and a poly(A) sequence or a polyadenylation signal and the use thereof for increasing the expression of an encoded protein. It also discloses its use for the preparation of a pharmaceutical composition, especially a vaccine, e.g. for use in the treatment of tumours and cancer diseases, cardiovascular diseases, infectious diseases, autoimmune diseases or genetic diseases, or in gene therapy. The present invention further describes an *in vitro* transcription method, *in vitro* methods for increasing the expression of a protein using the nucleic acid comprising or coding for a histone stem-loop and a poly(A) sequence or a polyadenylation signal and an *ex vivo* and *in vivo* method.

Apart from cardiovascular diseases and infectious diseases, the occurrence of tumours and cancer diseases is one of the most frequent causes of death in modern society and is in most cases associated with considerable costs in terms of therapy and subsequent rehabilitation measures. The treatment of tumours and cancer diseases is greatly dependent, for example, on the type of tumour that occurs, on the age, the distribution of cancer cells in the patient to be treated, etc. Cancer therapy is nowadays conventionally carried out by the use of radiation therapy or chemotherapy in addition to invasive operations. However, such conventional therapies typically place extraordinary stress on the immune system and can be used in some cases to only a limited extent. In addition, most of these conventional therapies require long intervals between the individual treatments to allow for regeneration of the immune system.

Therefore, supplementary strategies have been investigated in recent years in addition to such "conventional treatments" to avoid or at least reduce the impact on the immune system by such treatments. One such supplementary treatment in particular includes gene therapeutic approaches or genetic vaccination, which already have been found to be highly promising for treatment or for supporting such conventional therapies.

Gene therapy and genetic vaccination are methods of molecular medicine which already have been proven in the therapy and prevention of diseases and generally exhibit a considerable effect on daily medical practice, in particular on the treatment of diseases as mentioned above. Gene therapy is also used in further fields of medicine, e.g. in the case of genetic diseases, that is to say (inherited) diseases, that are caused by a defined gene defect and are inherited according to Mendel's laws. The most well known representatives of such genetic diseases include *inter alia* mucoviscidosis (cystic fibrosis) and sickle cell anaemia. Both methods, gene therapy and genetic vaccination, are based on the introduction of nucleic acids into the patient's cells or tissue and subsequent processing of the information coded for by the nucleic acid that has been introduced into the cells or tissue, that is to say the (protein) expression of the desired polypeptides.

In gene therapy approaches, typically DNA is used even though RNA is also known in recent developments. Importantly, in all these gene therapy approaches mRNA functions as messenger for the sequence information of the encoded protein, irrespectively if DNA, viral RNA or mRNA is used.

In general RNA is considered an unstable molecule: RNases are ubiquitous and notoriously difficult to inactivate. Furthermore, RNA is also chemically more labile than DNA. Thus, it is perhaps surprising that the "default state" of an mRNA in a eukaryotic cell is characterized by a relative stability and specific signals are required to accelerate the decay of individual mRNAs. The main reason for this finding appears to be that mRNA decay within cells is catalyzed almost exclusively by exonucleases. However, the ends of eukaryotic mRNAs are protected against these enzymes by specific terminal structures and their associated proteins: a m7GpppN CAP at the 5′ end and typically a poly(A) sequence at the 3′ end. Removal of these two terminal modifications is thus considered rate limiting for mRNA decay. Although a stabilizing element has been characterized in the 3′ UTR of the

alpha-globin mRNA, RNA sequences affecting turnover of eukaryotic mRNAs typically act as a promoter of decay usually by accelerating deadenylation (reviewed in Meyer, S., C. Temme, *et al.* (2004), Crit Rev Biochem Mol Biol 39(4): 197-216.).

As mentioned above, the 5′ ends of eukaryotic mRNAs are typically modified posttranscriptionally to carry a methylated CAP structure, e.g. m7GpppN. Aside from roles in RNA splicing, stabilization, and transport, the CAP structure significantly enhances the recruitment of the 40S ribosomal subunit to the 5′ end of the mRNA during translation initiation. The latter function requires recognition of the CAP structure by the eukaryotic initiation factor complex eIF4F. The poly(A) sequence additionally stimulates translation via increased 40S subunit recruitment to mRNAs, an effect that requires the intervention of poly(A) binding protein (PABP). PABP, in turn, was recently demonstrated to interact physically with eIF4G, which is part of the CAP-bound eIF4F complex. Thus, a closed loop model of translation initiation on capped, polyadenylated mRNAs was postulated (Michel, Y. M., D. Poncet, *et al.* (2000), J Biol Chem 275(41): 32268-76.).

Nearly all eukaryotic mRNAs end with such a poly(A) sequence that is added to their 3′ end by the ubiquitous cleavage/polyadenylation machinery. The presence of a poly(A) sequence at the 3′ end is one of the most recognizable features of eukaryotic mRNAs. After cleavage, most pre-mRNAs, with the exception of replication-dependent histone transcripts, acquire a polyadenylated tail. In this context, 3′ end processing is a nuclear co-transcriptional process that promotes transport of mRNAs from the nucleus to the cytoplasm and affects the stability and the translation of mRNAs. Formation of this 3′ end occurs in a two step reaction directed by the cleavage/polyadenylation machinery and depends on the presence of two sequence elements in mRNA precursors (pre-mRNAs); a highly conserved hexanucleotide AAUAAA (polyadenylation signal) and a downstream G/U-rich sequence. In a first step, pre-mRNAs are cleaved between these two elements. In a second step tightly coupled to the first step the newly formed 3′ end is extended by addition of a poly(A) sequence consisting of 200–250 adenylates which affects subsequently all aspects of mRNA metabolism, including mRNA export, stability and translation (Dominski, Z. and W. F. Marzluff (2007), Gene **396**(2): 373-90.).

The only known exception to this rule are the replication-dependent histone mRNAs which

end with a histone stem–loop instead of a poly(A) sequence. Exemplary histone stem-loop sequences are described in Lopez *et al.* (Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308.).

The stem-loops in histone pre-mRNAs are typically followed by a purine-rich sequence known as the histone downstream element (HDE). These pre-mRNAs are processed in the nucleus by a single endonucleolytic cleavage approximately 5 nucleotides downstream of the stem-loop, catalyzed by the U7 snRNP through base pairing of the U7 snRNA with the HDE.

Due to the requirement to package newly synthesized DNA into chromatin, histone synthesis is regulated in concert with the cell cycle. Increased synthesis of histone proteins during S phase is achieved by transcriptional activation of histone genes as well as posttranscriptional regulation of histone mRNA levels. It could be shown that the histone stem-loop is essential for all posttranscriptional steps of histone expression regulation. It is necessary for efficient processing, export of the mRNA into the cytoplasm, loading onto polyribosomes, and regulation of mRNA stability.

In the above context, a 32 kDa protein was identified, which is associated with the histone stem-loop at the 3′-end of the histone messages in both the nucleus and the cytoplasm. The expression level of this stem-loop binding protein (SLBP) is cell-cycle regulated and is highest during S-phase when histone mRNA levels are increased. SLBP is necessary for efficient 3′-end processing of histone pre-mRNA by the U7 snRNP. After completion of processing, SLBP remains associated with the stem–loop at the end of mature histone mRNAs and stimulates their translation into histone proteins in the cytoplasm. (Dominski, Z. and W. F. Marzluff (2007), Gene 396(2): 373-90). Interestingly, the RNA binding domain of SLBP is conserved throughout metazoa and protozoa (Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308) and it could be shown that its binding to the histone stem-loop sequence is dependent on the stem-loop structure and that the minimum binding site contains at least 3 nucleotides 5′ and 2 nucleotides 3′ of the stem-loop (Pandey, N. B., *et al.* (1994), *Molecular and Cellular Biology, 14*(3), 1709-1720 and Williams, A. S., & Marzluff, W. F., (1995), *Nucleic Acids Research, 23*(4), 654-662.).

Even though histone genes are generally classified as either "replication-dependent", giving rise to mRNA ending in a histone stem-loop, or "replacement-type", giving rise to mRNA bearing a poly(A)-tail instead, naturally occurring mRNAs containing both a histone stem-loop and poly(A) or oligo(A) 3' thereof have been identified in some very rare cases. Sanchez *et al.* examined the effect of naturally occurring oligo(A) tails appended 3' of the histone stem-loop of histone mRNA during Xenopus oogenesis using Luciferase as a reporter protein and found that the oligo(A) tail is an active part of the translation repression mechanism that silences histone mRNA during oogenesis and its removal is part of the mechanism that activates translation of histone mRNAs (Sanchez, R. and W. F. Marzluff (2004), Mol Cell Biol 24(6): 2513-25).

Furthermore, the requirements for regulation of replication dependent histones at the level of pre-mRNA processing and mRNA stability have been investigated using artificial constructs coding for the marker protein alpha Globin, taking advantage of the fact that the globin gene contains introns as opposed to the intron-less histone genes. For this purpose constructs were generated in which the alpha globin coding sequence was followed by a histone stem-loop signal (histone stem-loop followed by the histone downstream element) and a polyadenylation signal (Whitelaw, E., *et al.* (1986). Nucleic Acids Research, 14(17), 7059-7070.; Pandey, N. B., & Marzluff, W. F. (1987). Molecular and Cellular Biology, 7(12), 4557-4559.; Pandey, N. B., *et al.* (1990). Nucleic Acids Research, 18(11), 3161-3170).

In another approach Lüscher *et al.* investigated the cell-cycle dependent regulation of a recombinant histone H4 gene. Constructs were generated in which the H4 coding sequence was followed by a histone stem-loop signal and a polyadenylation signal, the two processing signals incidentally separated by a galactokinase coding sequence (Lüscher, B. *et al.*, (1985). Proc. Natl. Acad. Sci. USA, 82(13), 4389-4393).

Additionally, Stauber *et al.* identified the minimal sequence required to confer cell-cycle regulation on histone H4 mRNA levels. For these investigations constructs were used, comprising a coding sequence for the selection marker Xanthine:guanine phosphoribosyl transferase (GPT) preceding a histone stem-loop signal followed by a polyadenylation signal

(Stauber, C. *et al.*, (1986). EMBO J, 5(12), 3297-3303).

Examining histone pre-mRNA processing Wagner *et al.* identified factors required for cleavage of histone pre-mRNAs using a reporter construct placing EGFP between a histone stem-loop signal and a polyadenylation signal, such that EGFP was expressed only in case histone pre-mRNA processing was disrupted (Wagner, E. J. *et al.*, (2007). Mol Cell 28(4), 692-9).

To be noted, translation of polyadenylated mRNA usually requires the 3' poly(A) sequence to be brought into proximity of the 5' CAP. This is mediated through protein–protein interaction between the poly(A) binding protein and eukaryotic initiation factor eIF4G. With respect to replication-dependent histone mRNAs, an analogous mechanism has been uncovered. In this context, Gallie *et al.* show that the histone stem-loop is functionally similar to a poly(A) sequence in that it enhances translational efficiency and is co-dependent on a 5'-CAP in order to establish an efficient level of translation. They showed that the histone stem-loop is sufficient and necessary to increase the translation of a reporter mRNA in transfected Chinese hamster ovary cells but must be positioned at the 3'-terminus in order to function optimally. Therefore, similar to the poly(A) tail on other mRNAs, the 3' end of these histone mRNAs appears to be essential for translation *in vivo* and is functionally analogous to a poly(A) tail (Gallie, D. R., Lewis, N. J., & Marzluff, W. F. (1996), Nucleic Acids Research, 24(10), 1954-1962).

Additionally, it could be shown that SLBP is bound to the cytoplasmic histone mRNA and is required for its translation. Even though SLBP does not interact directly with eIF4G, the domain required for translation of histone mRNA interacts with the recently identified protein SLIP1. In a further step, SLIP1 interacts with eIF4G and allows to circularize histone mRNA and to support efficient translation of histone mRNA by a mechanism similar to the translation of polyadenylated mRNAs.

As mentioned above, gene therapy approaches normally use DNA to transfer the coding information into the cell which is then transcribed into mRNA, carrying the naturally occurring elements of an mRNA, particularly the 5'-CAP structure and the 3' poly(A) sequence to ensure expression of the encoded therapeutic protein.

However, in many cases expression systems based on the introduction of such nucleic acids into the patient's cells or tissue and the subsequent expression of the desired polypeptides coded for by these nucleic acids do not exhibit the desired, or even the required, level of expression which may allow for an efficient therapy, irrespective as to whether DNA or RNA is used.

In the prior art, different attempts have hitherto been made to increase the yield of the expression of an encoded protein, in particular by use of improved expression systems, both *in vitro* and/or *in vivo*. Methods for increasing expression described generally in the prior art are conventionally based on the use of expression vectors or cassettes containing specific promoters and corresponding regulation elements. As these expression vectors or cassettes are typically limited to particular cell systems, these expression systems have to be adapted for use in different cell systems. Such adapted expression vectors or cassettes are then usually transfected into the cells and typically treated in dependence of the specific cell line. Therefore, preference is given primarily to those nucleic acid molecules which are able to express the encoded proteins in a target cell by systems inherent in the cell, independent of promoters and regulation elements which are specific for particular cell types. In this context, there can be distinguished between mRNA stabilizing elements and elements which increase translation efficiency of the mRNA.

mRNAs which are optimized in their coding sequence and which are in general suitable for such a purpose are described in application WO 02/098443 (CureVac GmbH). For example, WO 02/098443 describes mRNAs that are stabilised in general form and optimised for translation in their coding regions. WO 02/098443 further discloses a method for determining sequence modifications. WO 02/098443 additionally describes possibilities for substituting adenine and uracil nucleotides in mRNA sequences in order to increase the guanine/cytosine (G/C) content of the sequences. According to WO 02/098443, such substitutions and adaptations for increasing the G/C content can be used for gene therapeutic applications but also genetic vaccines in the treatment of cancer or infectious diseases. In this context, WO 02/098443 generally mentions sequences as a base sequence for such modifications, in which the modified mRNA codes for at least one biologically active peptide or polypeptide, which is translated in the patient to be treated, for example,

either not at all or inadequately or with faults. Alternatively, WO 02/098443 proposes mRNAs coding for antigens e.g. tumour antigens or viral antigens as a base sequence for such modifications.

In a further approach to increase the expression of an encoded protein the application WO 2007/036366 describes the positive effect of long poly(A) sequences (particularly longer than 120 bp) and the combination of at least two 3' untranslated regions of the beta globin gene on mRNA stability and translational activity.

However, even though all these latter prior art documents already try to provide quite efficient tools for gene therapy approaches and additionally improved mRNA stability and translational activity, there still remains the problem of a generally lower stability of RNA-based applications versus DNA vaccines and DNA based gene therapeutic approaches. Accordingly, there still exists a need in the art to provide improved tools for gene therapy approaches and genetic vaccination or as a supplementary therapy for conventional treatments as discussed above, which allow for better provision of encoded proteins *in vivo*, e.g. via a further improved mRNA stability and/or translational activity, preferably for gene therapy.

The object underlying the present invention is, therefore, to provide additional and/or alternative methods to increase expression of an encoded protein, preferably via further stabilization of the mRNA and/or an increase of the translational efficiency of such an mRNA with respect to such nucleic acids known from the prior art for the use in therapeutic applications (e.g. gene therapy and genetic vaccination).

This object is solved by the subject matter of the attached claims. Particularly, the object underlying the present invention is solved according to a first embodiment by an inventive nucleic acid sequence comprising or coding for

a) a coding region, preferably encoding a peptide or protein;
b) at least one histone stem-loop, and
c) optionally a poly(A) sequence or a polyadenylation signal,

preferably for increasing the expression level of an encoded protein, wherein the encoded protein is preferably no histone protein, no reporter protein (e.g. Luciferase, GFP, EGFP, β-

Galactosidase, particularly EGFP) and no marker or selection protein (e.g. alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

In this context it is particularly preferred that the inventive nucleic acid according to the first embodiment of the present invention is produced at least partially by DNA or RNA synthesis or is an isolated nucleic acid.

The present invention is based on the surprising finding of the present inventors, that the combination of a poly(A) sequence or polyadenylation signal and at least one histone stem-loop, even though both representing alternative mechanisms in nature, acts synergistically as this combination increases the protein expression manifold above the level observed with either of the individual elements. The synergistic effect of the combination of poly(A) and at least one histone stem-loop is seen irrespective of the order of poly(A) and histone stem-loop and irrespective of the length of the poly(A) sequence.

Therefore it is particularly preferred that the inventive nucleic acid molecule comprises or codes for a) a coding region, preferably encoding a peptide or protein; b) at least one histone stem-loop, and c) a poly(A) sequence or polyadenylation sequence; preferably for increasing the expression level of an encoded protein, wherein the encoded protein is preferably no histone protein, no reporter protein (e.g. Luciferase, GFP, EGFP, β-Galactosidase, particularly EGFP) and/or no marker or selection protein (e.g. alpha-Globin, Galactokinase and Xanthine:Guanine phosphoribosyl transferase (GPT)).

In a further alternative aspect of the first embodiment of the present invention the inventive nucleic acid comprises no histone downstream element (HDE).

In this context it is particularly preferred that the inventive nucleic acid comprises or codes for in 5'- to 3'-direction:

a) a coding region, preferably encoding a peptide or protein;
b) at least one histone stem-loop, optionally without a histone downstream element 3' to the histone stem-loop
c) a poly(A) sequence or a polyadenylation signal.

The term "histone downstream element (HDE) refers to a purine-rich polynucleotide stretch of about 15 to 20 nucleotides 3' of naturally occurring stem-loops, which represents the binding site for the U7 snRNA involved in processing of histone pre-mRNA into mature histone mRNA. For example in sea urchins the HDE is CAAGAAAGA (Dominski, Z. and W. F. Marzluff (2007), Gene 396(2): 373-90).

Furthermore it is preferable that the inventive nucleic acid according to the first embodiment of the present invention does not comprise an intron.

In another particular preferred embodiment, the inventive nucleic acid sequence according to the first embodiment of the present invention comprises or codes for from 5' to 3':

a) a coding region, preferably encoding a peptide or protein;
c) a poly(A) sequence; and
b) at least one histone stem-loop.

The inventive nucleic acid sequence according to the first embodiment of the present invention comprise any suitable nucleic acid, selected e.g. from any (single-stranded or double-stranded) DNA, preferably, without being limited thereto, e.g. genomic DNA, single-stranded DNA molecules, double-stranded DNA molecules, or may be selected e.g. from any PNA (peptide nucleic acid) or may be selected e.g. from any (single-stranded or double-stranded) RNA, preferably a messenger RNA (mRNA); etc. The inventive nucleic acid molecule may also comprise a viral RNA (vRNA). Preferably, the inventive nucleic acid molecule is an RNA.

In particular aspects of the first embodiment of the present invention, the inventive nucleic acid is a nucleic acid sequence comprised in a nucleic acid suitable for *in vitro* transcription, particularly in an appropriate *in vitro* transcription vector (e.g. a plasmid or a linear nucleic acid sequence comprising specific promoters for *in vitro* transcription such as T3, T7 or Sp6 promoters).

In further particular preferred aspects of the first embodiment of the present invention, the inventive nucleic acid is comprised in a nucleic acid suitable for transcription and/or translation in an expression system (e.g. in an expression vector or plasmid), particularly a

prokaryotic (e.g. bacteria like *E. coli*) or eukaryotic (e.g. mammalian cells like CHO cells, yeast cells or insect cells or whole organisms like plants or animals) expression system.

The term "expression system" means a system (cell culture or whole organisms) which is suitable for production of peptides, proteins or RNA particularly mRNA.

