Claims

1. Nucleic acid sequence comprising or coding in 5'→3' direction for

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

or

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

wherein the at least one histone stem-loop in i) or ii) 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;

wherein at least one stem-loop binds to stem-loop binding protein (SLBP); and

wherein said peptide or protein comprises a pathogenic antigen or a fragment thereof having a length of at least six amino acid residues and having the specific antigenic property of the full-length native peptide or protein,

wherein the pathogenic antigen is associated with a bacterial infection, a viral infection, or a protozoan infection and wherein the pathogenic antigen is selected from a pathogenic antigen of the group consisting of

- Respiratory syncytial virus (RSV),
- Human immunodeficiency virus (HIV),
- Herpes simplex virus (HSV),
- Human Papillomavirus (HPV),
- Human parainfluenza virus (HPIV),
- Dengue virus,
- Hepatitis B virus (HBV),
- Influenza virus,
- Yellow fever virus,
- Rabies virus,
- Plasmodium,
- Cytomegalovirus (CMV),Staphylococcus,
- Mycobacterium tuberculosis,
- Chlamydia trachomatis,
- Rotavirus,

- Human metapneumovirus (hMPV),
- Crimean Congo Hemorrhagic Fever Virus (CCHFV),
- Ebola virus,
- Henipavirus,
- Norovirus,
- Lassa virus,
- Coronavirus,
- Rhinovirus,
- Flavivirus,
- Rift Valley Fever Virus, and
- Hand, foot and mouth disease virus.

2. The nucleic acid according to claim 1, wherein the pathogenic antigen is selected from the group consisting of

- HIV p24 antigen, HIV envelope proteins (Gp120, Gp41, Gp160), polyprotein GAG, negative factor protein Nef, trans-activator of transcription Tat, if the infectious disease is HIV, preferably an infection with Human immunodeficiency virus;
- capsid protein C, premembrane protein prM, membrane protein M, envelope protein E (domain I, domain II, domain III), protein NS1, protein NS2A, protein NS2B, protein NS3, protein NS4A, protein 2K, protein NS4B, protein NS5, if the infectious disease is Dengue fever, preferably an infection with Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4)–Flaviviruses;
- hepatitis B surface antigen HBsAg, Hepatitis B core antigen HbcAg, polymerase, protein Hbx, preS2 middle surface protein, surface protein L, large S protein, virus protein VP1, virus protein VP2, virus protein VP3, virus protein VP4, if the infectious disease is Hepatits B, preferably an infection with Hepatitis B Virus (HBV);
- Hemagglutinin (HA), Neuraminidase (NA), Nucleoprotein (NP), M1 protein, M2 protein, NS1 protein, NS2 protein (NEP protein: nuclear export protein), PA protein, PB1 protein (polymerase basic 1 protein), PB1-F2 protein and PB2 protein (Orthomyxoviridae family, Influenza virus (flu));

- nucleoprotein N, large structural protein L, phophoprotein P, matrix protein M, glycoprotein G, if the infectious disease is Rabies, preferably an infection with Rabies virus;
- genome polyprotein, protein E, protein M, capsid protein C, protease NS3, protein NS1, protein NS2A, protein AS2B, protein NS4A, protein NS4B, protein NS5, if the infectious disease is Yellow fever, perferable an infection with Yellow fever virus;
- thrombospondin-related anonymous protein TRAP, SSP2 Sporozoite surface protein 2, apical membrane antigen 1 AMA1, rhoptry membrane antigen RMA1, acidic basic repeat antigen ABRA, cell-traversal protein PF, protein Pvs25, merozoite surface protein 1 MSP-1, merozoite surface protein 2 MSP-2, ring-infected erythrocyte surface antigen RESALiver stage antigen 3 LSA-3, protein Eba-175, serine repeat antigen 5 SERA-5, circumsporozoite protein CS, merozoite surface protein 3 MSP3, merozoite surface protein 8 MSP8, enolase PF10, hepatocyte erythrocyte protein 17 kDa HEP17, erythrocyte membrane protein 1 EMP1, protein Kbetamerozoite surface protein 4/5 MSP 4/5, heat shock protein Hsp90, glutamate-rich protein GLURP, merozoite surface protein 4 MSP-4, protein STARP, circumsporozoite protein-related antigen precursor CRA (Plasmodium genus, Malaria).

