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KINDERMANN MAIK ET AL, "COVALENT AND SELECTIVE IMMOBILIZATION OF FUSION PROTEINS", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, ACS PUBLICATIONS, US, (20030702), vol. 125, no. 26, doi:10.1021/JA034145S, ISSN 0002-7863, pages 7810 - 7811

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(54) Title: MULTIPLEX IMMUNO SCREENING ASSAY

(57) Abstract: The present invention provides kits and assay methods for the early detection of pathogens, precise identification of the etiologic agent, and improved disease surveillance. More specifically, the present invention discloses an immunoassay leading to the rapid and simultaneous detection of antibodies to a wide range of infectious pathogens in biological fluids of infected patients. This immunoassay involves the covalent and oriented coupling of fusion proteins comprising an AGT enzyme and a viral antigen on an identifiable solid support (e.g. fluorescent microspheres), said support being previously coated with an AGT substrate. This coupling is mediated by the irreversible reaction of the AGT enzyme on its substrate. The thus obtained antigen-coupled microspheres show enhanced capture of specific antibodies as compared to antigen-coupled microspheres produced by standard amine coupling procedures. The methods of the invention possess the ability to multiplex, minimize the amount of biological sample, and have enhanced sensitivity and specificity toward target antibodies as compared with classical ELISA or Radio-Immunoprecipitation assays.

MULTIPLEX IMMUNO SCREENING ASSAY

Background of the invention

Infectious diseases and viral hemorrhagic fevers (VHFs) pose a significant public health problem, due to the severity of the diseases, high lethality, inter-human contagiousness of certain agents, and lack of effective treatment for most of them.

Some of them are caused by highly infectious RNA viruses from several families including the *Flaviviridae* (dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses) the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) and the *Filoviridae* (Ebola, Marburg). Transmission usually occurs by contact with infected animal reservoirs or arthropod vectors. Although the majority of those viruses have a higher occurrence in the tropics and subtropics, the geographic expansion of their natural reservoirs and vectors, and the increase in international travel have made the emergence of these agents in non-endemic areas highly probable. Control of epidemics crucially depends on the rapid detection and accurate identification of the agent, in order to define and implement timely and appropriate action. In this context, it is essential to produce and validate tools for early detection of outbreaks, precise identification of the etiologic agent, and improved disease surveillance.

In this respect, detection of antibodies in body fluids constitutes a major part of the diagnosis of virally induced diseases, autoimmune diseases and the detection of cancer. As a matter of fact, certain antibodies can serve as markers in diagnosis and can lead to prognosis and treatment, as their presence are known to correlate with the outbreak of a pathogen. This is particularly the case for the antibodies targeting viral antigens exclusively.

Current methods for detecting the presence of antibodies include diverse techniques such as immunofluorescence microscopy, chemiluminescence assay, Western blotting, Radio Immuno-Precipitation assay (RIP) and ELISA. For example, the team of Kim H-J. et al. recently developed a competitive ELISA for the detection of antibodies to Rift Valley Fever virus in goat and cattle (*The Journal of Veterinary Medical Science*, 2011). However, such techniques require measurement of each antibody separately, and thus are not useful for parallel, rapid, and high throughput analysis of multiple antibodies in a single sample of biological fluid. The parallel detection of several antibodies simultaneously may be particularly useful by minimizing the matrix effects that exist between individual assays, such as in ELISAs, because the calibrators and the antibodies are analyzed under the same conditions; it therefore will generate comparable results for the measurement of multiple antibodies present within the same sample.