The inventive nucleic acid sequence according to the first embodiment of the present invention comprises or codes for at least one histone stem-loop. In the context of the present invention, such a histone stem-loop is typically derived from histone genes and comprises an intramolecular base pairing of two neighbored entirely or partially reverse complementary sequences, thereby forming a stem-loop. A stem-loop can occur in single-stranded DNA or, more commonly, in RNA. The structure is also known as a hairpin or hairpin loop and usually consists of a stem and a (terminal) loop within a consecutive sequence, wherein the stem is formed by two neighbored entirely or partially reverse complementary sequences separated by a short sequence as sort of spacer, which builds the loop of the stem-loop structure. The two neighbored entirely or partially reverse complementary sequences may be defined as e.g. stem loop elements stem1 and stem2. The stem loop is formed when these two neighbored entirely or partially reverse complementary sequences, e.g. stem loop elements stem1 and stem2, form base-pairs with each other, leading to a double stranded nucleic acid sequence comprising an unpaired loop at its terminal ending formed by the short sequence located between stem loop elements stem1 and stem2 on the consecutive sequence. The unpaired loop thereby typically represents a region of the nucleic acid which is not capable of base pairing with either of these stem loop elements. The resulting lollipop-shaped structure is a key building block of many RNA secondary structures. The formation of a stem-loop structure is thus dependent on the stability of the resulting stem and loop regions, wherein the first prerequisite is typically the presence of a sequence that can fold back on itself to form a paired double strand. The stability of paired stem loop elements is determined by the length, the number of mismatches or bulges it contains (a small number of mismatches is typically tolerable, especially in a long double strand), and the base composition of the paired region. In the context of the present invention, optimal loop length is 3-10 bases, more preferably 3 to 8, 3 to 7, 3 to 6 or even more preferably 4 to 5 bases, and most preferably 4 bases.

According to one preferred aspect of the first inventive embodiment, the inventive nucleic acid sequence comprises or codes for at least one histone stem-loop sequence, preferably according to at least one of the 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):

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

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 or 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, e.g. by Watson-Crick base pairing of nucleotides A and U/T or G and C or by non-Watson-Crick base pairing e.g. wobble base pairing, reverse Watson-Crick base pairing, Hoogsteen base pairing, reverse Hoogsteen base pairing or are capable of base pairing with each other forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2, on the basis that one ore more bases in one stem do not have a complementary base in the reverse complementary sequence of the other stem.

In the above context, a wobble base pairing is typically a non-Watson-Crick base pairing between two nucleotides. The four main wobble base pairs in the present context, which may be used, are guanosine-uridine, inosine-uridine, inosine-adenosine, inosine-cytidine (G-U/T, I-U/T, I-A and I-C) and adenosine-cytidine (A-C).

Accordingly, in the context of the present invention, a wobble base is a base, which forms a wobble base pair with a further base as described above. Therefore non-Watson-Crick base pairing, e.g. wobble base pairing, may occur in the stem of the histone stem-loop structure according to the present invention.

In the above context a partially reverse complementary sequence comprises maximally 2, preferably only one mismatch in the stem-structure of the stem-loop sequence formed by base pairing of stem1 and stem2. In other words, stem1 and stem2 are preferably capable of (full) base pairing with each other throughout the entire sequence of stem1 and stem2 (100% of possible correct Watson-Crick or non-Watson-Crick base pairings), thereby forming a reverse complementary sequence, wherein each base has its correct Watson-Crick or non-Watson-Crick base pendant as a complementary binding partner. Alternatively, stem1 and stem2 are preferably capable of partial base pairing with each other throughout the entire sequence of stem1 and stem2, wherein at least about 70%, 75%, 80%, 85%, 90%, or 95% of the 100% possible correct Watson-Crick or non-Watson-Crick base pairings are occupied with the correct Watson-Crick or non-Watson-Crick base pairings and at most about 30%, 25%, 20%, 15%, 10%, or 5% of the remaining bases are unpaired.

According to a preferred aspect of the first inventive embodiment, the at least one histone stem-loop sequence (with stem bordering elements) of the inventive nucleic acid sequence as defined herein comprises a length of about 15 to about 45 nucleotides, preferably a length of about 15 to about 40 nucleotides, preferably a length of about 15 to about 35 nucleotides, preferably a length of about 15 to about 30 nucleotides and even more preferably a length of about 20 to about 30 and most preferably a length of about 24 to about 28 nucleotides.

According to a further preferred aspect of the first inventive embodiment, the at least one histone stem-loop sequence (without stem bordering elements) of the inventive nucleic acid sequence as defined herein comprises a length of about 10 to about 30 nucleotides, preferably a length of about 10 to about 20 nucleotides, preferably a length of about 12 to about 20 nucleotides, preferably a length of about 14 to about 20 nucleotides and even more preferably a length of about 16 to about 17 and most preferably a length of about 16 nucleotides.

According to a further preferred aspect of the first inventive embodiment, the inventive nucleic acid sequence according to the first embodiment of the present invention may comprise or code for at least one histone stem-loop sequence according to at least one of the following specific formulae (Ia) or (IIa):

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

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

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

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

wherein:

N, C, G, T and U	are as defined above.

According to a further more particularly preferred aspect of the first embodiment, the inventive nucleic acid sequence may comprise or code for at least one histone stem-loop sequence according to at least one of the following specific formulae (Ib) or (IIb):

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

$$\underbrace{[N_1GN_4]}_{\text{stem1}}\ \underbrace{[N_2(U/T)N_1]}_{\text{loop}}\ \underbrace{[N_4CN_1]}_{\text{stem2}}$$

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

$$\underbrace{N_{4-5}}_{\text{stem1 bordering element}}\ \underbrace{[N_1GN_4]}_{\text{stem1}}\ \underbrace{[N_2(U/T)N_1]}_{\text{loop}}\ \underbrace{[N_4CN_1]}_{\text{stem2}}\ \underbrace{N_{4-5}}_{\text{stem2 bordering element}}$$

wherein:

N, C, G, T and U	are as defined above.

According to an even more preferred aspect of the first inventive embodiment, the inventive nucleic acid sequence according to the first embodiment of the present invention may comprise or code for at least one histone stem-loop sequence according to at least one of the following specific formulae (Ic) to (Ih) or (IIc) to (IIh), shown alternatively in its stem-loop structure and as a linear sequence representing histone stem-loop sequences as generated according to Example 1:

formula (Ic): (metazoan and protozoan histone stem-loop consensus sequence without stem bordering elements):

N U
N N
N-N
N-N
N-N
N-N

G-C
N-N (stem-loop structure)

NGNNNNNNUNNNNNCN
(linear sequence) (SEQ ID NO: 1)

formula (IIc): (metazoan and protozoan histone stem-loop consensus sequence with stem bordering elements):

N U
N N
N-N
N-N
N-N
N-N
G-C
N*N*NNNN-NNNN*N*N* (stem-loop structure)

N*N*NNNNGNNNNNNUNNNNNCNNNN*N*N*
(linear sequence) (SEQ ID NO: 2)

formula (Id): (without stem bordering elements)

N U
N N
N-N
N-N
N-N
N-N
C-G
N-N (stem-loop structure)

NCNNNNNNUNNNNNGN
(linear sequence) (SEQ ID NO: 3)

formula (IId): (with stem bordering elements)

N U
N N

N-N
N-N
N-N
N-N
C-G
N*N*NNNN-NNNN*N*N* (stem-loop structure)

N*N*NNNNCNNNNNNUNNNNNGNNNN*N*N*
(linear sequence) (SEQ ID NO: 4)

formula (Ie): (protozoan histone stem-loop consensus sequence without stem bordering elements)

N U
N N
N-N
N-N
N-N
N-N
G-C
D-H (stem-loop structure)

DGNNNNNNUNNNNNCH
(linear sequence) (SEQ ID NO: 5)

formula (IIe): (protozoan histone stem-loop consensus sequence with stem bordering elements)

N U
N N
N-N
N-N
N-N
N-N
G-C
N*N*NNND-HNNN*N*N* (stem-loop structure)

N*N*NNNDGNNNNNNUNNNNNCHNNN*N*N*
(linear sequence) (SEQ ID NO: 6)

formula (If): (metazoan histone stem-loop consensus sequence without stem bordering elements)

N U
N N
Y-V
Y-N
B-D
N-N
G-C
N-N (stem-loop structure)

NGNBYYNNUNVNDNCN
(linear sequence) (SEQ ID NO: 7)

formula (IIf): (metazoan histone stem-loop consensus sequence with stem bordering elements)

N U
N N
Y-V
Y-N
B-D
N-N
G-C
N*N*NNNN-NNNN*N*N* (stem-loop structure)

N*N*NNNNGNBYYNNUNVNDNCNNNN*N*N*
(linear sequence) (SEQ ID NO: 8)

formula (Ig): (vertebrate histone stem-loop consensus sequence without stem bordering elements)

N U
D H
Y-A
Y-B
Y-R
H-D
G-C
N-N (stem-loop structure)

NGHYYYDNUHABRDCN
(linear sequence) (SEQ ID NO: 9)

formula (IIg): (vertebrate histone stem-loop consensus sequence with stem bordering elements)

N U
D H
Y-A
Y-B
Y-R
H-D
G-C
N*N*HNNN-NNNN*N*H* (stem-loop structure)

N*N*HNNNGHYYYDNUHABRDCNNNN*N*H*
(linear sequence) (SEQ ID NO: 10)

formula (Ih): (human histone stem-loop consensus sequence (Homo sapiens) without stem bordering elements)

Y U
D H
U-A
C-S
Y-R
H-R
G-C
D-C (stem-loop structure)

DGHYCUDYUHASRRCC
(linear sequence) (SEQ ID NO: 11)

formula (IIh): (human histone stem-loop consensus sequence (Homo sapiens) with stem bordering elements)

Y U
D H
U-A
C-S
Y-R
H-R

G-C
N*H*AAHD-CVHB*N*H* (stem loop structure)
N*H*AAHDGHYCUDYUHASRRCCVHB*N*H*
(linear sequence) (SEQ ID NO: 12)

wherein in each of above formulae (Ic) to (Ih) or (IIc) to (IIh):

N, C, G, A, T and U are as defined above;
each U may be replaced by T;
each (highly) conserved G or C in the stem elements 1 and 2 may be replaced by its complementary nucleotide base C or G, provided that its complementary nucleotide in the corresponding stem is replaced by its complementary nucleotide in parallel; and/or

G, A, T, U, C, R, Y, M, K, S, W, H, B, V, D, and N are nucleotide bases as defined in the following Table:

abbreviation	Nucleotide bases	remark
G	G	Guanine
A	A	Adenine
T	T	Thymine
U	U	Uracile
C	C	Cytosine
R	G or A	Purine
Y	T/U or C	Pyrimidine
M	A or C	Amino
K	G or T/U	Keto
S	G or C	Strong (3H bonds)
W	A or T/U	Weak (2H bonds)
H	A or C or T/U	Not G
B	G or T/U or C	Not A
V	G or C or A	Not T/U
D	G or A or T/U	Not C
N	G or C or T/U or A	Any base
*	Present or not	Base may be present or not

In this context it is particularly preferred that the histone stem-loop sequence according to at least one of the formulae (I) or (Ia) to (Ih) or (II) or (IIa) to (IIh) of the present invention is selected from a naturally occurring histone stem loop sequence, more particularly preferred from protozoan or metazoan histone stem-loop sequences, and even more particularly

preferred from vertebrate and mostly preferred from mammalian histone stem-loop sequences especially from human histone stem-loop sequences.

According to a particularly preferred aspect of the first embodiment, the histone stem-loop sequence according to at least one of the specific formulae (I) or (Ia) to (Ih) or (II) or (IIa) to (IIh) of the present invention is a histone stem-loop sequence comprising at each nucleotide position the most frequently occurring nucleotide, or either the most frequently or the second-most frequently occurring nucleotide of naturally occurring histone stem-loop sequences in metazoa and protozoa (Fig. 1), protozoa (Fig. 2), metazoa (Fig. 3), vertebrates (Fig. 4) and humans (Fig. 5) as shown in figure 1-5. In this context it is particularly preferred that at least 80%, preferably at least 85%, or most preferably at least 90% of all nucleotides correspond to the most frequently occurring nucleotide of naturally occurring histone stem-loop sequences.

In a further particular aspect of the first embodiment, the histone stem-loop sequence according to at least one of the specific formulae (I) or (Ia) to (Ih) of the present invention is selected from following histone stem-loop sequences (without stem-bordering elements) representing histone stem-loop sequences as generated according to Example 1:

VGYYYYHHTHRVVRCB (SEQ ID NO: 13 according to formula (Ic))
SGYYYTTYTMARRRCS (SEQ ID NO: 14 according to formula (Ic))
SGYYCTTTTMAGRRCS (SEQ ID NO: 15 according to formula (Ic))

DGNNNBNNTHVNNNCH (SEQ ID NO: 16 according to formula (Ie))
RGNNNYHBTHRDNNCY (SEQ ID NO: 17 according to formula (Ie))
RGNDBYHYTHRDHNCY (SEQ ID NO: 18 according to formula (Ie))

VGYYYTYHTHRVRRCB (SEQ ID NO: 19 according to formula (If))
SGYYCTTYTMAGRRCS (SEQ ID NO: 20 according to formula (If))
SGYYCTTTTMAGRRCS (SEQ ID NO: 21 according to formula (If))

GGYYCTTYTHAGRRCC (SEQ ID NO: 22 according to formula (Ig))
GGCYCTTYTMAGRGCC (SEQ ID NO: 23 according to formula (Ig))
GGCTCTTTTMAGRGCC (SEQ ID NO: 24 according to formula (Ig))

DGHYCTDYTHASRRCC (SEQ ID NO: 25 according to formula (Ih))
GGCYCTTTTHAGRGCC (SEQ ID NO: 26 according to formula (Ih))
GGCYCTTTTMAGRGCC (SEQ ID NO: 27 according to formula (Ih))

Furthermore in this context following histone stem-loop sequences (with stem bordering elements) as generated according to Example 1 according to one of specific formulae (II) or (IIa) to (IIh) are particularly preferred:

H*H*HHVVGYYYYHHTHRVVRCBVHH*N*N* (SEQ ID NO: 28 according to formula (IIc))
M*H*MHMSGYYYTTYTMARRRCSMCH*H*H* (SEQ ID NO: 29 according to formula (IIc))
M*M*MMMSGYYCTTTTMAGRRCSACH*M*H* (SEQ ID NO: 30 according to formula (IIc))

N*N*NNNDGNNNBNNTHVNNNCHNHN*N*N* (SEQ ID NO: 31 according to formula (IIe))
N*N*HHNRGNNNYHBTHRDNNCYDHH*N*N* (SEQ ID NO: 32 according to formula (IIe))
N*H*HHVRGNDBYHYTHRDHNCYRHH*H*H* (SEQ ID NO: 33 according to formula (IIe))

H*H*MHMVGYYYTYHTHRVRRCBVMH*H*N* (SEQ ID NO: 34 according to formula (IIf))
M*M*MMMSGYYCTTYTMAGRRCSMCH*H*H* (SEQ ID NO: 35 according to formula (IIf))
M*M*MMMSGYYCTTTTMAGRRCSACH*M*H* (SEQ ID NO: 36 according to formula (IIf))

H*H*MAMGGYYCTTYTHAGRRCCVHN*N*M* (SEQ ID NO: 37 according to formula (IIg))
H*H*AAMGGCYCTTYTMAGRGCCVCH*H*M* (SEQ ID NO: 38 according to formula (IIg))
M*M*AAMGGCTCTTTTMAGRGCCMCY*M*M* (SEQ ID NO: 39 according to formula (IIg))

N*H*AAHDGHYCTDYTHASRRCCVHB*N*H* (SEQ ID NO: 40 according to formula (IIh))
H*H*AAMGGCYCTTTTHAGRGCCVMY*N*M* (SEQ ID NO: 41 according to formula (IIh))
H*M*AAAGGCYCTTTTMAGRGCCRMY*H*M* (SEQ ID NO: 42 according to formula (IIh))

According to a further preferred aspect of the first inventive embodiment, the inventive nucleic acid sequence comprises or codes for at least one histone stem-loop sequence showing at least about 80%, preferably at least about 85%, more preferably at least about 90%, or even more preferably at least about 95%, sequence identity with the not to 100% conserved nucleotides

in the histone stem-loop sequences according to at least one of specific formulae (I) or (Ia) to (Ih) or (II) or (IIa) to (IIh) or with a naturally occurring histone stem-loop sequence.

The inventive nucleic acid sequence according to the first embodiment of the present invention may optionally comprise or code for a poly(A) sequence. When present, such a poly(A) sequence comprises a sequence of about 25 to about 400 adenosine nucleotides, preferably a sequence of about 50 to about 400 adenosine nucleotides, more preferably a sequence of about 50 to about 300 adenosine nucleotides, even more preferably a sequence of about 50 to about 250 adenosine nucleotides, most preferably a sequence of about 60 to about 250 adenosine nucleotides. In this context the term "about" refers to a deviation of ± 10% of the value(s) it is attached to.

Alternatively, according to the first embodiment of the present invention, the inventive nucleic sequence optionally comprises a polyadenylation signal which is defined herein as a signal which conveys polyadenylation to a (transcribed) mRNA by specific protein factors (e.g. cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I and II (CF I and CF II), poly(A) polymerase (PAP)). In this context a consensus polyadenylation signal is preferred comprising the NNUANA consensus sequence. In a particular preferred aspect the polyadenylation signal comprises one of the following sequences: AAUAAA or AUUAAA.

The inventive nucleic acid sequence according to the first embodiment of the present invention furthermore encode a protein or a peptide, which may be selected, without being restricted thereto, e.g. from therapeutically active proteins or peptides, including adjuvant proteins, from antigens, e.g. tumour antigens, pathogenic antigens (e.g. selected, from animal antigens, from viral antigens, from protozoal antigens, from bacterial antigens), allergenic antigens, autoimmune antigens, or further antigens, from allergens, from antibodies, from immunostimulatory proteins or peptides, from antigen-specific T-cell receptors, or from any other protein or peptide suitable for a specific (therapeutic) application, wherein the inventive nucleic acid may be transported into a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism and the protein may be expressed subsequently in this cell, tissue or organism.

The coding region of the inventive nucleic acid according to the first embodiment of the present invention may occur as a mono-, di-, or even multicistronic nucleic acid, i.e. a nucleic acid which carries the coding sequences of one, two or more proteins or peptides. Such coding sequences in di-, or even multicistronic nucleic acids may be separated by at least one internal ribosome entry site (IRES) sequence, e.g. as defined herein or by signal peptides which induce the cleavage of the resulting polypeptide which comprises several proteins or peptides.

In particular preferred aspects of the first embodiment of the present invention the encoded peptides or proteins are selected from human, viral, bacterial, protozoan proteins or peptides.

In the context of the present invention, therapeutically active proteins, encoded by the inventive nucleic acid molecule may be selected, without being restricted thereto, from proteins which have an effect on healing, prevent prophylactically or treat therapeutically a disease, preferably as defined herein, or are proteins of which an individual is in need of. Such proteins may be selected from any naturally or synthetically designed occurring recombinant or isolated protein known to a skilled person from the prior art. Without being restricted thereto therapeutically active proteins may comprise proteins, capable of stimulating or inhibiting the signal transduction in the cell, e.g. cytokines, lymphokines, monokines, growth factors, receptors, signal transduction molecules, transcription factors, etc; anticoagulants; antithrombins; antiallergic proteins; apoptotic factors or apoptosis related proteins, therapeutic active enzymes and any protein connected with any acquired disease or any hereditary disease.

Preferably, a therapeutically active protein, which may be encoded by the inventive nucleic acid molecule, may also be an adjuvant protein. In this context, an adjuvant protein is preferably to be understood as any protein, which is capable to elicit an innate immune response as defined herein. Preferably, such an innate immune response comprises activation of a pattern recognition receptor, such as e.g. a receptor selected from the Toll-like receptor (TLR) family, including e.g. a Toll like receptor selected from human TLR1 to TLR10 or from murine Toll like receptors TLR1 to TLR13. More preferably, the adjuvant protein is selected from human adjuvant proteins or from pathogenic adjuvant proteins,

selected from the group consisting of, without being limited thereto, bacterial proteins, protozoan proteins, viral proteins, or fungal proteins, animal proteins, in particular from bacterial adjuvant proteins. In addition, nucleic acids encoding human proteins involved in adjuvant effects (e.g. ligands of pattern recognition receptors, pattern recognition receptors, proteins of the signal transduction pathways, transcription factors or cytokines) may be used as well.