3. The nucleic acid sequence according to claim 1, wherein the pathogenic antigen is selected from the group consisting of

- fusion protein F, hemagglutinin-neuramidase HN, glycoprotein G, matrix protein M, phosphoprotein P, nucleoprotein N, polymerase L, if the infectious disease is Human parainfluenza virus infection, preferably an infection with Human parainfluenza viruses (HPIV);
- fusionprotein F, nucleoprotein N, matrix protein M, matrix protein M2-1, matrix protein M2-2, phophoprotein P, small hydrophobic protein SH, major surface glycoprotein G, polymerase L, non-structural protein 1 NS1, non-structural protein 2 NS2, if the infectious disease is Respiratory syncytial virus infection, preferably an infection with Respiratory syncytial virus (RSV);
- glycoprotein L UL1, uracil-DNA glycosylase UL2, protein UL3, protein UL4, DNA replication protein UL5, portal protein UL6, virion maturation protein

UL7, DNA helicase UL8, replication origin-binding protein UL9, glycoprotein M UL10, protein UL11, alkaline exonuclease UL12, serine-threonine protein kinase UL13, tegument protein UL14, terminase UL15, tegument protein UL16, protein UL17, capsid protein VP23 UL18, major capsid protein VP5 UL19, membrane protein UL20, tegument protein UL21, Glycoprotein H (UL22), Thymidine Kinase UL23, protein UL24, protein UL25, capsid protein P40 (UL26, VP24, VP22A), glycoprotein B (UL27), ICP18.5 protein (UL28), major DNA-binding protein ICP8 (UL29), DNA polymerase UL30, nuclear matrix protein UL31, envelope glycoprotein UL32, protein UL33, inner nuclear membrane protein UL34, capsid protein VP26 (UL35), large tegument protein UL36, capsid assembly protein UL37, VP19C protein (UL38), ribonucleotide reductase (Large subunit) UL39, ribonucleotide reductase (Small subunit) UL40, tegument protein/virion host shutoff VHS protein (UL41), DNA polymerase processivity factor UL42, membrane protein UL43, glycoprotein C (UL44), membrane protein UL45, tegument proteins VP11/12 (UL46), tegument protein VP13/14 (UL47), virion maturation protein VP16 (UL48, Alpha-TIF), envelope protein UL49, dUTP diphosphatase UL50, tegument protein UL51, DNA helicase/primase complex protein UL52, glycoprotein K (UL53), transcriptional regulation protein IE63 (ICP27, UL54), protein UL55, protein UL56, viral replication protein ICP22 (IE68, US1), protein US2, serine/threonine-protein kinase US3, glycoprotein G (US4), glycoprotein J (US5), glycoprotein D (US6), glycoprotein I (US7), glycoprotein E (US8), tegument protein US9, capsid/tegument protein US10, Vmw21 protein (US11), ICP47 protein (IE12, US12), major transcriptional activator ICP4 (IE175, RS1), E3 ubiquitin ligase ICP0 (IE110), latency-related protein 1 LRP1, latency-related protein 2 LRP2, neurovirulence factor RL1 (ICP34.5), latency-associated transcript LAT (Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Herpes simplex);

- replication protein E1, regulatory protein E2, protein E3, protein E4, protein E5, protein E6, protein E7, protein E8, major capsid protein L1, minor capsid protein L2 (Human papillomavirus (HPV), Human papillomavirus (HPV) infection);

- pp65 antigen, membrane protein pp15, capsid-proximal tegument protein pp150, protein M45, DNA polymerase UL54, helicase UL105, glycoprotein gM, glycoprotein gN, glcoprotein H, glycoprotein B gB, protein UL83, protein UL94, protein UL99 (Cytomegalovirus (CMV), Cytomegalovirus infection);
- heme-iron binding protein IsdB, collagen adhesin Cna, clumping factor A ClfA, protein MecA, fibronectin-binding protein A FnbA, enterotoxin type A EntA, enterotoxin type B EntB, enterotoxin type C EntC1, enterotoxin type C EntC2, enterotoxin type D EntD, enterotoxin type E EntE, Toxic shock syndrome toxin-1 TSST-1, Staphylokinase, Penicillin binding protein 2a PBP2a (MecA), secretory antigen SssA (Staphylococcus genus e.g. aureus, Staphylococcal infection);
- genome polyprotein, polymerase 3D, viral capsid protein VP1, viral capsid protein VP2, viral capsid protein VP3, viral capsid protein VP4, protease 2A, protease 3C (Rhinovirus, Rhinovirus infection);
- protein ESAT-6 (EsxA), 10 kDa filtrate antigen EsxB, secreted antigen 85-B FBPB, fibronectin-binding protein A FbpA (Ag85A), serine protease PepA, PPE family protein PPE18, fibronectin-binding protein D FbpD, immunogenic protein MPT64, secreted protein MPT51, catalase-peroxidase-peroxynitritase T KATG, periplasmic phosphate-binding lipoprotein PSTS3 (PBP-3, Phos-1), iron-regulated heparin binding hemagglutinin Hbha, PPE family protein PPE14, PPE family protein PPE68, protein Mtb72F, protein Apa, immunogenic protein MPT63, periplasmic phosphate-binding lipoprotein PSTS1 (PBP-1), molecular chaperone DnaK, cell surface lipoprotein Mpt83, lipoprotein P23, phosphate transport system permease protein pstA, 14 kDa antigen, fibronectin-binding protein C FbpC1, Alanine dehydrogenase TB43, Glutamine synthetase 1, ESX-1 protein, protein CFP10, TB10.4 protein, protein MPT83, protein MTB12, protein MTB8, Rpf-like proteins, protein MTB32, protein MTB39, crystallin, heat-shock protein HSP65, protein PST-S (usually Mycobacterium tuberculosis, Tuberculosis);
- nucleoprotein N, polymerase L, matrix protein Z, glycoprotein GP (Lassa virus, Lassa fever);