Complicating the straightforward identification of pathogenically relevant antibodies, however, is that normal sera contain large amounts of natural antibodies which manifest themselves in complex staining patterns (Avrameas S. *Immunol. Today* 1991). The presence of these natural antibodies can complicate the differentiation of disease-associated antibodies from the complex background of "auto-immune noise", i.e. naturally occurring autoantibodies. That's why most of previous studies evaluated one or a few specific disease-related antibodies and have screened only a limited number of purified homologous or heterologous proteins as antigens by means of ELISA or RIA. A diagnosis based on these antibodies was impossible to establish. On the other hand, Western blotting has evolved as the most important tool to detect antibodies because it permits simultaneous screening for a wide spectrum of different antigens. A recent new technique, capable of analyzing these complex staining patterns of Western blots simultaneously, is based on digital image analysis. This technique has been successfully used in studies of myasthenia gravis, Graves' disease and experimental uveitis (Zimmerman CW, *Electrophoresis* 1995). The antibodies may also be detected and measured on a protein chip array using surface-enhanced laser desorption/ionization (SELDI) or matrix assisted laser desorption/ionization mass spectrometry techniques, preferably SELDI mass spectrometry technique (US 2006/166268). Yet, these techniques use large cumbersome equipment that is complex and expensive to maintain, and requires

high amount of the biological samples to achieve the detection of antibodies being in a low amount.

In view of the foregoing, there exists a need for addressable systems and methods, which can provide additional improvements in high throughput, cost-effectiveness, and accuracy for molecular diagnosis of antibody-generating diseases. The present invention satisfies these and other needs.

Summary

The present invention as claimed herein is described in the following items 1 to 21:

1. An *in vitro* assay method for detecting at least two target antibodies in a biological sample comprising:
(a) contacting a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody with the biological sample, wherein said AGT substrate is further covalently coupled to said first solid support;
(b) contacting a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said first target antibody with the biological sample, wherein said AGT substrate is further covalently coupled to said second solid support and
(c) detecting the presence or absence of the two target antibodies.

2. The assay method of item 1, wherein said method is used to detect at least 5, more preferably at least 15 and even more preferably at least 50 target antibodies in a biological sample from a subject.

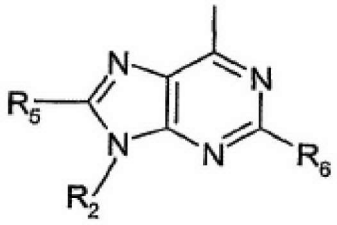
3. The assay method of any one of items 1 to 4, wherein said AGT polypeptide is the SNAP mutant of SEQ ID NO:2.

4. The assay method of any one of items 1 to 3, wherein said substrate of said AGT polypeptide is a O6 benzyl guanine derivative having the formula I:

R1-X-CH_2-R3-R4-Y

wherein:

- R1 is a heteroaromatic group containing 1 to 5 nitrogen atoms, preferably a purine radical of the formula:



wherein R5 is hydrogen, halogen, e. g. chloro or bromo, trifluoromethyl, or hydroxy; R6 is hydrogen, hydroxy or unsubstituted or substituted amino; and R2 is hydrogen, an alkyl of 1 to 10 carbon atoms, or a saccharide moiety;

- X is an oxygen or sulfur atom; preferably an oxygen atom;
- R3 is an aromatic or a heteroaromatic group, or an optionally substituted unsaturated alkyl, cycloalkyl or heterocyclyl group with the double bond connected to CH_2; preferably a phenyl, e.g. a phenyl substituted by R4 in para or meta position,
- R4 is a linker moiety, preferably -CH_2-NH-CO-NH-$[C_2H_4$-O$]_n$-, wherein n is comprised between 1 to 8, preferably 2 to 6;
- Y is a reactive group.

5. The assay method of any one of items 1 to 4, wherein said solid supports can be specifically identify by their specific location, size, diameter, weight, granulometry, and/or labeling.

6. The assay method of any one of items 1 to 5, wherein said solid supports are labeled with a fluorochrome, a chromophore, a radioisotope, and/or a mass tag.

7. The assay method of any one of items 1 to 5, wherein said solid supports are microparticles internally labeled with fluorescent dyes.

8. The assay method of any one of items 1 to 7, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

9. The assay method of any one of items 1 to 8, wherein said first and second fusion proteins that are coupled with said first and second solid supports are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

10. A kit for the detection of at least two target antibodies in a biological sample comprising:

(a) a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody, wherein said AGT substrate is further covalently coupled to said first solid support; and
(b) a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said first target antibody, wherein said AGT substrate is further covalently coupled to said first solid support,

and wherein the said solid supports are mixed together in at least one single compartment.