The inventive nucleic acid molecule may alternatively encode an antigen. According to the present invention, the term "antigen" refers to a substance which is recognized by the immune system and is capable of triggering an antigen-specific immune response, e.g. by formation of antibodies or antigen-specific T-cells as part of an adaptive immune response. In this context an antigenic epitope, fragment or peptide of a protein means particularly B cell and T cell epitopes which may be recognized by B cells, antibodies or T cells respectively.

In the context of the present invention, antigens, which may be encoded by the inventive nucleic acid molecule, typically comprise any antigen, antigenic epitope, antigenic fragment or antigenic peptide, falling under the above definition, more preferably protein and peptide antigens, e.g. tumour antigens, allergenic antigens or allergens, auto-immune self-antigens, pathogenic antigens, etc.

In particular antigens as encoded by the inventive nucleic acid molecule may be antigens generated outside the cell, more typically antigens not derived from the host organism (e.g. a human) itself (i.e. non-self antigens) but rather derived from host cells outside the host organism, e.g. viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens, allergenic antigens, etc. Allergenic antigens (allergy antigens or allergens) are typically antigens, which cause an allergy in a human and may be derived from either a human or other sources. Additionally, antigens as encoded by the inventive nucleic acid molecule may be furthermore antigens generated inside the cell, the tissue or the body. Such antigens include antigens derived from the host organism (e.g. a human) itself, e.g. tumour antigens, self-antigens or auto-antigens, such as auto-immune self-antigens, etc., but also (non-self) antigens as defined herein, which have been originally been derived from

host cells outside the host organism, but which are fragmented or degraded inside the body, tissue or cell, e.g. by (protease) degradation, metabolism, etc.

One class of antigens, which may be encoded by the inventive nucleic acid molecule comprises tumour antigens. "Tumour antigens" are preferably located on the surface of the (tumour) cell. Tumour antigens may also be selected from proteins, which are overexpressed in tumour cells compared to a normal cell. Furthermore, tumour antigens also include antigens expressed in cells which are (were) not themselves (or originally not themselves) degenerated but are associated with the supposed tumour. Antigens which are connected with tumour-supplying vessels or (re)formation thereof, in particular those antigens which are associated with neovascularization, e.g. growth factors, such as VEGF, bFGF etc., are also included herein. Antigens connected with a tumour furthermore include antigens from cells or tissues, typically embedding the tumour. Further, some substances (usually proteins or peptides) are expressed in patients suffering (knowingly or not-knowingly) from a cancer disease and they occur in increased concentrations in the body fluids of said patients. These substances are also referred to as "tumour antigens", however they are not antigens in the stringent meaning of an immune response inducing substance. The class of tumour antigens can be divided further into tumour-specific antigens (TSAs) and tumour-associated-antigens (TAAs). TSAs can only be presented by tumour cells and never by normal "healthy" cells. They typically result from a tumour specific mutation. TAAs, which are more common, are usually presented by both tumour and healthy cells. These antigens are recognized and the antigen-presenting cell can be destroyed by cytotoxic T cells. Additionally, tumour antigens can also occur on the surface of the tumour in the form of, e.g., a mutated receptor. In this case, they can be recognized by antibodies.

According to another alternative, one further class of antigens, which may be encoded by the inventive nucleic acid molecule, comprises allergenic antigens. Such allergenic antigens may be selected from antigens derived from different sources, e.g. from animals, plants, fungi, bacteria, etc. Allergens in this context include e.g. grasses, pollens, molds, drugs, or numerous environmental triggers, etc. Allergenic antigens typically belong to different classes of compounds, such as nucleic acids and their fragments, proteins or peptides and their fragments, carbohydrates, polysaccharides, sugars, lipids, phospholipids, etc. Of particular interest in the context of the present invention are antigens, which may be

encoded by the inventive nucleic acid molecule as defined herein, i.e. protein or peptide antigens and their fragments or epitopes, or nucleic acids and their fragments, particularly nucleic acids and their fragments, encoding such protein or peptide antigens and their fragments or epitopes.

According to a further alternative, the inventive nucleic acid molecule may encode an antibody or an antibody fragment. According to the present invention, such an antibody may be selected from any antibody, e.g. any recombinantly produced or naturally occurring antibodies, known in the art, in particular antibodies suitable for therapeutic, diagnostic or scientific purposes, or antibodies which have been identified in relation to specific cancer diseases. Herein, the term "antibody" is used in its broadest sense and specifically covers monoclonal and polyclonal antibodies (including agonist, antagonist, and blocking or neutralizing antibodies) and antibody species with polyepitopic specificity. According to the invention, the term "antibody" typically comprises any antibody known in the art (e.g. IgM, IgD, IgG, IgA and IgE antibodies), such as naturally occurring antibodies, antibodies generated by immunization in a host organism, antibodies which were isolated and identified from naturally occurring antibodies or antibodies generated by immunization in a host organism and recombinantly produced by biomolecular methods known in the art, as well as chimeric antibodies, human antibodies, humanized antibodies, bispecific antibodies, intrabodies, i.e. antibodies expressed in cells and optionally localized in specific cell compartments, and fragments and variants of the aforementioned antibodies. In general, an antibody consists of a light chain and a heavy chain both having variable and constant domains. The light chain consists of an N-terminal variable domain, V_L, and a C-terminal constant domain, C_L. In contrast, the heavy chain of the IgG antibody, for example, is comprised of an N-terminal variable domain, V_H, and three constant domains, C_H1, C_H2 und C_H3.

In the context of the present invention, antibodies as encoded by the inventive nucleic acid molecule may preferably comprise full-length antibodies, i.e. antibodies composed of the full heavy and full light chains, as described above. However, derivatives of antibodies such as antibody fragments, variants or adducts may also be encoded by the inventive nucleic acid molecule. Antibody fragments are preferably selected from Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd and Fv fragments of the aforementioned (full-length) antibodies. In general,

antibody fragments are known in the art. For example, a Fab ("fragment, antigen binding") fragment is composed of one constant and one variable domain of each of the heavy and the light chain. The two variable domains bind the epitope on specific antigens. The two chains are connected via a disulfide linkage. A scFv ("single chain variable fragment") fragment, for example, typically consists of the variable domains of the light and heavy chains. The domains are linked by an artificial linkage, in general a polypeptide linkage such as a peptide composed of 15-25 glycine, proline and/or serine residues.

In the present context it is preferable that the different chains of the antibody or antibody fragment are encoded by a multicistronic nucleic acid molecule. Alternatively, the different strains of the antibody or antibody fragment are encoded by several monocistronic nucleic acid(s) (sequences).

According to the first embodiment of the present invention, the inventive nucleic acid sequence comprises a coding region, preferably encoding a peptide or protein. Preferably, the encoded protein is no histone protein. In the context of the present invention such a histone protein is typically a strongly alkaline protein found in eukaryotic cell nuclei, which package and order the DNA into structural units called nucleosomes. Histone proteins are the chief protein components of chromatin, act as spools around which DNA winds, and play a role in gene regulation. Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to one in human DNA). For example, each human cell has about 1.8 meters of DNA, but wound on the histones it has about 90 millimeters of chromatin, which, when duplicated and condensed during mitosis, result in about 120 micrometers of chromosomes. More preferably, in the context of the present invention such a histone protein is typically defined as a highly conserved protein selected from one of the following five major classes of histones: H1/H5, H2A, H2B, H3, and H4", preferably selected from mammalian histone, more preferably from human histones or histone proteins. Such histones or histone proteins are typically organised into two super-classes defined as core histones, comprising histones H2A, H2B, H3 and H4, and linker histones, comprising histones H1 and H5.

In this context, linker histones, preferably excluded from the scope of protection of the pending invention, preferably mammalian linker histones, more preferably human linker

histones, are typically selected from H1, including H1F, particularly including H1F0, H1FNT, H1FOO, H1FX, and H1H1, particularly including HIST1H1A, HIST1H1B, HIST1H1C, HIST1H1D, HIST1H1E, HIST1H1T; and

Furthermore, core histones, preferably excluded from the scope of protection of the pending invention, preferably mammalian core histones, more preferably human core histones, are typically selected from H2A, including H2AF, particularly including H2AFB1, H2AFB2, H2AFB3, H2AFJ, H2AFV, H2AFX, H2AFY, H2AFY2, H2AFZ, and H2A1, particularly including HIST1H2AA, HIST1H2AB, HIST1H2AC, HIST1H2AD, HIST1H2AE, HIST1H2AG, HIST1H2AI, HIST1H2AJ, HIST1H2AK, HIST1H2AL, HIST1H2AM, and H2A2, particularly including HIST2H2AA3, HIST2H2AC; H2B, including H2BF, particularly including H2BFM, H2BFO, H2BFS, H2BFWT H2B1, particularly including HIST1H2BA, HIST1H2BB, HIST1H2BC, HIST1H2BD, HIST1H2BE, HIST1H2BF, HIST1H2BG, HIST1H2BH, HIST1H2BI, HIST1H2BJ, HIST1H2BK, HIST1H2BL, HIST1H2BM, HIST1H2BN, HIST1H2BO, and H2B2, particularly including HIST2H2BE; H3, including H3A1, particularly including HIST1H3A, HIST1H3B, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J, and H3A2, particularly including HIST2H3C, and H3A3, particularly including HIST3H3; H4, including H41, particularly including HIST1H4A, HIST1H4B, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4G, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L, and H44, particularly including HIST4H4, and H5.

According to the first embodiment of the present invention, the inventive nucleic acid sequence comprises a coding region, preferably encoding a peptide or protein. Preferably, the encoded protein is no reporter protein (e.g. Luciferase, Green Fluorescent Protein (GFP), Enhanced Green Fluorescent Protein (EGFP), β-Galactosidase) and no marker or selection protein (e.g. alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

The inventive nucleic acid as define above, comprises or codes for a) a coding region, preferably encoding a peptide or protein; b) at least one histone stem-loop, and c) optionally a poly(A) sequence or polyadenylation signal; preferably for increasing the expression level of an encoded protein, wherein the encoded protein is preferably no

histone protein, no reporter protein and/or no marker or selection protein, as defined above. The elements b) to c) of the inventive nucleic acid may occur in the inventive nucleic acid in any order, i.e. the elements a), b) and c) may occur in the order a), b) and c) or a), c) and b) from 5′ to 3′ direction in the inventive nucleic acid sequence, wherein further elements as described herein, may also be contained, such as a 5′-CAP structure, a poly(C) sequence, stabilization sequences, IRES sequences, etc. Each of the elements a) to c) of the inventive nucleic acid, particularly a) in di- or multicistronic constructs and/or each of the elements b) and c), more preferably element b) may also be repeated at least once, preferably twice or more in the inventive nucleic acid. As an example, the inventive nucleic acid may show its sequence elements a), b) and optionally c) in e.g. the following order:

5′ – coding region – histone stem-loop – 3′; or

5′ – coding region – coding region - histone stem-loop – 3′; or

5′ – coding region – IRES - coding region - histone stem-loop – 3′; or

5′ – coding region – histone stem-loop – poly(A) sequence – 3′; or

5′ – coding region – histone stem-loop – polyadenylation signal – 3′; or

5′ – coding region – coding region - histone stem-loop – polyadenylation signal - 3′; or

5′ – coding region – histone stem-loop – histone stem-loop – 3′; or

5′ – coding region – histone stem-loop – histone stem-loop – poly(A) sequence – 3′; or

5′ – coding region – histone stem-loop – histone stem-loop – polyadenylation signal– 3′; or

5′ – coding region – histone stem-loop – poly(A) sequence – histone stem-loop – 3′; or

5′ – coding region– poly(A) sequence – histone stem-loop – 3′; or

5′ – coding region – poly(A) sequence – histone stem-loop – histone stem-loop – 3′;etc.

In this context it is particularly preferred that the inventive nucleic acid molecule comprises or codes for a) a coding region, preferably encoding a peptide or protein; b) at least one histone stem-loop, and c) a poly(A) sequence or polyadenylation sequence; preferably for increasing the expression level of an encoded protein, wherein the encoded protein is preferably no histone protein, no reporter protein (e.g. Luciferase, GFP, EGFP, β-Galactosidase, particularly EGFP) and/or no marker or selection protein (e.g. alpha-Globin, Galactokinase and Xanthine:Guanine phosphoribosyl transferase (GPT)).

In a further preferred aspect of the first embodiment the inventive nucleic acid molecule as defined herein may also occur in the form of a modified nucleic acid.

According to one aspect of the first embodiment, the inventive nucleic acid molecule as defined herein may be provided as a "stabilized nucleic acid", preferably as a stabilized RNA, more preferably as a RNA that is essentially resistant to *in vivo* degradation (e.g. by an exo- or endo-nuclease).

In this context, the inventive nucleic acid molecule as defined herein may contain nucleotide analogues/modifications e.g. backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present invention is a modification in which phosphates of the backbone of the nucleotides contained in inventive nucleic acid molecule as defined herein are chemically modified. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of the inventive nucleic acid molecule as defined herein. Furthermore, a base modification in connection with the present invention is a chemical modification of the base moiety of the nucleotides of the nucleic acid molecule of the inventive nucleic acid molecule. In this context nucleotide analogues or modifications are preferably selected from nucleotide analogues which are applicable for transcription and/or translation.

In a particular preferred aspect of the first embodiment of the present invention the herein defined nucleotide analogues/modifications are selected from base modifications which additionally increase the expression of the encoded protein and which are preferably selected from 2-amino-6-chloropurineriboside-5'-triphosphate, 2-aminoadenosine-5'-triphosphate, 2-thiocytidine-5'-triphosphate, 2-thiouridine-5'-triphosphate, 4-thiouridine-5'-triphosphate, 5-aminoallylcytidine-5'-triphosphate, 5-aminoallyluridine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, 5-bromouridine-5'-triphosphate, 5-iodocytidine-5'-triphosphate, 5-iodouridine-5'-triphosphate, 5-methylcytidine-5'-triphosphate, 5-methyluridine-5'-triphosphate, 6-azacytidine-5'-triphosphate, 6-azauridine-5'-triphosphate, 6-chloropurineriboside-5'-triphosphate, 7-deazaadenosine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 8-azaadenosine-5'-triphosphate, 8-azidoadenosine-5'-triphosphate, benzimidazole-riboside-5'-triphosphate, N1-methyladenosine-5'-triphosphate, N1-methylguanosine-5'-triphosphate, N6-methyladenosine-5'-triphosphate, O6-methylguanosine-5'-triphosphate, pseudouridine-5'-triphosphate, or puromycin-5'-

triphosphate, xanthosine-5'-triphosphate. Particular preference is given to nucleotides for base modifications selected from the group of base-modified nucleotides consisting of 5-methylcytidine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, and pseudouridine-5'-triphosphate.

According to a further aspect, the inventive nucleic acid molecule as defined herein can contain a lipid modification. Such a lipid-modified nucleic acid typically comprises a nucleic acid as defined herein. Such a lipid-modified nucleic acid molecule of the inventive nucleic acid molecule as defined herein typically further comprises at least one linker covalently linked with that nucleic acid molecule, and at least one lipid covalently linked with the respective linker. Alternatively, the lipid-modified nucleic acid molecule comprises at least one nucleic acid molecule as defined herein and at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid molecule. According to a third alternative, the lipid-modified nucleic acid molecule comprises a nucleic acid molecule as defined herein, at least one linker covalently linked with that nucleic acid molecule, and at least one lipid covalently linked with the respective linker, and also at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid molecule. In this context it is particularly preferred that the lipid modification is present at the terminal ends of a linear inventive nucleic acid sequence.

According to another preferred aspect of the first embodiment of the invention, the inventive nucleic acid molecule as defined herein, particularly if provided as an (m)RNA, can therefore be stabilized against degradation by RNases by the addition of a so-called "5' CAP" structure.

According to a further preferred aspect of the first embodiment of the invention, the inventive nucleic acid molecule as defined herein, can be modified by a sequence of at least 10 cytidines, preferably at least 20 cytidines, more preferably at least 30 cytidines (so-called "poly(C) sequence"). Particularly, the inventive nucleic acid molecule may contain or code for a poly(C) sequence of typically about 10 to 200 cytidine nucleotides, preferably about 10 to 100 cytidine nucleotides, more preferably about 10 to 70 cytidine nucleotides or even more preferably about 20 to 50 or even 20 to 30 cytidine nucleotides. This poly(C)

sequence is preferably located 3′ of the coding region comprised in the inventive nucleic acid according to the first embodiment of the present invention.

According to another preferred aspect of the first embodiment of the invention, the inventive nucleic acid molecule as defined herein, preferably has at least one 5' and/or 3' stabilizing sequence. These stabilizing sequences in the 5′ and/or 3′ untranslated regions have the effect of increasing the half-life of the nucleic acid in the cytosol. These stabilizing sequences can have 100% sequence identity to naturally occurring sequences which occur in viruses, bacteria and eukaryotes, but can also be partly or completely synthetic. The untranslated sequences (UTR) of the (alpha-)globin gene, e.g. from *Homo sapiens* or *Xenopus laevis* may be mentioned as an example of stabilizing sequences which can be used in the present invention for a stabilized nucleic acid. Another example of a stabilizing sequence has the general formula (C/U)CCAN$_x$CCC(U/A)Py$_x$UC(C/U)CC (SEQ ID NO: 55), which is contained in the 3′-UTRs of the very stable RNAs which code for (alpha-)globin, type(I)-collagen, 15-lipoxygenase or for tyrosine hydroxylase (cf. Holcik *et al.*, Proc. Natl. Acad. Sci. USA 1997, 94: 2410 to 2414). Such stabilizing sequences can of course be used individually or in combination with one another and also in combination with other stabilizing sequences known to a person skilled in the art. In this context it is particularly preferred that the 3′ UTR sequence of the alpha globin gene is located 3′ of the coding sequence comprised in the inventive nucleic acid according to the first embodiment of the present invention.

Substitutions, additions or eliminations of bases are preferably carried out with the inventive nucleic acid molecule as defined herein, using a DNA matrix for preparation of the nucleic acid molecule by techniques of the well known site directed mutagenesis or with an oligonucleotide ligation strategy (see e.g. Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., Cold Spring Harbor, NY, 2001). In such a process, for preparation of the inventive nucleic acid molecule as defined herein, especially if the nucleic acid is in the form of an mRNA, a corresponding DNA molecule may be transcribed *in vitro*. This DNA matrix preferably comprises a suitable promoter, e.g. a T7 or SP6 promoter, for *in vitro* transcription, which is followed by the desired nucleotide sequence for the nucleic acid molecule, e.g. mRNA, to be prepared and a termination signal for *in vitro* transcription. The DNA molecule, which forms the matrix of the at least

one RNA of interest, may be prepared by fermentative proliferation and subsequent isolation as part of a plasmid which can be replicated in bacteria. Plasmids which may be mentioned as suitable for the present invention are e.g. the plasmids pT7Ts (GenBank accession number U26404; Lai *et al.*, Development 1995, 121: 2349 to 2360), pGEM® series, e.g. pGEM®-1 (GenBank accession number X65300; from Promega) and pSP64 (GenBank accession number X65327); cf. also Mezei and Storts, Purification of PCR Products, in: Griffin and Griffin (ed.), PCR Technology: Current Innovation, CRC Press, Boca Raton, FL, 2001.

Nucleic acid molecules used according to the present invention as defined herein may be prepared using any method known in the art, including synthetic methods such as e.g. solid phase synthesis, as well as *in vitro* methods, such as *in vitro* transcription reactions or *in vivo* reactions, such as *in vivo* propagation of DNA plasmids in bacteria.