- sprike proteins S, envelope proteins E, membrane proteins M, nucleocapsid proteins N (usually rhinoviruses and coronaviruses;
- glycoprotein GP, nucleoprotein NP, minor matrix protein VP24, major matrix protein VP40, transcription activator VP30, polymerase cofactor VP35, RNA polymerase L (Ebolavirus (EBOV), Ebola hemorrhagic fever);
- envelope protein Gc, envelope protein Gn, nucleocapsid proteins (Crimean-Congo hemorrhagic fever virus, Crimean-Congo hemorrhagic fever (CCHF));
- glycoprotein G, matrix protein M, nucleoprotein N, fusion protein F, polymerase L, protein W, proteinC, phosphoprotein p, non-structural protein V (Henipavirus (Hendra virus Nipah virus), Henipavirus infections); and
- envelope glycoprotein GP, polymerase L, nucleoprotein N, non-structural protein NSS (Rift Valley fever virus, Rift Valley fever (RVF)).

4. The nucleic acid sequence according to claim 1, wherein the pathogenic antigen is selected from the group consisting of

- non-structural protein 6 NS6, non-structural protein 2 NS2, intermediate capsid protein VP6, inner capsid protein VP2, non-structural protein 3 NS3, RNA-directed RNA polymerase L, protein VP3, non-structural protein 1 NS1, non-structural protein 5 NS5, outer capsid glycoprotein VP7, non-structural glycoprotein 4 NS4, outer capsid protein VP4 (Rotavirus, Rotavirus infection).

5. The nucleic acid sequence according to claim 1, wherein the pathogenic antigen is selected from the group consisting of

- protein NS1, small hydrophobic protein NS2, SH protein, fusion protein F, glycoprotein G, matrix protein M, matrix protein M2-1, matrix protein M2-2, phosphoprotein P, nucleoprotein N, polymerase L (Human metapneumovirus (hMPV)).

6. The nucleic acid according to claims 1 to 5, wherein the at least one histone stem loop is heterologous to the coding region encoding the at least one peptide or

protein, preferably, wherein the coding region does not encode a histone protein or fragment, derivate of variant thereof having histone or histone-like function.

7. Nucleic acid sequence according to any of claims 1 to 6, wherein the nucleic acid is an RNA, preferably an mRNA.

8. Nucleic acid sequence according to any of claims 1 to 7, 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)

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

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

or

one of the following 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):

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

bordering element — bordering element

9. Nucleic acid sequence according to any of claims 1 to 8, 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.

10. A composition comprising a plurality or more than one of nucleic acid sequences each according to any of claims 1 to 9.

11. Nucleic acid sequence as defined according to any of claims 1 to 8 or composition as defined according to claim 10 for use as a medicament or vaccine.

12. Pharmaceutical composition comprising a nucleic acid sequence as defined according to any of claims 1 to 9 or a composition as defined according to claim 10 and optionally a pharmaceutically acceptable carrier.

13. Use of a nucleic acid sequence as defined according to any of claims 1 to 9 or a composition as defined according to claim 10 for increasing the expression of said encoded peptide or protein *in vitro*.

14. An *in vitro or ex vivo* method for increasing the expression of an encoded peptide or protein comprising the steps:

a) providing the nucleic acid sequence as defined according to any of claims 1 to 9 or the composition as defined according to claim 10,

b) applying or administering the nucleic acid sequence or the composition to a cell-free expression system, a cell or a tissue.