11. The kit according to item 10, comprising at least 10, preferably at least 50, more preferably at least 100 differently coupled-solid supports.

12. The kit according to any one of items 10 to 11, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

13. The kit according to any one of items 10-12, comprising at least two solid supports coated with at least two fusion proteins that are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

14. Use of the kit as defined in any one of the items 10 to 13, for detecting at least two target antibodies in a biological sample from a subject.

15. Use as defined in item 14**,** for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum.*

16. An *in vitro* method for diagnosing at least one target disease in a subject, said target disease being known to induce the synthesis of at least one target antibody in said subject, comprising performing the assay method as defined in any of the items 1 to 9, wherein said subject is diagnosed to be suffering from said at least one target disease if the amount of said at least one target antibody is higher than a control value.

17. The *in vitro* diagnosis method according to item 16, wherein said method is used to diagnose at least 5, more preferably at least 15 and even more preferably at least 50 viral infections in said subject.

18. The assay method according to any one of items 1 to 9, wherein the first and the second solid supports are obtained by covalently coupling the AGT polypeptide comprised in the fusion protein as defined in item 1 to a first and a second functionalized solid supports, by performing a method comprising the following steps:
a) activating the said functionalized solid support,
b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support,
c) contacting the said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer, DTT being preferably at a concentration of 1mM in the PBS/DTT buffer, wherein unbound molecules are washed out after steps b) and c).

19. A method for manufacturing a kit as defined in any one of items 10 to 13, said method comprising the steps of:
(a) providing a least a first fusion protein comprising :
- a polypeptide comprising a first epitope that is recognized by a first target antibody and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,
(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,
(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,
(d) providing at least a second fusion protein comprising :
- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,
(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,
(f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody,

wherein said at least first and at least second solid supports can be specifically identified from each other.

20. A multiplex immuno screening assay method comprising at least 2, 25, 50, or 96 solid supports as defined in item 1 or 18 and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

21. A multiplex immuno screening assay method comprising:
a) contacting one or several biological sample(s) with at least 2, 25, 50, or 96 solid supports as defined in item 1 or 19 and wherein each of the solid supports emits a different and distinguishable wave length after excitation, and
b) detecting the presence or absence of target antibodies.

Figure legends

Figure 1 represents the oriented coupling of chimeric AGT-antigen proteins to substrate-coated microspheres. First step of coupling consists of coupling the AGT substrate BG-PEG-NH2 to the activated microspheres by amine coupling. The second step consists of contacting the substrate-coated microspheres with fusion proteins containing AGT (for example the SNAP mutant), said enzyme being intended to covalently attach to its BG-PEG-NH2 substrate, that is, to the microspheres.

Figure 2 shows the coupling efficiency of chimeric SNAP-viral antigens proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-ZIKA), as followed by anti-SNAP antibody.

Figure 3 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV2 antibody on chimeric SNAP-DV2.EDIII protein conjugated to microspheres via the substrate of the hAGT protein (coupling of the invention) or coupled through a standard amine coupling procedure, *e.g.* Bio-Plex Amine Coupling Kit, BIORAD.

Figure 4 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV1 antibody on chimeric SNAP-DV1.EDIII protein conjugated to microspheres, either in a singleplex or in a multiplex fomat with other chimeric SNAP-viral Ags proteins (SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE) coupled to microspheres.

Figure 5 shows the reactivity and specificity of the multiplex immunoassay experiment for the detection of dilutions of purified monoclonal anti-WNV antibody on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE) coupled to microspheres.