Any of the above modifications may be applied to the inventive nucleic acid molecule as defined herein and further to any nucleic acid as used in the context of the present invention and may be, if suitable or necessary, be combined with each other in any combination, provided, these combinations of modifications do not interfere with each other in the respective nucleic acid. A person skilled in the art will be able to take his choice accordingly.

The inventive nucleic acid molecule as defined herein as well as proteins or peptides as encoded by this nucleic acid molecule may comprise fragments or variants of those sequences. Such fragments or variants may typically comprise a sequence having a sequence identity with one of the above mentioned nucleic acids, or with one of the proteins or peptides or sequences, if encoded by the at least one nucleic acid molecule, of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, preferably at least 70%, more preferably at least 80%, equally more preferably at least 85%, even more preferably at least 90% and most preferably at least 95% or even 97%, 98% or 99%, to the entire wild type sequence, either on nucleic acid level or on amino acid level.

In a further preferred aspect of the first embodiment of the present invention the inventive nucleic acid sequence is associated with a vehicle, transfection or complexation agent for

increasing the transfection efficiency of the inventive nucleic acid sequence. Particularly preferred agents in this context suitable for increasing the transfection efficiency are cationic or polycationic compounds, including protamine, nucleoline, spermine or spermidine, or other cationic peptides or proteins, such as poly-L-lysine (PLL), poly-arginine, basic polypeptides, cell penetrating peptides (CPPs), including HIV-binding peptides, HIV-1 Tat (HIV), Tat-derived peptides, Penetratin, VP22 derived or analog peptides, HSV VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, Calcitonin peptide(s), Antennapedia-derived peptides (particularly from *Drosophila antennapedia*), pAntp, plsl, FGF, Lactoferrin, Transportan, Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, or histones. Additionally, preferred cationic or polycationic proteins or peptides may be selected from the following proteins or peptides having the following total formula: $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$, wherein l + m + n +o + x = 8-15, and l, m, n or o independently of each other may be any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, provided that the overall content of Arg, Lys, His and Orn represents at least 50% of all amino acids of the oligopeptide; and Xaa may be any amino acid selected from native (= naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and x may be any number selected from 0, 1, 2, 3 or 4, provided, that the overall content of Xaa does not exceed 50 % of all amino acids of the oligopeptide. Particularly preferred cationic peptides in this context are e.g. Arg_7, Arg_8, Arg_9, H_3R_9, R_9H_3, $H_3R_9H_3$, $YSSR_9SSY$, $(RKH)_4$, $Y(RKH)_2R$, etc. Further preferred cationic or polycationic compounds, which can be used as transfection agent may include cationic polysaccharides, for example chitosan, polybrene, cationic polymers, e.g. polyethyleneimine (PEI), cationic lipids, e.g. DOTMA: [1-(2,3-sioleyloxy)propyl)]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleyl phosphatidylethanol-amine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglicylspermin, DIMRI: Dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethanolamine chloride, CLIP1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium, oligofectamine, or

cationic or polycationic polymers, e.g. modified polyaminoacids, such as β-aminoacid-polymers or reversed polyamides, etc., modified polyethylenes, such as PVP (poly(N-ethyl-4-vinylpyridinium bromide)), etc., modified acrylates, such as pDMAEMA (poly(dimethylaminoethyl methylacrylate)), etc., modified Amidoamines such as pAMAM (poly(amidoamine)), etc., modified polybetaaminoester (PBAE), such as diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, etc., dendrimers, such as polypropylamine dendrimers or pAMAM based dendrimers, etc., polyimine(s), such as PEI: poly(ethyleneimine), poly(propyleneimine), etc., polyallylamine, sugar backbone based polymers, such as cyclodextrin based polymers, dextran based polymers, chitosan, etc., silan backbone based polymers, such as PMOXA-PDMS copolymers, etc., blockpolymers consisting of a combination of one or more cationic blocks (e.g. selected from a cationic polymer as mentioned above) and of one or more hydrophilic or hydrophobic blocks (e.g polyethyleneglycole); etc.

According to a further embodiment, the present invention also provides a method for increasing the expression level of an encoded protein/peptide comprising the steps, e.g. a) providing the inventive nucleic acid as defined herein, b) applying or administering the inventive nucleic acid encoding a protein or peptide as defined herein to an expression system, e.g. to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism. The method may be applied for laboratory, for research, for diagnostic, for commercial production of peptides or proteins and/or for therapeutic purposes. In this context, typically after preparing the inventive nucleic acid as defined herein, it is typically applied or administered to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism, preferably in naked form or as a pharmaceutical composition or vaccine as described herein, preferably via transfection or by using any of the administration modes as described herein. The method may be carried out *in vitro*, *in vivo* or *ex vivo*. The method may furthermore be carried out in the context of the treatment of a specific disease, preferably as defined herein.

In this context *in vitro* is defined herein as transfection or transduction of the inventive nucleic acid into cells in culture outside of an organism; *in vivo* is defined herein as transfection or transduction of the inventive nucleic acid into cells by application of the inventive nucleic acid to the whole organism or individual and *ex vivo* is defined herein as

transfection or transduction of the inventive nucleic acid into cells outside of an organism or individual and subsequent application of the transfected cells to the organism or individual.

Likewise, according to another embodiment, the present invention also provides the use of the inventive nucleic acid as defined herein, preferably for diagnostic or therapeutic purposes, for increasing the expression level of an encoded protein/peptide, e.g. by applying or administering the inventive nucleic acid encoding a protein or peptide as defined herein, e.g. to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism. The use may be applied for laboratory, for research, for diagnostic for commercial production of peptides or proteins and/or for therapeutic purposes. In this context, typically after preparing the inventive nucleic acid as defined herein, it is typically applied or administered to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism, preferably in naked form or complexed form, or as a pharmaceutical composition or vaccine as described herein, preferably via transfection or by using any of the administration modes as described herein. The use may be carried out *in vitro*, *in vivo* or *ex vivo*. The use may furthermore be carried out in the context of the treatment of a specific disease, preferably as defined herein.

In yet another embodiment the present invention also relates to an inventive expression system comprising an inventive nucleic acid or expression vector or plasmid according to the first embodiment of the present invention. In this context the expression system may be a cell-free expression system (e.g. an *in vitro* transcription/translation system), a cellular expression system (e.g. mammalian cells like CHO cells, insect cells, yeast cells, bacterial cells like *E. coli*) or organisms used for expression of peptides or proteins (e.g. plants or animals like cows).

Additionally, according to another embodiment, the present invention also relates to the use of the inventive nucleic acid as defined herein for the preparation of a pharmaceutical composition for increasing the expression level of an encoded protein/peptide, e.g. for treating a disease as defined herein, e.g. applying or administering the inventive nucleic acid as defined herein to a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism, preferably in naked form or complexed form or as a pharmaceutical composition

or vaccine as described herein, more preferably using any of the administration modes as described herein.

Accordingly, in a particular preferred embodiment, the present invention also provides a pharmaceutical composition, comprising the inventive nucleic acid as defined herein and optionally a pharmaceutically acceptable carrier and/or vehicle.

As a first ingredient, the inventive pharmaceutical composition comprises the inventive nucleic acid as defined herein.

As a second ingredient the inventive pharmaceutical composition may comprise at least one additional pharmaceutically active component. A pharmaceutically active component in this connection is a compound that has a therapeutic effect to heal, ameliorate or prevent a particular indication or disease as mentioned herein, preferably cancer diseases, autoimmune disease, allergies or infectious diseases, cardiovascular diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, neoplasms, immune deficiencies, endocrine, nutritional and metabolic diseases, neural diseases, eye diseases, ear diseases and hereditary diseases. Such compounds include, without implying any limitation, peptides or proteins, preferably as defined herein, nucleic acids, preferably as defined herein, (therapeutically active) low molecular weight organic or inorganic compounds (molecular weight less than 5000, preferably less than 1000), sugars, antigens or antibodies, preferably as defined herein, therapeutic agents already known in the prior art, antigenic cells, antigenic cellular fragments, cellular fractions; cell wall components (e.g. polysaccharides), modified, attenuated or de-activated (e.g. chemically or by irradiation) pathogens (virus, bacteria etc.), adjuvants, preferably as defined herein, etc.

Furthermore, the inventive pharmaceutical composition may comprise a pharmaceutically acceptable carrier and/or vehicle. In the context of the present invention, a pharmaceutically acceptable carrier typically includes the liquid or non-liquid basis of the inventive pharmaceutical composition. If the inventive pharmaceutical composition is provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or buffered (aqueous) solutions, e.g phosphate, citrate etc. buffered solutions. The injection

buffer may be hypertonic, isotonic or hypotonic with reference to the specific reference medium, i.e. the buffer may have a higher, identical or lower salt content with reference to the specific reference medium, wherein preferably such concentrations of the afore mentioned salts may be used, which do not lead to damage of cells due to osmosis or other concentration effects. Reference media are e.g. liquids occurring in "*in vivo*" methods, such as blood, lymph, cytosolic liquids, or other body liquids, or e.g. liquids, which may be used as reference media in "*in vitro*" methods, such as common buffers or liquids. Such common buffers or liquids are known to a skilled person. Ringer-Lactate solution is particularly preferred as a liquid basis.

However, one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well for the inventive pharmaceutical composition, which are suitable for administration to a patient to be treated. The term "compatible" as used here means that these constituents of the inventive pharmaceutical composition are capable of being mixed with the inventive nucleic acid as defined herein in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the inventive pharmaceutical composition under typical use conditions.

According to a specific aspect, the inventive pharmaceutical composition may comprise an adjuvant. In this context, an adjuvant may be understood as any compound, which is suitable to initiate or increase an immune response of the innate immune system, i.e. a non-specific immune response. With other words, when administered, the inventive pharmaceutical composition preferably elicits an innate immune response due to the adjuvant, optionally contained therein. Preferably, such an adjuvant may be selected from an adjuvant known to a skilled person and suitable for the present case, i.e. supporting the induction of an innate immune response in a mammal, e.g. an adjuvant protein as defined above or an adjuvant as defined in the following.

Particularly preferred as adjuvants suitable for depot and delivery are cationic or polycationic compounds as defined above for the inventive nucleic acid sequence as vehicle, transfection or complexation agent.

The inventive pharmaceutical composition can additionally contain one or more auxiliary substances in order to increase its immunogenicity or immunostimulatory capacity, if desired. A synergistic action of the inventive nucleic acid as defined herein and of an auxiliary substance, which may be optionally contained in the inventive pharmaceutical composition, is preferably achieved thereby. Depending on the various types of auxiliary substances, various mechanisms can come into consideration in this respect. For example, compounds that permit the maturation of dendritic cells (DCs), for example lipopolysaccharides, TNF-alpha or CD40 ligand, form a first class of suitable auxiliary substances. In general, it is possible to use as auxiliary substance any agent that influences the immune system in the manner of a "danger signal" (LPS, GP96, etc.) or cytokines, such as GM-CFS, which allow an immune response to be enhanced and/or influenced in a targeted manner. Particularly preferred auxiliary substances are cytokines, such as monokines, lymphokines, interleukins or chemokines, that further promote the innate immune response, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, G-CSF, M-CSF, LT-beta or TNF-alpha, growth factors, such as hGH.

Further additives which may be included in the inventive pharmaceutical composition are emulsifiers, such as, for example, Tween®; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; taste-imparting agents, pharmaceutical carriers; tablet-forming agents; stabilizers; antioxidants; preservatives.

The inventive pharmaceutical composition can also additionally contain any further compound, which is known to be immunostimulating due to its binding affinity (as ligands) to human Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, or due to its binding affinity (as ligands) to murine Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13.

The inventive pharmaceutical composition may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic,

intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques.

Preferably, the inventive pharmaceutical composition may be administered by parenteral injection, more preferably by subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or via infusion techniques. Particularly preferred is intradermal and intramuscular injection. Sterile injectable forms of the inventive pharmaceutical compositions may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents.

The inventive pharmaceutical composition as defined herein may also be administered orally in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions.

The inventive pharmaceutical composition may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, e.g. including diseases of the skin or of any other accessible epithelial tissue. Suitable topical formulations are readily prepared for each of these areas or organs. For topical applications, the inventive pharmaceutical composition may be formulated in a suitable ointment, containing the inventive nucleic acid as defined herein suspended or dissolved in one or more carriers.

The inventive pharmaceutical composition typically comprises a "safe and effective amount" of the components of the inventive pharmaceutical composition, particularly of the inventive nucleic acid as defined herein. As used herein, a "safe and effective amount" means an amount of the inventive nucleic acid as defined herein as such that is sufficient to significantly induce a positive modification of a disease or disorder as defined herein. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects and to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment.

The inventive pharmaceutical composition may be used for human and also for veterinary medical purposes, preferably for human medical purposes, as a pharmaceutical composition in general or as a vaccine.

According to another particularly preferred embodiment, the inventive pharmaceutical composition (or the inventive nucleic acid as defined herein) may be provided or used as a vaccine. Typically, such a vaccine is as defined above for pharmaceutical compositions. Additionally, such a vaccine typically contains the inventive nucleic acid as defined herein, which preferably encodes an antigen as defined above. Alternatively, such a vaccine may contain the inventive nucleic acid as defined herein and additional an antigen, preferably as a protein or peptide or as a nucleic acid encoding an antigen, e.g. as defined herein, or as any antigenic format as defined herein or all possible combinations thereof.

The inventive vaccine may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined herein for the inventive pharmaceutical composition. In the specific context of the inventive vaccine, the choice of a pharmaceutically acceptable carrier is determined in principle by the manner in which the inventive vaccine is administered. The inventive vaccine can be administered, for example, systemically or locally. Routes for systemic administration in general include, for example, transdermal, oral, parenteral routes, including subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal injections and/or intranasal administration routes. Routes for local administration in general include, for example, topical administration routes but also intradermal, transdermal, subcutaneous, or intramuscular injections or intralesional, intracranial, intrapulmonal, intracardial, and sublingual injections. More preferably, vaccines may be administered by an intradermal, subcutaneous, or intramuscular route. Inventive vaccines are therefore preferably formulated in liquid (or sometimes in solid) form.

The inventive vaccine can additionally contain one or more auxiliary substances in order to increase its immunogenicity or immunostimulatory capacity, if desired. Particularly preferred are adjuvants as auxiliary substances or additives as defined for the pharmaceutical composition.

The present invention furthermore provides several applications and uses of the inventive nucleic acid as defined herein, the inventive pharmaceutical composition, the inventive vaccine, both comprising the inventive nucleic acid as defined herein or of kits comprising same.

According to one specific embodiment, the present invention is directed to the first medical use of the inventive nucleic acid as defined herein as a medicament, preferably as an immunostimulating agent, adjuvant or vaccine or in the field of gene therapy.

According to another embodiment, the present invention is directed to the second medical use of the nucleic acid as defined herein, for the treatment of diseases as defined herein, preferably to the use of inventive nucleic acid as defined herein, of a pharmaceutical composition or vaccine comprising same or of kits comprising same for the preparation of a medicament for the prophylaxis, treatment and/or amelioration of various diseases as defined herein. Preferably, the pharmaceutical composition or a vaccine is used or to be administered to a patient in need thereof for this purpose.

Preferably, diseases as mentioned herein are selected from cancer or tumour diseases, infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, diseases which have a genetic inherited background and which are typically caused by a defined gene defect and are inherited according to Mendel's laws, cardiovascular diseases, neuronal diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, neoplasms, immune deficiencies, endocrine, nutritional and metabolic diseases, eye diseases, ear diseases and any disease which can be influenced by the present invention.

Cancer or tumour diseases as mentioned above preferably include e.g. colon carcinomas, melanomas, renal carcinomas, lymphomas, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), gastrointestinal tumours, pulmonary carcinomas, gliomas, thyroid tumours, mammary carcinomas, prostate tumours, hepatomas, various virus-induced

tumours such as, for example, papilloma virus-induced carcinomas (e.g. cervical carcinoma), adenocarcinomas, herpes virus-induced tumours (e.g. Burkitt's lymphoma, EBV-induced B-cell lymphoma), heptatitis B-induced tumours (hepatocell carcinoma), HTLV-1- and HTLV-2-induced lymphomas, acoustic neuromas/neurinomas, cervical cancer, lung cancer, pharyngeal cancer, anal carcinomas, glioblastomas, lymphomas, rectal carcinomas, astrocytomas, brain tumours, stomach cancer, retinoblastomas, basaliomas, brain metastases, medulloblastomas, vaginal cancer, pancreatic cancer, testicular cancer, melanomas, thyroidal carcinomas, bladder cancer, Hodgkin's syndrome, meningiomas, Schneeberger disease, bronchial carcinomas, hypophysis tumour, Mycosis fungoides, oesophageal cancer, breast cancer, carcinoids, neurinomas, spinaliomas, Burkitt's lymphomas, laryngeal cancer, renal cancer, thymomas, corpus carcinomas, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumours, oligodendrogliomas, vulval cancer, intestinal cancer, colon carcinomas, oesophageal carcinomas, wart involvement, tumours of the small intestine, craniopharyngeomas, ovarian carcinomas, soft tissue tumours/sarcomas, ovarian cancer, liver cancer, pancreatic carcinomas, cervical carcinomas, endometrial carcinomas, liver metastases, penile cancer, tongue cancer, gall bladder cancer, leukaemia, plasmocytomas, uterine cancer, lid tumour, prostate cancer, etc.

Additionally, in the above context, infectious diseases are preferably selected from influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, Leishmaniasis, anthrax, meningitis, viral infectious diseases such as AIDS, Condyloma acuminata, hollow warts, Dengue fever, three-day fever, Ebola virus, cold, early summer meningoencephalitis (FSME), flu, shingles, hepatitis, herpes simplex type I, herpes simplex type II, Herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot-and-mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (childhood lameness), pseudo-croup, fifth disease, rabies, warts, West Nile fever, chickenpox, cytomegalic virus (CMV), from bacterial infectious diseases such as miscarriage (prostate inflammation), anthrax, appendicitis, borreliosis, botulism, Camphylobacter, Chlamydia trachomatis (inflammation of the urethra, conjunctivitis), cholera, diphtheria, donavanosis, epiglottitis, typhus fever, gas gangrene, gonorrhoea, rabbit fever, Heliobacter pylori, whooping cough, climatic bubo, osteomyelitis, Legionnaire's disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, otitis

media, Mycoplasma hominis, neonatal sepsis (Chorioamnionitis), noma, paratyphus, plague, Reiter's syndrome, Rocky Mountain spotted fever, Salmonella paratyphus, Salmonella typhus, scarlet fever, syphilis, tetanus, tripper, tsutsugamushi disease, tuberculosis, typhus, vaginitis (colpitis), soft chancre, and from infectious diseases caused by parasites, protozoa or fungi, such as amoebiasis, bilharziosis, Chagas disease, athlete's foot, yeast fungus spots, scabies, malaria, onchocercosis (river blindness), or fungal diseases, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral Leishmaniosis, nappy/diaper dermatitis, schistosomiasis, fish poisoning (Ciguatera), candidosis, cutaneous Leishmaniosis, lambliasis (giardiasis), or sleeping sickness, or from infectious diseases caused by Echinococcus, fish tapeworm, fox tapeworm, canine tapeworm, lice, bovine tapeworm, porcine tapeworm, miniature tapeworm.