Figure 6 shows the reactivity and specificity of anti-DV3 IgG detection in mouse polyclonal serum against DV3 (A) and anti-YF IgG detection in mouse polyclonal serum against YF (B) in multiplex immunoassays on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-WSL, SNAP-ROCIO, SNAP-MVE, SNAP-SLE, SNAP-ZIKA) coupled to microspheres

Figure 7 shows the reactivity and specificity of anti-DV1 IgM detection (A) and anti-DV1 IgG detection (B) in DV1-infected serum of a human patient in multiplex immunoassays on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-WSL, SNAP-ROCIO, SNAP-MVE, SNAP-SLE, SNAP-ZIKA, SNAP-TBE) coupled to microspheres.

Figure 8 discloses the structure of the pDeSNAPuniv cassette.

Figure 9 discloses the structure of the pDeSNAPuniv/SBV.N cassette.

Figure 10 shows (A) an immunoblot assay performed on the supernatants of S2/SNAP-SBV.N cells induced for 10 days with Cd^{2+} (+) or non induced (-). The secreted chimeric protein SNAP-SBV.N (theorical MW 50 kDa) was detected using an anti-His_{tag} antibody, in comparison to define amounts of highly purified chimeric protein SNAP-TOS.N (theorical MW 49 kDa). (B) Immunoblot performed on fractions of size-exclusion chromatography column (Coomassie blue staining of PAGE-SDS) corresponding to the final purification step of secreted SNAP+SBV.N protein from induced S2/SNAP+SBV.N cells for 10 days.

Figure 11 shows an example of a device containing the antigen-coated microspheres of the invention.

Detailed description of the invention

The 6-alkylguanine-DNA-alkyltransferase enzyme (AGT, also known as ATase or MGMT, and hereafter referred to as "AGT") is numbered EC 2.1.1.63 in the IUBMB enzyme nomenclature. It is a 6-alkylguanine-DNA-alkyltransferase DNA repair enzyme of 207 amino acid residues whose function in the cells is to repair alkylated DNA. More precisely, AGT acts on O^6-methylated guanine in DNA by irreversibly transferring the methyl group in an S_N2 reaction to a reactive cysteine residue (Cys 145). Recently, a number of O6-benzylguanine derivatives have been shown to irreversibly react with said enzyme by transferring their benzyl group to the active site cysteine of the AGT enzyme (cf. Damoiseaux et al., *ChemBiochem.*, 2001, WO 2004/031404 and WO 2005/085470).

The present inventors have developed and validated immunoassays leading to rapid and simultaneous detection of several antibodies generated by a wide range of diseases, in particular arboviral diseases and VHFs, in biological fluids.

To achieve both optimal sensitivity and specificity for the detection of low amount of antibodies, an oriented antigen coupling procedure has been developed. This oriented antigen coupling procedure is based on the covalent interaction between the AGT enzymes and their substrates, the O6-benzylguanine derivatives, which irreversibly react with AGT enzymes by transferring their benzyl group to the active site cysteine of the enzyme. Accordingly, a number of target antigens can be fused to an AGT enzyme moiety, resulting in different chimeric fusion proteins (hereafter referred to as [AGT-Antigen] fusion proteins), that can be used as capture reagents for the antibodies present in a biological sample. The present inventors have shown that this antibody capture is enhanced when these fusion proteins are bound to solid supports thanks to the specific AGT-substrate interaction. Coating the said solid supports with AGT-substrate is thus an essential step of the immunoassay of the invention.

More precisely, in the context of the invention, the method for coupling antigens to solid supports comprises the two following steps: i) the coating of solid surfaces with an AGT substrate (e.g. BG-PEG-amino), and ii) the covalent immobilization of chimeric [AGT-Antigen] fusion proteins using the AGT substrate as an anchor (see Figure 1). Before

being coated with said AGT substrate, the solid surfaces are advantageously functionalized, preferably by using an optimized two-step carbodiimide process (Kufer SK, *Eur. Biophys.J.* 2005), so that the AGT substrate is covalently attached to the solid surfaces. Once these steps have been performed, the solid surfaces carry AGT substrates that are irreversibly linked to the chimeric [AGT-antigen] fusion proteins. Due to the high specificity of this reaction, the fusion protein is exclusively coupled via the cysteine-containing domain of the AGT enzyme, thus leaving the antigen accessible for its interactions with antibodies.

This coupling procedure is very advantageous as it allows the binding of the antigen in an oriented manner on the solid supports. Also, this antigen coupling procedure advantageously enables to obtain a multimeric antigen organization on a solid surface, so as to enhance immunoglobulin G, and potentially immunoglobulin M, capture efficiency. Consequently, the antigen-coupled microspheres developed in the experimental part of the application have shown enhanced capture of specific antibodies as compared to antigen-coupled microspheres produced by standard non-oriented amine coupling procedures (see the experimental part below and figure 3). Finally, this antigen coupling procedure enables to obtain a high coupling efficiency and a long-term stability of the antigen-conjugated microspheres (>6 months at 4°C).

Importantly, the solid supports used in the immunoassays of the invention should be intrinsically identifiable, so that it is possible to determine precisely which antigen is carried by which solid support. The antigen-coupled and identifiable solid supports are then used as capture reagents for specific human immunoglobulins and are therefore contacted with the biological sample of the patient.

The final step of the method of the invention involves the detection of the solid supports which are effectively bound to immunoglobulins. The identification of immunoglobulin-coated solid support(s) enables to diagnose which pathogen was infecting the patient (as each solid support matches with a defined pathogenic antigen). This final detection step is performed by any usual means, for example by using labeled detection antibodies and by identifying the nature of the solid support.

Advantageously, the method of the invention involves only the detection of the presence of antibodies in diseased patients, but knowledge about the identity of those antibodies is not required.

As shown in the experimental part of the application, the inventors have used the antigen-coupling procedure of the invention to generate a number of different antigen-coated fluorescent microspheres. Presently, 16 distinct sets of microspheres have been coupled with 16 purified chimeric [AGT-Antigen] fusion proteins, allowing titration of 16 serum antibodies specific to different proteins of the dengue serotypes 1 to 4, West Nile, yellow fever, Japanese encephalitis, tick-borne encephalitis, Saint-Louis encephalitis, Murray Valley encephalitis, Wesselsbron, Zika, Rocio, Usutu, Rift Valley fever, and chikungunya virus. These 16 distinct sets of microspheres have been mixed in a single sample without affecting the sensitivity and specificity of the detection (see figure 5). The production of this system is highly time- and cost-effective, as only a very small amount of recombinant antigen (< 50 μg) is required to produce one set of antigen-coupled microspheres (~1.25 x 10^6 microspheres), such set being sufficient to perform 500 individual assays.

In a first aspect, the present invention relates to a method for detecting at least two target antibodies in a biological sample comprising:

(a) contacting a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody with the biological sample;

(b) contacting a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said first target antibody with the biological sample; and

(c) detecting the presence or absence of the two target antibodies.

More precisely, the present invention relates to an *in vitro* assay method for detecting at least two different target antibodies present in a biological sample from a subject, said method comprising the steps of:

(a) providing a first fusion protein comprising :
- a polypeptide comprising a first epitope that is recognized by a first target antibody and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,

(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,

(d) providing a second fusion protein comprising :
- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,

(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody, wherein said first and second solid supports can be specifically identified from each other,

(g) contacting said biological sample with the first and second solid supports obtained in steps (c) and (f),

(h) detecting the presence of said at least two target antibodies.

As used hereafter, the terms "an antibody", "a fusion protein", "an epitope", "an antigen", "an AGT polypeptide", "a solid support" and the like have obviously to be understood as usual in the art, that is, in a broad manner. In particular, they encompass not only particular single molecules but a number of said molecules. For example, the term "solid support" encompasses a subset of numerous identical solid supports, the term "microparticle" encompasses a subset of numerous identical microparticles, and the term "fusion protein" encompasses a number of identical single protein molecules. In the context of the present invention, it is noteworthy that a solid support carries a number of identical fusion proteins, said fusion proteins containing, apart from the AGT polypeptide, identical antigen, and therefore identical epitopes, so that the antibodies which will be detected on the solid support can be unambiguously identified.