Furthermore, in the above context, allergies normally result in a local or systemic inflammatory response to these antigens or allergens and leading to immunity in the body against these allergens. Allergens in this context include e.g. grasses, pollens, molds, drugs, or numerous environmental triggers, etc. Without being bound to theory, several different disease mechanisms are supposed to be involved in the development of allergies. According to a classification scheme by P. Gell and R. Coombs the word "allergy" was restricted to type I hypersensitivities, which are caused by the classical IgE mechanism. Type I hypersensitivity is characterised by excessive activation of mast cells and basophils by IgE, resulting in a systemic inflammatory response that can result in symptoms as benign as a runny nose, to life-threatening anaphylactic shock and death. Well known types of allergies include, without being limited thereto, allergic asthma (leading to swelling of the nasal mucosa), allergic conjunctivitis (leading to redness and itching of the conjunctiva), allergic rhinitis ("hay fever"), anaphylaxis, angiodema, atopic dermatitis (eczema), urticaria (hives), eosinophilia, allergies to insect stings, skin allergies (leading to and including various rashes, such as eczema, hives (urticaria) and (contact) dermatitis), food allergies, allergies to medicine, etc. With regard to the present invention, e.g. an inventive nucleic acid, pharmaceutical composition or vaccine is provided, which encodes or contains an allergen (e.g. from a cat allergen, a dust allergen, a mite antigen, a plant antigen (e.g. a birch antigen) etc.) either as a protein, a nucleic acid encoding that protein allergen in combination with a nucleic acid of the invention as defined above or as an inventive nucleic acid. A pharmaceutical composition of the present invention may shift the

(exceeding) immune response to a stronger TH1 response, thereby suppressing or attenuating the undesired IgE response.

Additionally, autoimmune diseases can be broadly divided into systemic and organ-specific or localised autoimmune disorders, depending on the principal clinico-pathologic features of each disease. Autoimmune disease may be divided into the categories of systemic syndromes, including SLE, Sjögren's syndrome, Scleroderma, Rheumatoid Arthritis and polymyositis or local syndromes which may be endocrinologic (DM Type 1, Hashimoto's thyroiditis, Addison's disease etc.), dermatologic (pemphigus vulgaris), haematologic (autoimmune haemolytic anaemia), neural (multiple sclerosis) or can involve virtually any circumscribed mass of body tissue. The autoimmune diseases to be treated may be selected from the group consisting of type I autoimmune diseases or type II autoimmune diseases or type III autoimmune diseases or type IV autoimmune diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, type I diabetes (Diabetes mellitus), systemic lupus erythematosus (SLE), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, type I allergy diseases, type II allergy diseases, type III allergy diseases, type IV allergy diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, Myasthenia gravis, neurodermitis, Polymyalgia rheumatica, progressive systemic sclerosis (PSS), psoriasis, Reiter's syndrome, rheumatic arthritis, psoriasis, vasculitis, etc, or type II diabetes. While the exact mode as to why the immune system induces an immune reaction against autoantigens has not been elucidated so far, there are several findings with regard to the etiology. Accordingly, the autoreaction may be due to a T-Cell Bypass. A normal immune system requires the activation of B-cells by T-cells before the former can produce antibodies in large quantities. This requirement of a T-cell can be by-passed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of B-cells, or even of T-cells, by directly binding to one subunit of T-cell receptors in a non-specific fashion. Another explanation deduces autoimmune diseases from a molecular mimicry. An exogenous antigen may share structural similarities with certain host antigens; thus, any antibody produced against this antigen (which mimics the self-antigens) can also, in theory, bind to the host antigens and amplify the immune response. The most striking form of molecular mimicry is observed in Group A beta-haemolytic streptococci, which shares antigens with human myocardium, and is responsible for the cardiac manifestations of

rheumatic fever. The present invention allows therefore provision of a nucleic acid or pharmaceutical composition or vaccine as defined herein encoding or containing e.g. an autoantigen (as protein, mRNA or DNA encoding an autoantigen protein) which typically allows the immune system to be desensitized.

According to an additional embodiment, the present invention is directed to the second medical use of the inventive nucleic acid as defined herein, for the treatment of diseases as defined herein by means of gene therapy.

In a further preferred embodiment, the inventive nucleic acid may be used for the preparation of a pharmaceutical composition or a vaccine, particularly for purposes as defined herein.

The inventive pharmaceutical composition or vaccine may furthermore be used for the treatment of a disease or a disorder as defined herein.

According to a final embodiment, the present invention also provides kits, particularly kits of parts. Such kits, particularly kits of parts, typically comprise as components alone or in combination with further components as defined herein at least one inventive nucleic acid as defined herein, the inventive pharmaceutical composition or vaccine comprising the inventive nucleic acid. The at least one inventive nucleic acid as defined herein, optionally in combination with further components as defined herein, the inventive pharmaceutical composition and/or the inventive vaccine may occur in one or different parts of the kit. As an example, e.g. at least one part of the kit may comprise at least one inventive nucleic acid as defined herein, and at least one further part of the kit at least one other component as defined herein, e.g. at least one other part of the kit may comprise at least one pharmaceutical composition or vaccine or a part thereof, e.g. at least one part of the kit may comprise the inventive nucleic acid as defined herein, at least one further part of the kit at least one other component as defined herein, at least one further part of the kit at least one component of the inventive pharmaceutical composition or vaccine or the inventive pharmaceutical composition or vaccine as a whole, and at least one further part of the kit e.g. at least one antigen, at least one pharmaceutical carrier or vehicle, etc. The kit or kit of parts may furthermore contain technical instructions with information on the administration

and dosage of the inventive nucleic acid, the inventive pharmaceutical composition or the inventive vaccine or of any of its components or parts, e.g. if the kit is prepared as a kit of parts.

In the present invention, if not otherwise indicated, different features of alternatives and embodiments may be combined with each other. Furthermore, the term "comprising" shall not be construed as meaning "consisting of", if not specifically mentioned. However, in the context of the present invention, term "comprising" may be substituted with the term "consisting of", where applicable.

Figures:

The following Figures are intended to illustrate the invention further and shall not be construed to limit the present invention thereto.

Figure 1: shows the histone stem-loop consensus sequence generated from metazoan and protozoan stem loop sequences (as reported by Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308). 4001 histone stem-loop sequences from metazoa and protozoa were aligned and the quantity of the occurring nucleotides is indicated for every position in the stem-loop sequence. The generated consensus sequence representing all nucleotides present in the sequences analyzed is given using the single-letter nucleotide code. In addition to the consensus sequence, sequences are shown representing at least 99%, 95% and 90% of the nucleotides present in the sequences analyzed.

Figure 2: shows the histone stem-loop consensus sequence generated from protozoan stem loop sequences (as reported by Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308). 131 histone stem-loop sequences from protozoa were aligned and the quantity of the occurring nucleotides is indicated for every position in the stem-loop sequence. The generated consensus sequence representing all nucleotides present in the sequences analyzed is given using the single-letter nucleotide code. In addition to the consensus sequence, sequences are shown representing at least 99%, 95% and 90% of the nucleotides present in the sequences analyzed.

Figure 3: shows the histone stem-loop consensus sequence generated from metazoan stem loop sequences (as reported by Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308). 3870 histone stem-loop sequences from metazoa were aligned and the quantity of the occurring nucleotides is indicated for every position in the stem-loop

sequence. The generated consensus sequence representing all nucleotides present in the sequences analyzed is given using the single-letter nucleotide code. In addition to the consensus sequence, sequences are shown representing at least 99%, 95% and 90% of the nucleotides present in the sequences analyzed.

Figure 4: shows the histone stem-loop consensus sequence generated from vertebrate stem loop sequences (as reported by Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308). 1333 histone stem-loop sequences from vertebrates were aligned and the quantity of the occurring nucleotides is indicated for every position in the stem-loop sequence. The generated consensus sequence representing all nucleotides present in the sequences analyzed is given using the single-letter nucleotide code. In addition to the consensus sequence, sequences are shown representing at least 99%, 95% and 90% of the nucleotides present in the sequences analyzed.

Figure 5: shows the histone stem-loop consensus sequence generated from human (Homo sapiens) stem loop sequences (as reported by Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308). 84 histone stem-loop sequences from humans were aligned and the quantity of the occurring nucleotides is indicated for every position in the stem-loop sequence. The generated consensus sequence representing all nucleotides present in the sequences analyzed is given using the single-letter nucleotide code. In addition to the consensus sequence, sequences are shown representing at least 99%, 95% and 90% of the nucleotides present in the sequences analyzed.

Figures 6 to 17: show mRNAs from *in vitro* transcription.

Given are the designation and the sequence of mRNAs obtained by *in vitro* transcription. The following abbreviations are used:

ppLuc (GC): GC-enriched mRNA sequence coding for *Photinus pyralis* luciferase

ag:	3' untranslated region (UTR) of the alpha globin gene
A64:	poly(A)-sequence with 64 adenylates
A120:	poly(A)-sequence wit 120 adenylates
histoneSL:	histone stem-loop
aCPSL:	stem loop which has been selected from a library for its specific binding of the αCP-2KL protein
PolioCL:	5' clover leaf from Polio virus genomic RNA
G30:	poly(G) sequence with 30 guanylates
U30:	poly(U) sequence with 30 uridylates
SL:	unspecific/artificial stem-loop
N32:	unspecific sequence of 32 nucleotides

Within the sequences, the following elements are highlighted: ppLuc(GC) ORF (capital letters), ag (bold), histoneSL (underlined), further distinct sequences tested (italic).

Figure 6: shows the mRNA sequence of ppLuc(GC) – ag (SEQ ID NO: 43).
By linearization of the original vector at the restriction site immediately following the alpha-globin 3'-UTR (ag), mRNA is obtained lacking a poly(A) sequence.

Figure 7: shows the mRNA sequence of ppLuc(GC) – ag – A64 (SEQ ID NO: 44).
By linearization of the original vector at the restriction site immediately following the A64 poly(A)-sequence, mRNA is obtained ending with an A64 poly(A) sequence.

Figure 8: shows the mRNA sequence of ppLuc(GC) – ag – histoneSL (SEQ ID NO: 45).
The A64 poly(A) sequence was replaced by a histoneSL. The histone stem-loop sequence used in the examples was obtained from Cakmakci *et al.* (2008). Molecular and Cellular Biology, 28(3), 1182-1194.

Figure 9: shows the mRNA sequence of ppLuc(GC) – ag – A64 – histoneSL (SEQ ID NO: 46).
The histoneSL was appended 3' of A64 poly(A).

Figure 10: shows the mRNA sequence of ppLuc(GC) – ag – A120 (SEQ ID NO: 47).
The A64 poly(A) sequence was replaced by an A120 poly(A) sequence.

Figure 11: shows the mRNA sequence of ppLuc(GC) – ag – A64 – ag (SEQ ID NO: 48). A second alpha-globin 3'-UTR was appended 3' of A64 poly(A).

Figure 12: shows the mRNA sequence of ppLuc(GC) – ag – A64 – aCPSL (SEQ ID NO: 49).
A stem loop was appended 3' of A64 poly(A). The stem loop has been selected from a library for its specific binding of the αCP-2KL protein (Thisted *et al.*, (2001), The Journal of Biological Chemistry, 276(20), 17484-17496). αCP-2KL is an isoform of αCP-2, the most strongly expressed αCP protein (alpha-globin mRNA poly(C) binding protein) (Makeyev *et al.*, (2000), Genomics, 67(3), 301-316), a group of RNA binding proteins, which bind to the alpha-globin 3'-UTR (Chkheidze *et al.*, (1999), Molecular and Cellular Biology, 19(7), 4572-4581).

Figure 13: shows the mRNA sequence of ppLuc(GC) – ag – A64 – PolioCL (SEQ ID NO: 50).
The 5' clover leaf from Polio virus genomic RNA was appended 3' of A64 poly(A).

Figure 14: shows the mRNA sequence of ppLuc(GC) – ag – A64 – G30 (SEQ ID NO: 51)
A stretch of 30 guanylates was appended 3' of A64 poly(A).

Figure 15: shows the mRNA sequence of ppLuc(GC) – ag – A64 – U30 (SEQ ID NO: 52)
A stretch of 30 uridylates was appended 3' of A64 poly(A).

Figure 16: shows the mRNA sequence of ppLuc(GC) – ag – A64 – SL (SEQ ID NO: 53)
A stem loop was appended 3' of A64 poly(A). The upper part of the stem and the loop were taken from (Babendure *et al.*, (2006), RNA (New York, N.Y.), 12(5), 851-861). The stem loop consists of a 17 base pair long, CG-rich stem and a 6 base long loop.

Figure 17: shows ppLuc(GC) – ag – A64 – N32 (SEQ ID NO: 54)

By linearization of the original vector at an alternative restriction site, mRNA is obtained with 32 additional nucleotides following poly(A).

Figure 18: shows that the combination of poly(A) and histoneSL increases protein expression from mRNA in a synergistic manner.

The effect of poly(A) sequence, histoneSL, and the combination of poly(A) and histoneSL on luciferase expression from mRNA was examined. Therefore different mRNAs were electroporated into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after transfection. Little luciferase is expressed from mRNA having neither poly(A) sequence nor histoneSL. Both a poly(A) sequence or the histoneSL increase the luciferase level. Strikingly however, the combination of poly(A) and histoneSL further strongly increases the luciferase level, manifold above the level observed with either of the individual elements, thus acting synergistically. Data are graphed as mean RLU ± SD (relative light units ± standard deviation) for triplicate transfections. Specific RLU are summarized in Example 8.2.

Figure 19: shows that the combination of poly(A) and histoneSL increases protein expression from mRNA irrespective of their order.

The effect of poly(A) sequence, histoneSL, the combination of poly(A) and histoneSL, and their order on luciferase expression from mRNA was examined. Therefore different mRNAs were lipofected into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after the start of transfection. Both an A64 poly(A) sequence or the histoneSL give rise to comparable luciferase levels. Increasing the length of the poly(A) sequence from A64 to A120 or to A300 increases the luciferase level moderately. In contrast, the combination of poly(A) and histoneSL increases the luciferase level much further than lengthening of the poly(A) sequence. The combination of poly(A) and histoneSL acts synergistically as it increases the luciferase level manifold above the level observed with either of the individual elements. The synergistic effect of the combination of poly(A) and histoneSL is seen irrespective of the order of poly(A) and histoneSL and irrespective of the length of poly(A) with A64-histoneSL or histoneSL-A250

mRNA. Data are graphed as mean RLU ± SD for triplicate transfections. Specific RLU are summarized in Example 8.3.

Figure 20: shows that the rise in protein expression by the combination of poly(A) and histoneSL is specific.

The effect of combining poly(A) and histoneSL or poly(A) and alternative sequences on luciferase expression from mRNA was examined. Therefore different mRNAs were electroporated into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after transfection. Both a poly(A) sequence or the histoneSL give rise to comparable luciferase levels. The combination of poly(A) and histoneSL strongly increases the luciferase level, manifold above the level observed with either of the individual elements, thus acting synergistically. In contrast, combining poly(A) with any of the other sequences is without effect on the luciferase level compared to mRNA containing only a poly(A) sequence. Thus, the combination of poly(A) and histoneSL acts specifically and synergistically. Data are graphed as mean RLU ± SD for triplicate transfections. Specific RLU are summarized in Example 8.4.

Examples:

The following Examples are intended to illustrate the invention further and shall not be construed to limit the present invention thereto.

1. Generation of histone-stem-loop consensus sequences

Prior to the experiments, histone stem-loop consensus sequences were determined on the basis of metazoan and protozoan histone stem-loop sequences. Sequences were taken from the supplement provided by Lopez *et al.* (Dávila López, M., & Samuelsson, T. (2008), RNA (New York, N.Y.), 14(1), 1-10. doi:10.1261/rna.782308), who identified a large number of natural histone stem-loop sequences by searching genomic sequences and expressed sequence tags. First, all sequences from metazoa and protozoa (4001 sequences), or all sequences from protozoa (131 sequences) or alternatively from metazoa (3870 sequences), or from vertebrates (1333 sequences) or from humans (84 sequences) were grouped and aligned. Then, the quantity of the occurring nucleotides was determined for every position. Based on the tables thus obtained, consensus sequences for the 5 different groups of sequences were generated representing all nucleotides present in the sequences analyzed. In addition, more restrictive consensus sequences were also obtained, increasingly emphasizing conserved nucleotides

2. Preparation of DNA-templates

A vector for *in vitro* transcription was constructed containing a T7 promoter followed by a GC-enriched sequence coding for *Photinus pyralis* luciferase (ppLuc(GC)), the center part of the 3' untranslated region (UTR) of alpha-globin (ag), and a poly(A) sequence. The poly(A) sequence was immediately followed by a restriction site used for linearization of the vector before *in vitro* transcription in order to obtain mRNA ending in an A64 poly(A) sequence. mRNA obtained from this vector accordingly by *in vitro* transcription is designated as „ppLuc(GC) – ag – A64".

Linearization of this vector at alternative restriction sites before *in vitro* transcription allowed to obtain mRNA either extended by additional nucleotides 3' of A64 or

lacking A64. In addition, the original vector was modified to include alternative sequences. In summary, the following mRNAs were obtained from these vectors by *in vitro* transcription (mRNA sequences are given in Figures 6 to 17):

ppLuc(GC) – ag	(SEQ ID NO: 43)
ppLuc(GC) – ag – A64	(SEQ ID NO: 44)
ppLuc(GC) – ag – histoneSL	(SEQ ID NO: 45)
ppLuc(GC) – ag – A64 – histoneSL	(SEQ ID NO: 46)
ppLuc(GC) – ag – A120	(SEQ ID NO: 47)
ppLuc(GC) – ag – A64 – ag	(SEQ ID NO: 48)
ppLuc(GC) – ag – A64 – aCPSL	(SEQ ID NO: 49)
ppLuc(GC) – ag – A64 – PolioCL	(SEQ ID NO: 50)
ppLuc(GC) – ag – A64 – G30	(SEQ ID NO: 51)
ppLuc(GC) – ag – A64 – U30	(SEQ ID NO: 52)
ppLuc(GC) – ag – A64 – SL	(SEQ ID NO: 53)
ppLuc(GC) – ag – A64 – N32	(SEQ ID NO: 54)

3. In vitro transcription

The DNA-template according to Example 2 was linearized and transcribed *in vitro* using T7-Polymerase. The DNA-template was then digested by DNase-treatment. All mRNA-transcripts contained a 5'-CAP structure obtained by adding an excess of N7-Methyl-Guanosine-5'-Triphosphate-5'-Guanosine to the transcription reaction. mRNA thus obtained was purified and resuspended in water.

4. Enzymatic adenylation of mRNA

Two mRNAs were enzymatically adenylated:

ppLuc(GC) – ag – A64 and ppLuc(GC) – ag – histoneSL.

To this end, RNA was incubated with *E. coli* Poly(A)-polymerase and ATP (Poly(A) Polymerase Tailing Kit, Epicentre, Madison, USA) following the manufacturer's instructions. mRNA with extended poly(A) sequence was purified and resuspended in water. The length of the poly(A) sequence was determined via agarose gel electrophoresis. Starting mRNAs were extended by approximately 250 adenylates, the mRNAs obtained are designated as

ppLuc(GC) – ag – A300 and ppLuc(GC) – ag – histoneSL – A250, respectively.

5. Luciferase expression by mRNA electroporation

HeLa cells were trypsinized and washed in opti-MEM. $1x10^5$ cells in 200 µl of opti-MEM each were electroporated with 0.5 µg of ppLuc-encoding mRNA. As a control, mRNA not coding for ppLuc was electroporated separately. Electroporated cells were seeded in 24-well plates in 1 ml of RPMI 1640 medium. 6, 24, or 48 hours after transfection, medium was aspirated and cells were lysed in 200 µl of lysis buffer (25 mM Tris, pH 7.5 (HCl), 2 mM EDTA, 10% glycerol, 1% Triton X-100, 2 mM DTT, 1 mM PMSF). Lysates were stored at -20°C until ppLuc activity was measured.

6. Luciferase expression by mRNA lipofection

HeLa cells were seeded in 96 well plates at a density of $2x10^4$ cells per well. The following day, cells were washed in opti-MEM and then transfected with 0.25 µg of Lipofectin-complexed ppLuc-encoding mRNA in 150 µl of opti-MEM. As a control, mRNA not coding for ppLuc was lipofected separately. In some wells, opti-MEM was aspirated and cells were lysed in 200 µl of lysis buffer 6 hours after the start of transfection. In the remaining wells, opti-MEM was exchanged for RPMI 1640 medium at that time. In these wells, medium was aspirated and cells were lysed in 200 µl of lysis buffer 24 or 48 hours after the start of transfection. Lysates were stored at -20°C until ppLuc activity was measured.

7. Luciferase measurement

ppLuc activity was measured as relative light units (RLU) in a BioTek SynergyHT plate reader at 5 seconds measuring time using 50 µl of lysate and 200 µl of luciferin buffer (25 mM Glycylglycin, pH 7.8 (NaOH), 15 mM $MgSO_4$, 2 mM ATP, 75 µM luciferin). Specific RLU were calculated by subtracting RLU of the control RNA from total RLU.

8. RESULTS

8.1 Histone stem-loop sequences:

In order to characterize histone stem-loop sequences, sequences from metazoa and protozoa (4001 sequences), or from protozoa (131 sequences) or alternatively from metazoa (3870 sequences), or from vertebrates (1333 sequences) or from humans (84 sequences) were grouped and aligned. Then, the quantity of the occurring

nucleotides was determined for every position. Based on the tables thus obtained, consensus sequences for the 5 different groups of sequences were generated representing all nucleotides present in the sequences analyzed. Within the consensus sequence of metazoa and protozoa combined, 3 nucleotides are conserved, a T/U in the loop and a G and a C in the stem, forming a base pair. Structurally, typically a 6 base-pair stem and a loop of 4 nucleotides is formed. However, deviating structures are common: Of 84 human histone stem-loops, two contain a stem of only 5 nucleotides comprising 4 base-pairs and one mismatch. Another human histone stem-loop contains a stem of only 5 base-pairs. Four more human histone stem-loops contain a 6 nucleotide long stem, but include one mismatch at three different positions, respectively. Furthermore, four human histone stem-loops contain one wobble base-pair at two different positions, respectively. Concerning the loop, a length of 4 nucleotides seems not to be strictly required, as a loop of 5 nucleotides has been identified in *D. discoideum*.

In addition to the consensus sequences representing all nucleotides present in the sequences analyzed, more restrictive consensus sequences were also obtained, increasingly emphasizing conserved nucleotides. In summary, the following sequences were obtained:

(Cons): represents all nucleotides present
(99%): represents at least 99% of all nucleotides present
(95%): represents at least 95% of all nucleotides present
(90%): represents at least 90% of all nucleotides present

The results of the analysis of histone stem-loop sequences are summarized in the following Tables 1 to 5 (see also Fig. 1 to 5):

Table 1: Metzoan and protozoan histone stem-loop consensus sequence: (based on an alignment of 4001 metazoan and protozoan histone stem-loop sequences) (see also Fig. 1)

						<	<	<	<	<	<	•	•	•	•	>	>	>	>	>	>					
# A	2224	1586	3075	2872	1284	184	0	13	12	9	1	47	59	0	675	3818	195	1596	523	0	14	3727	61	771	2012	2499
# T	172	188	47	205	19	6	0	569	1620	199	3947	3830	3704	4001	182	1	21	15	11	0	179	8	64	557	201	690
# C	1557	2211	875	918	2675	270	0	3394	2342	3783	51	119	227	0	3140	7	50	31	16	4001	3543	154	3870	2636	1744	674
# G	25	16	4	6	23	3541	4001	25	27	10	2	5	11	0	4	175	3735	2359	3451	0	265	112	4	37	43	138
Cons	N*	N*	N	N	N	N	G	N	N	N	N	N	N	T	N	N	N	N	N	C	N	N	N	N*	N*	N*
99%	H*	H*	H	H	V	V	G	Y	Y	Y	Y	H	H	T	H	R	V	V	R	C	B	V	H	H*	N*	N*
95%	M*	H*	M	H	M	S	G	Y	Y	Y	T	T	Y	T	M	A	R	R	R	C	S	M	C	H*	H*	H*
90%	M*	M*	M	M	M	S	G	Y	Y	C	T	T	T	T	M	A	G	R	R	C	S	A	C	H*	M*	H*

Table 2: Protozoan histone stem-loop consensus sequence: (based on an alignment of 131 protozoan histone stem-loop sequences) (see also Fig. 2)

						<	<	<	<	<	<	•	•	•	•	>	>	>	>	>	>					
# A	52	32	71	82	76	13	0	12	12	9	1	46	3	0	75	82	53	79	20	0	4	94	17	35	74	56
# T	20	32	37	21	8	3	0	21	85	58	86	70	65	131	28	1	17	13	10	0	15	7	31	32	20	28
# C	45	59	20	25	38	0	0	86	8	54	42	13	58	0	27	2	6	31	10	131	112	5	82	58	30	40
# G	14	8	3	3	9	115	131	12	26	10	2	2	5	0	1	46	55	8	91	0	0	25	1	6	7	7
Cons	N*	N*	N	N	N	D	G	N	N	N	N	N	N	T	N	N	N	N	N	C	H	N	N	N*	N*	N*
99%	N*	N*	N	N	N	D	G	N	N	N	B	N	N	T	H	V	N	N	N	C	H	N	H	N*	N*	N*
95%	N*	N*	H	H	N	R	G	N	N	N	Y	H	B	T	H	R	D	N	N	C	Y	D	H	H*	N*	N*
90%	N*	H*	H	H	V	R	G	N	D	B	Y	H	Y	T	H	R	D	H	N	C	Y	R	H	H*	H*	H*

Table 3: Metazoan histone stem-loop consensus sequence: (based on an alignment of 3870 (including 1333 vertebrate sequences) metazoan histone stem-loop sequences) (see also Fig. 3)

						<	<	<	<	<	<	•	•	•	•	>	>	>	>	>	>					
# A	2172	1554	3004	2790	1208	171	0	1	0	0	0	1	56	0	600	3736	142	1517	503	0	10	3633	44	736	1938	2443
# T	152	156	10	184	11	3	0	548	1535	141	3861	3760	3639	3870	154	0	4	2	1	0	164	1	33	525	181	662
# C	1512	2152	855	893	2637	270	0	3308	2334	3729	9	106	169	0	3113	5	44	0	6	3870	3431	149	3788	2578	1714	634
# G	11	8	1	3	14	3426	3870	13	1	0	0	3	6	0	3	129	3680	2351	3360	0	265	87	3	31	36	131
Cons	N*	N*	N	N	N	N	G	N	B	Y	Y	N	N	T	N	V	N	D	N	C	N	N	N	N*	N*	N*
99%	H*	H*	M	H	M	V	G	Y	Y	Y	T	Y	H	T	H	R	V	R	R	C	B	V	M	H*	H*	N*
95%	M*	M*	M	M	M	S	G	Y	Y	C	T	T	Y	T	M	A	G	R	R	C	S	M	C	H*	H*	H*
90%	M*	M*	M	M	M	S	G	Y	Y	C	T	T	T	T	M	A	G	R	R	C	S	A	C	H*	M*	H*

Table 4: Vertebrate histone stem-loop consensus sequence: (based on an alignment of 1333 vertebrate histone stem-loop sequences) (see also Fig. 4)

						<	<	<	<	<	<	•	•	•	•	>	>	>	>	>	>					
# A	661	146	1315	1323	920	8	0	1	0	0	0	1	4	0	441	1333	0	1199	21	0	1	1126	26	81	380	960
# T	63	121	2	2	6	2	0	39	1217	2	1331	1329	1207	1333	30	0	1	0	1	0	2	1	22	91	91	12
# C	601	1062	16	6	403	1	0	1293	116	1331	2	0	121	0	862	0	2	0	0	1333	1328	128	1284	1143	834	361
# G	8	4	0	2	4	1322	1333	0	0	0	0	3	1	0	0	0	1330	134	1311	0	2	78	1	18	28	0
Cons	N*	N*	H	N	N	N	G	H	Y	Y	Y	D	N	T	H	A	B	R	D	C	N	N	N	N*	N*	H*
99%	H*	H*	M	A	M	G	G	Y	Y	C	T	T	Y	T	H	A	G	R	R	C	C	V	H	N*	N*	M*
95%	H*	H*	A	A	M	G	G	C	Y	C	T	T	Y	T	M	A	G	R	G	C	C	V	C	H*	H*	M*
90%	M*	M*	A	A	M	G	G	C	T	C	T	T	T	T	M	A	G	R	G	C	C	M	C	Y*	M*	M*

Table 5: *Homo sapiens* histone stem-loop consensus sequence: (based on an alignment of 84 human histone stem-loop sequences) (see also Fig. 5)

						<	<	<	<	<	<	•	•	•	•	>	>	>	>	>	>					
# A	10	17	84	84	76	1	0	1	0	0	0	1	0	0	12	84	0	65	3	0	0	69	5	0	10	64
# T	8	6	0	0	2	2	0	1	67	0	84	80	81	84	5	0	0	0	0	0	0	0	4	25	24	3
# C	62	61	0	0	6	0	0	82	17	84	0	0	3	0	67	0	1	0	0	84	84	5	75	57	44	17
# G	4	0	0	0	0	81	84	0	0	0	0	3	0	0	0	0	83	19	81	0	0	10	0	2	6	0
Cons	N*	H*	A	A	H	D	G	H	Y	C	T	D	Y	T	H	A	S	R	R	C	C	V	H	B*	N*	H*
99%	N*	H*	A	A	H	D	G	H	Y	C	T	D	Y	T	H	A	S	R	R	C	C	V	H	B*	N*	H*
95%	H*	H*	A	A	M	G	G	C	Y	C	T	T	T	T	H	A	G	R	G	C	C	V	M	Y*	N*	M*
90%	H*	M*	A	A	A	G	G	C	Y	C	T	T	T	T	M	A	G	R	G	C	C	R	M	Y*	H*	M*

Wherein the used abbreviations were defined as followed:

abbreviation	Nucleotide bases	remark
G	G	Guanine
A	A	Adenine
T	T	Thymine
U	U	Uracile
C	C	Cytosine
R	G or A	Purine
Y	T/U or C	Pyrimidine
M	A or C	Amino
K	G or T/U	Keto
S	G or C	Strong (3H bonds)
W	A or T/U	Weak (2H bonds)
H	A or C or T/U	Not G
B	G or T/U or C	Not A
V	G or C or A	Not T/U
D	G or A or T/U	Not C
N *	G or C or T/U or A present or not	Any base Base may be present or not

8.2 **The combination of poly(A) and histoneSL increases protein expression from mRNA in a synergistic manner.**

To investigate the effect of the combination of poly(A) and histoneSL on protein expression from mRNA, mRNAs with different sequences 3′ of the alpha-globin 3′-UTR were synthesized: mRNAs either ended just 3′ of the 3′-UTR, thus lacking both poly(A) sequence and histoneSL, or contained either an A64 poly(A) sequence or a histoneSL instead, or both A64 poly(A) and histoneSL 3′ of the 3′-UTR. Luciferase-encoding mRNAs or control mRNA were electroporated into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after transfection (see following Table 6 and Figure 18).

Table 6:

mRNA	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
ppLuc(GC)-ag-A64-histoneSL	466553	375169	70735
ppLuc(GC)-ag-histoneSL	50947	3022	84
ppLuc(GC)-ag-A64	10471	19529	4364
ppLuc(GC)-ag	997	217	42

Little luciferase was expressed from mRNA having neither poly(A) sequence nor histoneSL. Both a poly(A) sequence or the histoneSL increased the luciferase level to a similar extent. Either mRNA gave rise to a luciferase level much higher than did mRNA lacking both poly(A) and histoneSL. Strikingly however, the combination of poly(A) and histoneSL further strongly increased the luciferase level, manifold above the level observed with either of the individual elements. The magnitude of the rise in luciferase level due to combining poly(A) and histoneSL in the same mRNA demonstrates that they are acting synergistically.

The synergy between poly(A) and histoneSL was quantified by dividing the signal from poly(A)-histoneSL mRNA (+/+) by the sum of the signals from histoneSL mRNA (-/+) plus poly(A) mRNA (+/-) (see following Table 7).

Table 7:

	A64	histoneSL	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
	+	+	466553	375169	70735
	—	+	50947	3022	84
	+	—	10471	19529	4364
Synergy			7.6	16.6	15.9

The factor thus calculated specifies how much higher the luciferase level from mRNA combining poly(A) and histoneSL is than would be expected if the effects of poly(A) and histoneSL were purely additive. The luciferase level from mRNA combining poly(A) and histoneSL was up to 16.6 times higher than if their effects were purely additive. This result confirms that the combination of poly(A) and histoneSL effects a markedly synergistic increase in protein expression.

8.3 **The combination of poly(A) and histoneSL increases protein expression from mRNA irrespective of their order.**

The effect of the combination of poly(A) and histoneSL might depend on the length of the poly(A) sequence and the order of poly(A) and histoneSL. Thus, mRNAs with increasing poly(A) sequence length and mRNA with poly(A) and histoneSL in reversed order were synthesized: Two mRNAs contained 3′ of the 3′-UTR either an

A120 or an A300 poly(A) sequence. One further mRNA contained 3' of the 3'-UTR first a histoneSL followed by an A250 poly(A) sequence. Luciferase-encoding mRNAs or control mRNA were lipofected into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after the start of transfection (see following Table 8 and Figure 19).

Table 8:

mRNA	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
ppLuc(GC)-ag-histoneSL-A250	98472	734222	146479
ppLuc(GC)-ag-A64-histoneSL	123674	317343	89579
ppLuc(GC)-ag-histoneSL	7291	4565	916
ppLuc(GC)-ag-A300	4357	38560	11829
ppLuc(GC)-ag-A120	4371	45929	10142
ppLuc(GC)-ag-A64	1928	26781	537

Both an A64 poly(A) sequence or the histoneSL gave rise to comparable luciferase levels. In agreement with the previous experiment did the combination of A64 and histoneSL strongly increase the luciferase level, manifold above the level observed with either of the individual elements. The magnitude of the rise in luciferase level due to combining poly(A) and histoneSL in the same mRNA demonstrates that they are acting synergistically. The synergy between A64 and histoneSL was quantified as before based on the luciferase levels of A64-histoneSL, A64, and histoneSL mRNA (see following Table 9). The luciferase level from mRNA combining A64 and histoneSL was up to 61.7 times higher than if the effects of poly(A) and histoneSL were purely additive.

Table 9:

	A64	histoneSL	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
	+	+	123674	317343	89579
	—	+	7291	4565	916
	+	—	1928	26781	537
Synergy			13.4	10.1	61.7

In contrast, increasing the length of the poly(A) sequence from A64 to A120 or to A300 increased the luciferase level only moderately (see Table 8 and Figure 19). mRNA with the longest poly(A) sequence, A300, was also compared to mRNA in which a poly(A) sequence of similar length was combined with the histoneSL, histoneSL-A250. In addition to having a long poly(A) sequence, the order of histoneSL and poly(A) is reversed in this mRNA relative to A64-histoneSL mRNA. The combination of A250 and histoneSL strongly increased the luciferase level, manifold above the level observed with either histoneSL or A300. Again, the synergy between A250 and histoneSL was quantified as before comparing RLU from histoneSL-A250 mRNA to RLU from A300 mRNA plus histoneSL mRNA (see following Table 10). The luciferase level from mRNA combining A250 and histoneSL was up to 17.0 times higher than if the effects of poly(A) and histoneSL were purely additive.

Table 10:

	histoneSL	A250/A300	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
	+	+	98472	734222	146479
	+	—	7291	4565	916
	—	+	4357	38560	11829
Synergy			8.5	17.0	11.5

In summary, a highly synergistic effect of the combination of histoneSL and poly(A) on protein expression from mRNA has been demonstrated for substantially different lengths of poly(A) and irrespective of the order of poly(A) and histoneSL.

8.4 The rise in protein expression by the combination of poly(A) and histoneSL is specific

To investigate whether the effect of the combination of poly(A) and histoneSL on protein expression from mRNA is specific, mRNAs with alternative sequences in combination with poly(A) were synthesized: These mRNAs contained 3′ of A64 one of seven distinct sequences, respectively. Luciferase-encoding mRNAs or control mRNA were electroporated into HeLa cells. Luciferase levels were measured at 6, 24, and 48 hours after transfection (see following Table 11 and Figure 20).

Table 11:

mRNA	RLU at 6 hours	RLU at 24 hours	RLU at 48 hours
ppLuc(GC)-ag-A64-N32	33501	38979	2641
ppLuc(GC)-ag-A64-SL	28176	20364	874
ppLuc(GC)-ag-A64-U30	41632	54676	3408
ppLuc(GC)-ag-A64-G30	46763	49210	3382
ppLuc(GC)-ag-A64-PolioCL	46428	26090	1655
ppLuc(GC)-ag-A64-aCPSL	34176	53090	3338
ppLuc(GC)-ag-A64-ag	18534	18194	989
ppLuc(GC)-ag-A64-histoneSL	282677	437543	69292
ppLuc(GC)-ag-histoneSL	27597	3171	0
ppLuc(GC)-ag-A64	14339	48414	9357

Both a poly(A) sequence or the histoneSL gave rise to comparable luciferase levels. Again, the combination of poly(A) and histoneSL strongly increased the luciferase level, manifold above the level observed with either of the individual elements, thus acting synergistically. In contrast, combining poly(A) with any of the alternative sequences was without effect on the luciferase level compared to mRNA containing only a poly(A) sequence. Thus, the combination of poly(A) and histoneSL increases protein expression from mRNA in a synergistic manner, and this effect is specific.

Claims

1. Nucleic acid sequence comprising or coding for
   a) a coding region, preferably encoding a peptide or protein; provided that the coding region does not code for histone proteins, reporter proteins selected from EGFP and Luciferase and marker or selection proteins selected from alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT),
   b) at least one histone stem-loop, and
   c) a poly(A) sequence or a polyadenylation signal.

2. Nucleic acid according to claim 1, wherein the nucleic acid is an RNA, preferably an mRNA.

3. Nucleic acid according to any of claims 1 or 2, 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):

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

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.

4. Nucleic acid according to claim 3, 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)

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

bordering element bordering element

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

5. Nucleic acid according to any of claims 1 to 4, wherein the poly(A) sequence comprises a sequence of about 25 to about 400 adenosine nucleotides, preferably a sequence of about 50 to about 400 adenosine nucleotides, more preferably a sequence of about 50 to about 300 adenosine nucleotides, even more preferably a sequence of about 50 to about 250 adenosine nucleotides, most preferably a sequence of about 60 to about 250 adenosine nucleotides.

6. Nucleic acid according to any of claims 1 to 4, wherein the polyadenylation signal comprises the consensus sequence NNUANA, preferably AAUAAA or AUUAAA.

7. Nucleic acid according to any of claims 1 to 6, wherein the nucleic acid encodes a therapeutically active protein or peptide, an adjuvant protein, an antigen, a tumour antigen, a pathogenic antigen, an animal antigen, a viral antigen, a protozoal antigen, a bacterial antigen, an allergenic antigen, an autoimmune antigen, an allergen, an antibody, an immunostimulatory protein or peptide, or an antigen-specific T-cell receptor.

8. Nucleic acid according to any of claims 1 to 7, wherein the nucleic acid is a monocistronic, bicistronic or even multicistronic nucleic acid.

9. Use of a nucleic acid as defined according to any of claims 1 to 8 for increasing the expression level of an encoded peptide or protein.

10. Nucleic acid as defined according to any of claims 1 to 8 for use as a medicament.

11. Pharmaceutical composition comprising a nucleic acid as defined according to any of claims 1 to 8 and optionally a pharmaceutically acceptable carrier.

12. Vaccine comprising a nucleic acid according to any of claims 1-8 coding for an antigen or comprising an antigen.

13. Vaccine according to claim 12, wherein the antigen is selected from tumour antigens, allergenic antigens, auto-immune self-antigens, pathogenic antigens, viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens, or allergenic antigens.

14. Use of a nucleic acid as defined according to any of claims 1 to 8 for increasing the expression of a protein in treatment of cancer or tumour diseases, infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, diseases which have a genetic inherited background and which are typically caused by a defined gene defect and are inherited according to Mendel's laws, cardiovascular diseases, neuronal diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, neoplasms, immune deficiencies, endocrine, nutritional and metabolic diseases, eye diseases or ear diseases.

15. *In vitro* or *ex vivo* method for increasing the expression of a protein comprising the steps:

a) providing the nucleic acid as defined according to any of claims 1 to 8,

b) applying or administering the nucleic acid to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism.

#A	#T	#C	#G	Cons	99%	95%	90%	
2224	172	1557	25	N*	H*	M*	M*	
1586	188	2211	16	N*	H*	H*	M*	
3075	47	875	4	N	H	M	M	
2872	205	918	6	N	H	H	M	
1284	19	2675	23	N	V	M	M	
184	6	270	3541	N	V	S	S	<
0	0	0	4001	G	G	G	G	<
13	569	3394	25	N	Y	Y	Y	<
12	1620	2342	27	N	Y	Y	Y	<
9	199	3783	10	N	Y	Y	C	<
1	3947	51	2	N	Y	T	T	<
47	3830	119	5	N	H	T	T	•
59	3704	227	11	N	H	Y	T	•
0	4001	0	0	T	T	T	T	•
675	182	3140	4	N	H	M	M	•
3818	1	7	175	N	R	A	A	>
195	21	50	3735	N	V	R	G	>
1596	15	31	2359	N	V	R	R	>
523	11	16	3451	N	R	R	R	>
0	0	4001	0	C	C	C	C	>
14	179	3543	265	N	B	S	S	>
3727	8	154	112	N	V	M	A	
61	64	3870	4	N	H	C	C	
771	557	2636	37	N*	H*	H*	H*	
2012	201	1744	43	N*	N*	H*	M*	
2499	690	674	138	N*	N*	H*	H*	

Stem 1

Loop

Stem 2

Figure 1

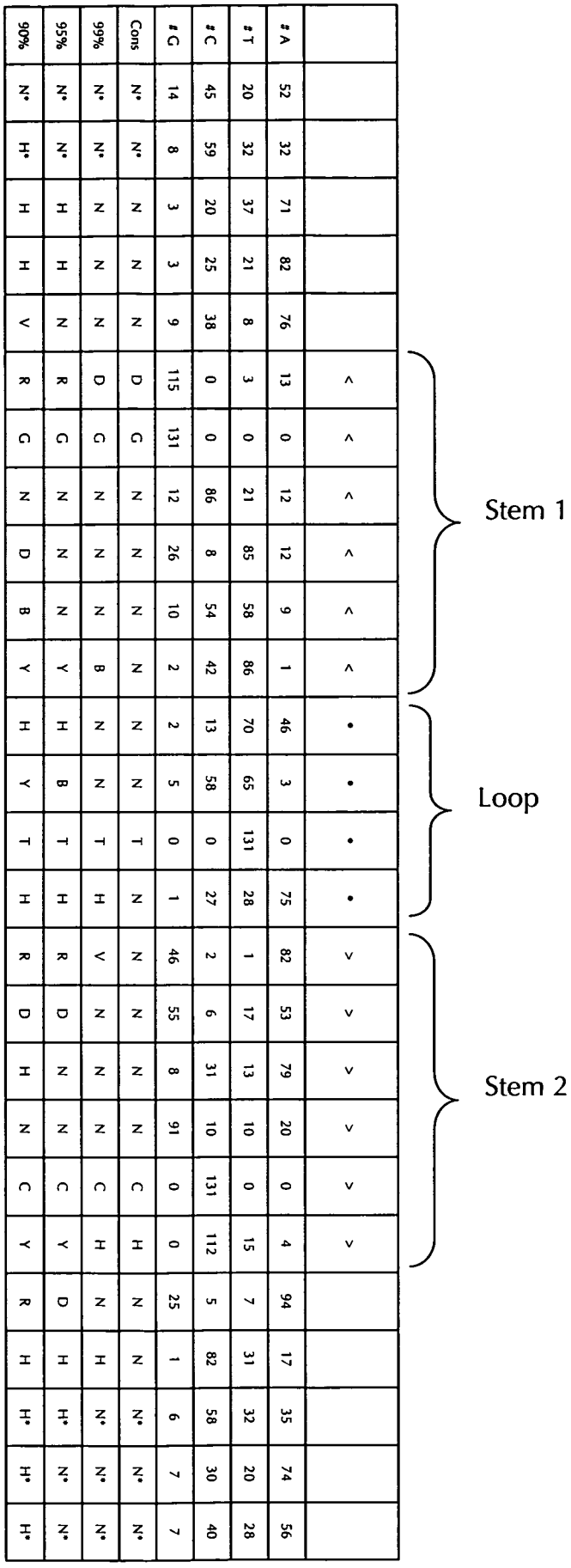

Figure 2

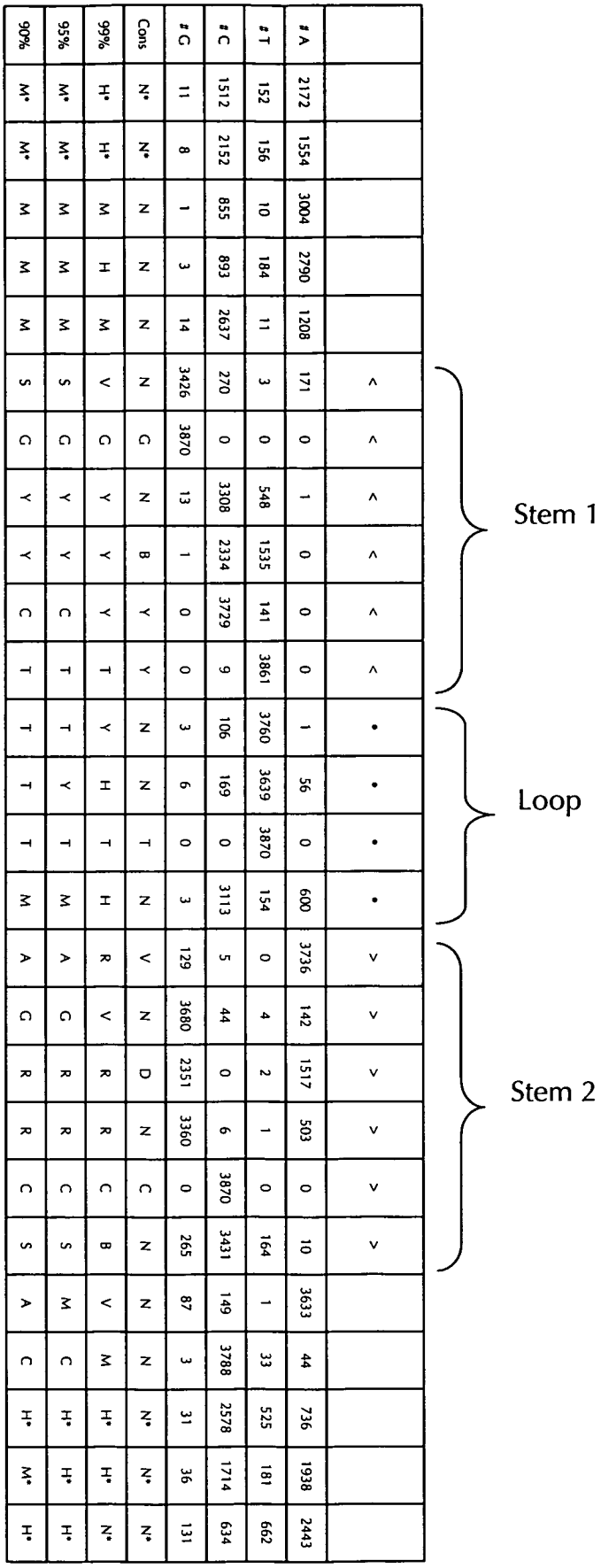

Figure 3

	# A	# T	# C	# G	Cons	99%	95%	90%
	661	63	601	8	N*	H*	H*	M*
	146	121	1062	4	N*	H*	H*	M*
	1315	2	16	0	H	M	A	A
	1323	2	6	2	N	A	A	A
	920	6	403	4	N	M	M	M
<	8	2	1	1322	N	G	G	G
<	0	0	0	1333	G	G	G	G
<	1	39	1293	0	H	Y	C	C
<	0	1217	116	0	Y	Y	Y	T
<	0	2	1331	0	Y	C	C	C
<	0	1331	2	0	Y	T	T	T
•	1	1329	0	3	D	T	T	T
•	4	1207	121	1	N	Y	Y	T
•	0	1333	0	0	T	T	T	T
•	441	30	862	0	H	H	M	M
>	1333	0	0	0	A	A	A	A
>	0	1	2	1330	B	G	G	G
>	1199	0	0	134	R	R	R	R
>	21	1	0	1311	D	R	G	G
>	0	0	0	1333	C	C	C	C
>	1	2	1	1328	C	C	C	C
	1126	1	128	78	N	V	V	M
	26	22	1284	1	N	H	C	C
	81	91	1143	18	N*	N*	H*	Y*
	380	91	834	28	N*	N*	H*	M*
	960	12	361	0	M*	M*	M*	M*

Stem 1

Loop

Stem 2

Figure 4

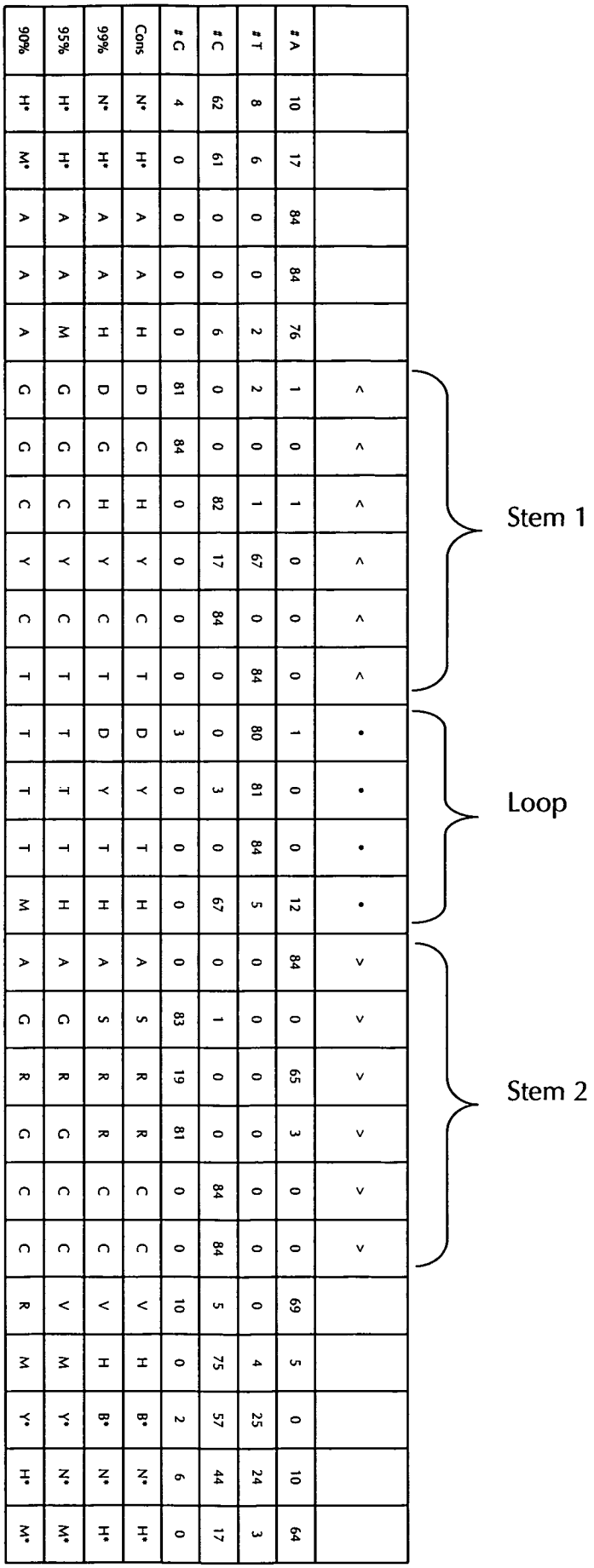

Figure 5

ppLuc(GC) – ag

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCUCCUUGCACCG**agauua
auagauc-3′

Figure 6

ppLuc(GC) – ag – A64

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA-3′

Figure 7

ppLuc(GC) – ag – histoneSL

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auagaucuCAAAGGCUCUUUUCAGAGCCACCA-3′

Figure 8

ppLuc(GC) – ag – A64 – histoneSL

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcauCAAAGGCUCUUUUCAGAGCCACCA-3′

Figure 9

ppLuc(GC) – ag – A120

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCUCCUUGCACCG**agauua
auagaucuAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAA-3′

Figure 10

ppLuc(GC) – ag – A64 – ag

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau**CCUGCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**3′

Figure 11

ppLuc(GC) – ag – A64 – aCPSL

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau*CAAUUCCUACACGUGAGGCGCUGUGAUUCCCUAUCCCCCUUCAUUCCCU*
AUACAUUAGCACAGCGCCAUUGCAUGUAGGAAUU–3′

Figure 12

ppLuc(GC) – ag – A64 – PolioCL

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau*CAAUUCUAAAACAGCUCUGGGGUUGUACCCACCCCAGAGGCCCACGUGG*
CGGCUAGUACUCCGGUAUUGCGGUACCCUUGUACGCCUGUUUUAGAAUU–3′

Figure 13

ppLuc(GC) – ag – A64 – G30

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau*GGGGGGGGGGGGGGGGGGGGGGGGGGGG*-3′

Figure 14

ppLuc(GC) – ag – A64 – U30

gggagaaagcuugaggAUGGAGGACGCCAAGAACATCAAGAAGGGCCCGGCGCCCTTCTA
CCCGCTGGAGGACGGGACCGCCGGCGAGCAGCTCCACAAGGCCATGAAGCGGTACGCCCT
GGTGCCGGGCACGATCGCCTTCACCGACGCCCACATCGAGGTCGACATCACCTACGCGGA
GTACTTCGAGATGAGCGTGCGCCTGGCCGAGGCCATGAAGCGGTACGGCCTGAACACCAA
CCACCGGATCGTGGTGTGCTCGGAGAACAGCCTGCAGTTCTTCATGCCGGTGCTGGGCGC
CCTCTTCATCGGCGTGGCCGTCGCCCCGGCGAACGACATCTACAACGAGCGGGAGCTGCT
GAACAGCATGGGGATCAGCCAGCCGACCGTGGTGTTCGTGAGCAAGAAGGGCCTGCAGAA
GATCCTGAACGTGCAGAAGAAGCTGCCCATCATCCAGAAGATCATCATCATGGACAGCAA
GACCGACTACCAGGGCTTCCAGTCGATGTACACGTTCGTGACCAGCCACCTCCCGCCGGG
CTTCAACGAGTACGACTTCGTCCCGGAGAGCTTCGACCGGGACAAGACCATCGCCCTGAT
CATGAACAGCAGCGGCAGCACCGGCCTGCCGAAGGGGGTGGCCCTGCCGCACCGGACCGC
CTGCGTGCGCTTCTCGCACGCCCGGGACCCCATCTTCGGCAACCAGATCATCCCGGACAC
CGCCATCCTGAGCGTGGTGCCGTTCCACCACGGCTTCGGCATGTTCACGACCCTGGGCTA
CCTCATCTGCGGCTTCCGGGTGGTCCTGATGTACCGGTTCGAGGAGGAGCTGTTCCTGCG
GAGCCTGCAGGACTACAAGATCCAGAGCGCGCTGCTCGTGCCGACCCTGTTCAGCTTCTT
CGCCAAGAGCACCCTGATCGACAAGTACGACCTGTCGAACCTGCACGAGATCGCCAGCGG
GGGCGCCCCGCTGAGCAAGGAGGTGGGCGAGGCCGTGGCCAAGCGGTTCCACCTCCCGGG
CATCCGCCAGGGCTACGGCCTGACCGAGACCACGAGCGCGATCCTGATCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGTGGGCAAGGTGGTCCCGTTCTTCGAGGCCAAGGTGGTGGA
CCTGGACACCGGCAAGACCCTGGGCGTGAACCAGCGGGGCGAGCTGTGCGTGCGGGGGCC
GATGATCATGAGCGGCTACGTGAACAACCCGGAGGCCACCAACGCCCTCATCGACAAGGA
CGGCTGGCTGCACAGCGGCGACATCGCCTACTGGGACGAGGACGAGCACTTCTTCATCGT
CGACCGGCTGAAGTCGCTGATCAAGTACAAGGGCTACCAGGTGGCGCCGGCCGAGCTGGA
GAGCATCCTGCTCCAGCACCCCAACATCTTCGACGCCGGCGTGGCCGGGCTGCCGGACGA
CGACGCCGGCGAGCTGCCGGCCGCGGTGGTGGTGCTGGAGCACGGCAAGACCATGACGGA
GAAGGAGATCGTCGACTACGTGGCCAGCCAGGTGACCACCGCCAAGAAGCTGCGGGGCGG
CGTGGTGTTCGTGGACGAGGTCCCGAAGGGCCTGACCGGGAAGCTCGACGCCCGGAAGAT
CCGCGAGATCCTGATCAAGGCCAAGAAGGGCGGCAAGATCGCCGTGTAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugca*UUUUUUUUUUUUUUUUUUUUUUUUUUUUUU*-3′

Figure 15

ppLuc(GC) – ag – A64 – SL

gggagaaagcuugaggAUGGAGGACGCCAAGAACAUCAAGAAGGGCCCGGCGCCCUUCUA
CCCGCUGGAGGACGGGACCGCCGGCGAGCAGCUCCACAAGGCCAUGAAGCGGUACGCCCU
GGUGCCGGGCACGAUCGCCUUCACCGACGCCCACAUCGAGGUCGACAUCACCUACGCGGA
GUACUUCGAGAUGAGCGUGCGCCUGGCCGAGGCCAUGAAGCGGUACGGCCUGAACACCAA
CCACCGGAUCGUGGUGUGCUCGGAGAACAGCCUGCAGUUCUUCAUGCCGGUGCUGGGCGC
CCUCUUCAUCGGCGUGGCCGUCGCCCCGGCGAACGACAUCUACAACGAGCGGGAGCUGCU
GAACAGCAUGGGGAUCAGCCAGCCGACCGUGGUGUUCGUGAGCAAGAAGGGCCUGCAGAA
GAUCCUGAACGUGCAGAAGAAGCUGCCCAUCAUCCAGAAGAUCAUCAUCAUGGACAGCAA
GACCGACUACCAGGGCUUCCAGUCGAUGUACACGUUCGUGACCAGCCACCUCCCGCCGGG
CUUCAACGAGUACGACUUCGUCCCGGAGAGCUUCGACCGGGACAAGACCAUCGCCCUGAU
CAUGAACAGCAGCGGCAGCACCGGCCUGCCGAAGGGGGUGGCCCUGCCGCACCGGACCGC
CUGCGUGCGCUUCUCGCACGCCCGGGACCCCAUCUUCGGCAACCAGAUCAUCCCGGACAC
CGCCAUCCUGAGCGUGGUGCCGUUCCACCACGGCUUCGGCAUGUUCACGACCCUGGGCUA
CCUCAUCUGCGGCUUCCGGGUGGUCCUGAUGUACCGGUUCGAGGAGGAGCUGUUCCUGCG
GAGCCUGCAGGACUACAAGAUCCAGAGCGCGCUGCUCGUGCCGACCCUGUUCAGCUUCUU
CGCCAAGAGCACCCUGAUCGACAAGUACGACCUGUCGAACCUGCACGAGAUCGCCAGCGG
GGGCGCCCCGCUGAGCAAGGAGGUGGGCGAGGCCGUGGCCAAGCGGUUCCACCUCCCGGG
CAUCCGCCAGGGCUACGGCCUGACCGAGACCACGAGCGCGAUCCUGAUCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGUGGGCAAGGUGGUCCCGUUCUUCGAGGCCAAGGUGGUGGA
CCUGGACACCGGCAAGACCCUGGGCGUGAACCAGCGGGGCGAGCUGUGCGUGCGGGGGCC
GAUGAUCAUGAGCGGCUACGUGAACAACCCGGAGGCCACCAACGCCCUCAUCGACAAGGA
CGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCAUCGU
CGACCGGCUGAAGUCGCUGAUCAAGUACAAGGGCUACCAGGUGGCGCCGGCCGAGCUGGA
GAGCAUCCUGCUCCAGCACCCCAACAUCUUCGACGCCGGCGUGGCCGGGCUGCCGGACGA
CGACGCCGGCGAGCUGCCGGCCGCGGUGGUGGUGCUGGAGCACGGCAAGACCAUGACGGA
GAAGGAGAUCGUCGACUACGUGGCCAGCCAGGUGACCACCGCCAAGAAGCUGCGGGGCGG
CGUGGUGUUCGUGGACGAGGUCCCGAAGGGCCUGACCGGGAAGCUCGACGCCCGGAAGAU
CCGCGAGAUCCUGAUCAAGGCCAAGAAGGGCGGCAAGAUCGCCGUGUAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau*UAUGGCGGCCGUGUCCACCACGGAUAUCACCGUGGUGGACGCGGCC*-3′

Figure 16

ppLuc(GC) – ag – A64 – N32

gggagaaagcuugaggAUGGAGGACGCCAAGAACATCAAGAAGGGCCCGGCGCCCTTCTA
CCCGCTGGAGGACGGGACCGCCGGCGAGCAGCTCCACAAGGCCATGAAGCGGTACGCCCT
GGTGCCGGGCACGATCGCCTTCACCGACGCCCACATCGAGGTCGACATCACCTACGCGGA
GTACTTCGAGATGAGCGTGCGCCTGGCCGAGGCCATGAAGCGGTACGGCCTGAACACCAA
CCACCGGATCGTGGTGTGCTCGGAGAACAGCCTGCAGTTCTTCATGCCGGTGCTGGGCGC
CCTCTTCATCGGCGTGGCCGTCGCCCCGGCGAACGACATCTACAACGAGCGGGAGCTGCT
GAACAGCATGGGGATCAGCCAGCCGACCGTGGTGTTCGTGAGCAAGAAGGGCCTGCAGAA
GATCCTGAACGTGCAGAAGAAGCTGCCCATCATCCAGAAGATCATCATCATGGACAGCAA
GACCGACTACCAGGGCTTCCAGTCGATGTACACGTTCGTGACCAGCCACCTCCCGCCGGG
CTTCAACGAGTACGACTTCGTCCCGGAGAGCTTCGACCGGGACAAGACCATCGCCCTGAT
CATGAACAGCAGCGGCAGCACCGGCCTGCCGAAGGGGGTGGCCCTGCCGCACCGGACCGC
CTGCGTGCGCTTCTCGCACGCCCGGGACCCCATCTTCGGCAACCAGATCATCCCGGACAC
CGCCATCCTGAGCGTGGTGCCGTTCCACCACGGCTTCGGCATGTTCACGACCCTGGGCTA
CCTCATCTGCGGCTTCCGGGTGGTCCTGATGTACCGGTTCGAGGAGGAGCTGTTCCTGCG
GAGCCTGCAGGACTACAAGATCCAGAGCGCGCTGCTCGTGCCGACCCTGTTCAGCTTCTT
CGCCAAGAGCACCCTGATCGACAAGTACGACCTGTCGAACCTGCACGAGATCGCCAGCGG
GGGCGCCCCGCTGAGCAAGGAGGTGGGCGAGGCCGTGGCCAAGCGGTTCCACCTCCCGGG
CATCCGCCAGGGCTACGGCCTGACCGAGACCACGAGCGCGATCCTGATCACCCCCGAGGG
GGACGACAAGCCGGGCGCCGTGGGCAAGGTGGTCCCGTTCTTCGAGGCCAAGGTGGTGGA
CCTGGACACCGGCAAGACCCTGGGCGTGAACCAGCGGGGCGAGCTGTGCGTGCGGGGGCC
GATGATCATGAGCGGCTACGTGAACAACCCGGAGGCCACCAACGCCCTCATCGACAAGGA
CGGCTGGCTGCACAGCGGCGACATCGCCTACTGGGACGAGGACGAGCACTTCTTCATCGT
CGACCGGCTGAAGTCGCTGATCAAGTACAAGGGCTACCAGGTGGCGCCGGCCGAGCTGGA
GAGCATCCTGCTCCAGCACCCCAACATCTTCGACGCCGGCGTGGCCGGGCTGCCGGACGA
CGACGCCGGCGAGCTGCCGGCCGCGGTGGTGGTGCTGGAGCACGGCAAGACCATGACGGA
GAAGGAGATCGTCGACTACGTGGCCAGCCAGGTGACCACCGCCAAGAAGCTGCGGGGCGG
CGTGGTGTTCGTGGACGAGGTCCCGAAGGGCCTGACCGGGAAGCTCGACGCCCGGAAGAT
CCGCGAGATCCTGATCAAGGCCAAGAAGGGCGGCAAGATCGCCGTGTAAgacuaguuaua
agacugacua**GCCCGAUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCG**agauua
auAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAugcau*CCCCCUCUAGACAAUUGGAAUUCCAUA*-3′

Figure 17

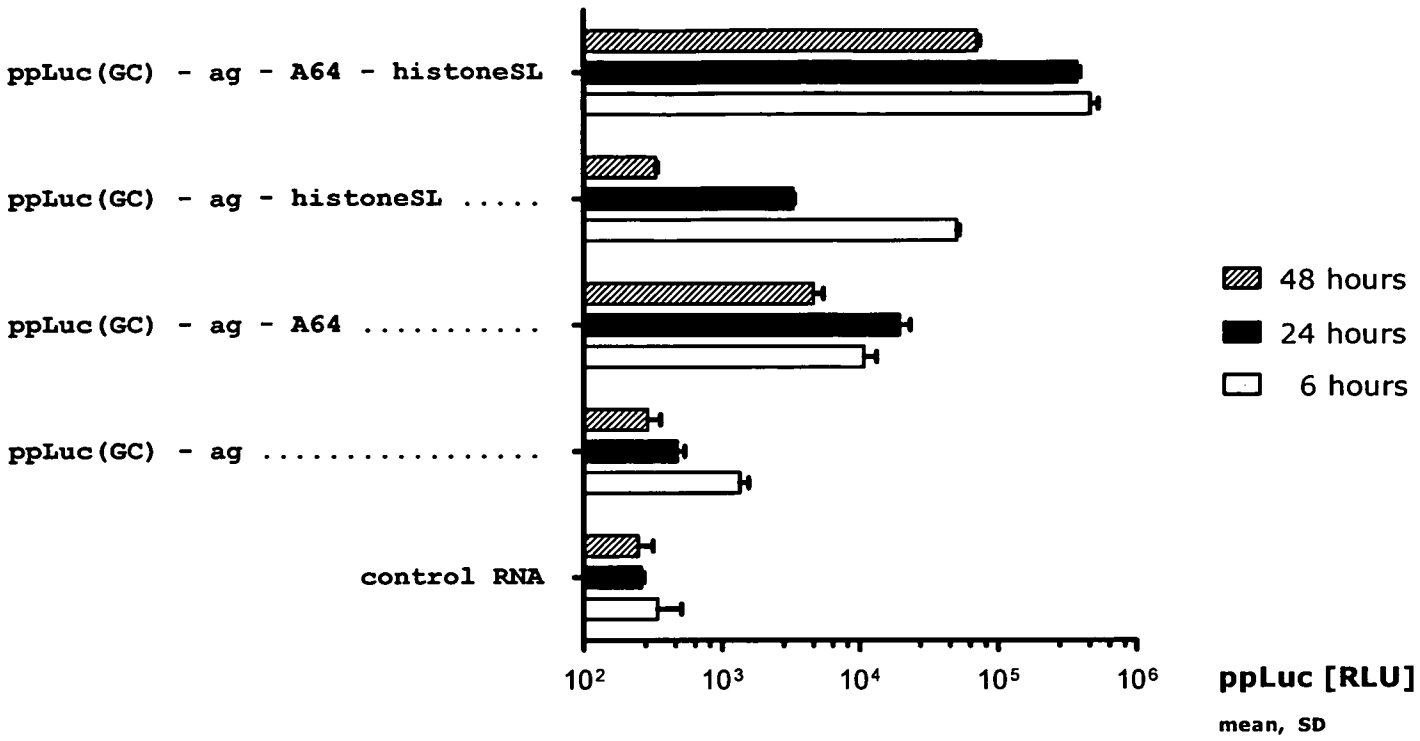
ppLuc(GC) - ag - A64 - histoneSL
ppLuc(GC) - ag - histoneSL .....
ppLuc(GC) - ag - A64 .....
ppLuc(GC) - ag .....
control RNA
48 hours
24 hours
6 hours
10²
10³
10⁴
10⁵
10⁶
ppLuc [RLU]
mean, SD

Figure 18

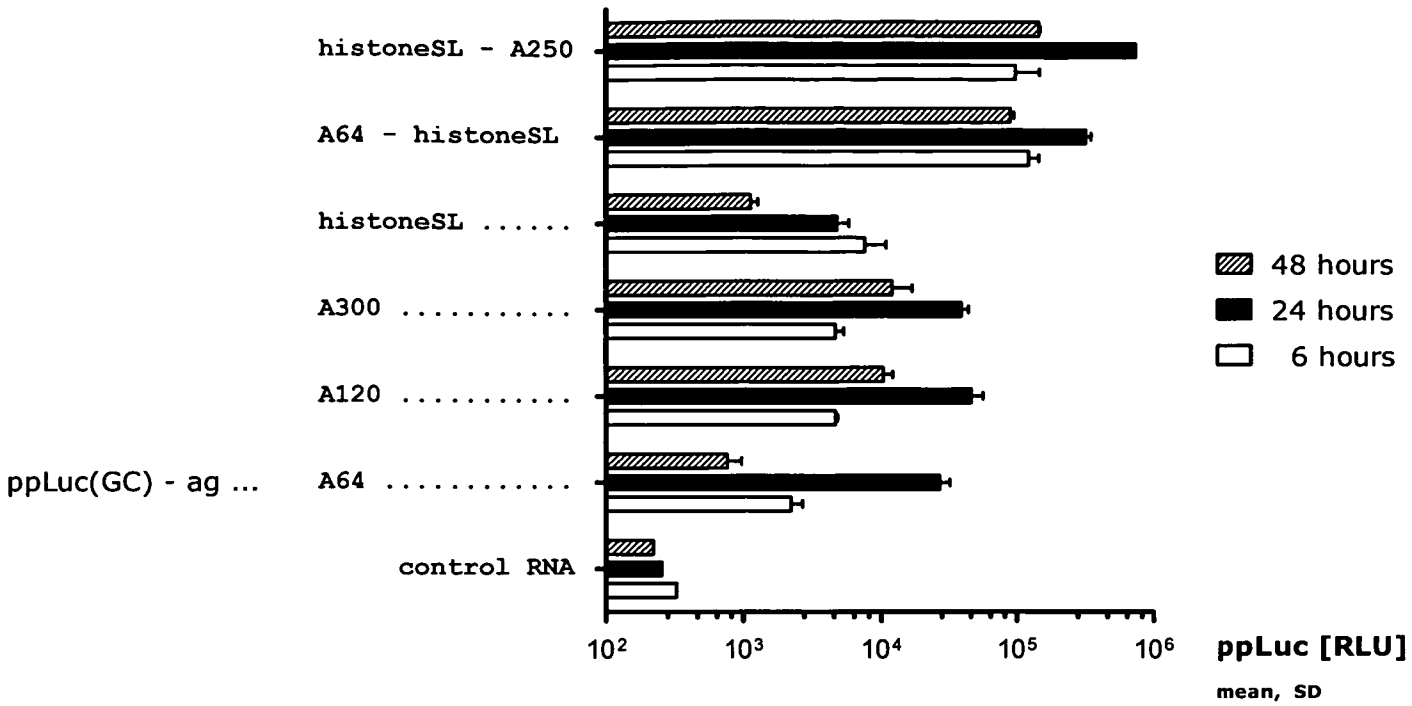
histoneSL - A250
A64 - histoneSL
histoneSL .....
A300 .....
A120 .....
ppLuc(GC) - ag ...
A64 .....
control RNA
48 hours
24 hours
6 hours
10²
10³
10⁴
10⁵
10⁶
ppLuc [RLU]
mean, SD

Figure 19

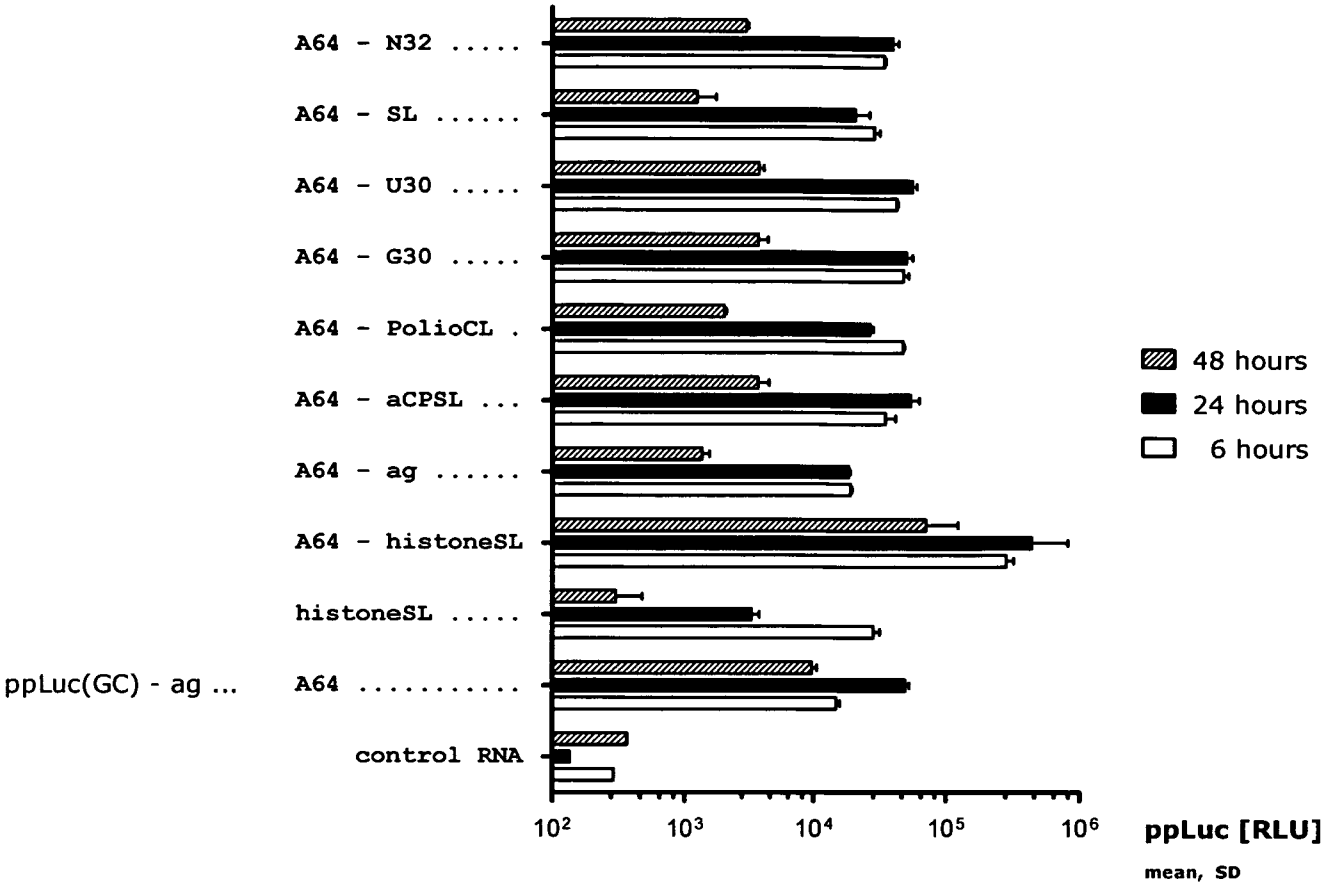
A64 - N32 .....
A64 - SL .....
A64 - U30 .....
A64 - G30 .....
A64 - PolioCL .
A64 - aCPSL ...
A64 - ag .....
A64 - histoneSL
histoneSL .....
ppLuc(GC) - ag ...
A64 .....
control RNA
48 hours
24 hours
6 hours
10²
10³
10⁴
10⁵
10⁶
ppLuc [RLU]
mean, SD

Figure 20

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/004998

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/67
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/172949 A9 (LIU DAKAI [US] ET AL) 26 July 2007 (2007-07-26) * abstract paragraph [0146]; example 2 paragraph [0012] paragraphs [0038], [0049], [0058], [0059]	1-4,7,8, 10
A	WO 95/15394 A1 (UNIV CONNECTICUT [US]) 8 June 1995 (1995-06-08) * abstract page 5, lines 13-28 page 7, lines 11-13	1-15

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2011	28/01/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Montrone, Marco

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/12824 A1 (COMMW SCIENT IND RES ORG [AU]; WANG MING BO [AU]; WATERHOUSE PETER [AU) 22 February 2001 (2001-02-22) * abstract page 6, line 20 - page 7, line 23 page 19, lines 6-16 page 19, line 28 - page 20, line 6 page 22, lines 8-13 page 39, lines 10,11; sequence 7 example 4	1-4,7,8, 10
A	LING JUN ET AL: "The histone 3'-terminal stem-loop-binding protein enhances translation through a functional and physical interaction with eukaryotic initiation factor 4G (eIF4G) and eIF3", MOLECULAR AND CELLULAR BIOLOGY, vol. 22, no. 22, November 2002 (2002-11), pages 7853-7867, XP002616164, ISSN: 0270-7306 * abstract page 7854, column 1, paragraph 2; figure 1 page 7861, column 1, paragraph 2	1-15
A	GALLIE DANIEL R ET AL: "The histone 3'-terminal stem-loop is necessary for translation in Chinese hamster ovary cells", NUCLEIC ACIDS RESEARCH, vol. 24, no. 10, 1996, pages 1954-1962, XP002616165, ISSN: 0305-1048 * abstract page 1954, column 1, paragraph 2 - column 2, paragraph 2 figures 1-3	1-15
X	STAUBER C ET AL: "A SIGNAL REGULATING MOUSE HISTONE H-4 MESSENGER RNA LEVELS IN A MAMMALIAN CELL CYCLE MUTANT AND SEQUENCES CONTROLLING RNA 3' PROCESSING ARE BOTH CONTAINED WITHIN THE SAME 80-BP FRAGMENT", EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 5, no. 12, 1986, pages 3297-3304, XP009141603, ISSN: 0261-4189 * abstract page 3297, column 2, paragraph 4 - page 3298, column 1, paragraph 1; figure 1 page 3301, column 2, paragraph 2-3	1-8

-/--

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANCHEZ RICARDO ET AL: "The oligo(A) tail on histone mRNA plays an active role in translational silencing of histone mRNA during Xenopus oogenesis.", MOLECULAR AND CELLULAR BIOLOGY, vol. 24, no. 6, March 2004 (2004-03), pages 2513-2525, XP002616167, ISSN: 0270-7306 * abstract figure 1 page 2515, column 2, paragraph 5 figure 4 -----	1-8
X	PANDEY N B ET AL: "INTRONS IN HISTONE GENES ALTER THE DISTRIBUTION OF 3' ENDS", NUCLEIC ACIDS RESEARCH, vol. 18, no. 11, 1990, pages 3161-3170, XP1525377, ISSN: 0305-1048 * abstract page 3162, column 2, paragraph 2 - page 3163; figure 1 figure 5 -----	1-8

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/004998

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [X] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [X] subsequently to this Authority for the purpose of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/004998

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007172949 A9	26-07-2007	US 2004038405 A1	26-02-2004
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