As used herein, the term "fusion protein" means a polypeptide containing a protein or a polypeptide created through the artificial joining of two or more polypeptides. In the immunoassays of the invention, said fusion proteins contain a AGT polypeptide and an antigen, containing at least one epitope. Fusion proteins can be obtained through genetic engineering of a fusion gene. This typically involves removing the stop codon from a cDNA sequence coding for the first protein, then appending the cDNA sequence of the second protein in frame through ligation or overlap extension PCR. That DNA sequence will then be expressed by a cell as a single protein. The protein can be engineered to include the full sequence of both original proteins, or only a portion of either. If the two entities are proteins, a linker (or "spacer") peptides can be added, which makes it more likely that the proteins fold independently and behave as expected. In particular, the fusion proteins of the invention can be obtained by providing vectors comprising AGT encoding sequences in frame with an epitope or antigen encoding sequences, either attached to the N-terminal or to the C-terminal side of the AGT DNA sequence. These vectors may be introduced in prokaryotic hosts, including eubacteria such as *E.coli* bacteria, or eukaryotic hosts, e.g., yeast, insect cells or mammalian cells and the recombinant fusion proteins may be produced under appropriate conditions. Typical constructions are presented in the experimental part of this application.

The term "antibody" as used herein is intended to include monoclonal antibodies, polyclonal antibodies, and chimeric antibodies. Preferably, the antibodies which are to be detected by the immunoassays of the invention are polyclonal antibodies, which are present in biological samples of diseased patients, and have therefore been generated from different B cell sources. As such, they recognize different epitopes exhibited by a pathogenic antigen (on the other hand, monoclonal antibodies are derived from a single cell line and recognize the same epitope).

An antibody (or "immunoglobulin") consists of a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (or domain) (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain, CL. The VH and VL regions can be further

subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR) or "hypervariable regions", which are primarily responsible for binding an epitope of an antigen, and which are interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g. effector cells) and the first component (Clq) of the classical complement system.

Antibody can be of different isotypes (namely IgA, IgD, IgE, IgG or IgM). Both IgG and IgM type antibodies can be detected by the present method. Of note, these isotypes are composed of two identical heavy chains and two identical light chains that are joined by disulfide bonds. Importantly, IgM antibodies form polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer but also as a hexamer, so that they have a molecular mass of approximately 900 kDa (in their pentamer form). Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. Typically, however, IgM antibodies cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites. Due to its polymeric nature, IgM possesses high avidity.

Antibody fragments can also be detected thanks to the present method. This term is intended to include Fab, Fab', F(ab')2, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, and multimers thereof and bispecific antibody fragments.

Monoclonal antibodies can be used in the present immunoassays; for example for detecting the immunoglobulins that are bound to the solid supports. As used herein, "monoclonal antibody" defines an antibody arising from a homogeneous antibody population. More particularly, the individual antibodies of a population are identical except for a few possible naturally-occurring mutations which can be found in minimal proportions. In other words, a monoclonal antibody consists of a homogeneous antibody arising from the growth of a single cell clone (for example a hybridoma, a eukaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, a prokaryotic host cell transfected with a

DNA molecule coding for the homogeneous antibody, etc.) and is generally characterized by heavy chains of one and only one class and subclass, and light chains of only one type. Monoclonal antibodies are highly specific and are directed against a single antigen. In addition, in contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

The term "antigen" herein means any substance that causes the immune system to produce antibodies against the said substance. An "immunogenic" antigen is a specific type of antigen which is able to stimulate an adaptive immune response if injected on its own. At the molecular level, an antigen is thus characterized by its ability to be "bound" to the antigen-binding site of an antibody.

In the context of the present invention, an antibody is said to "bind" a define antigen (or epitope) or to "recognize" said antigen (or epitope) if said antibody has an affinity constant K_a (which is the inverted dissociation constant, i.e. $1/K_d$) higher than 10^5 M^{-1}, preferably higher than 10^6 M^{-1}, more preferably higher than 10^7 M^{-1} for said antigen (or epitope). This affinity can be measured for example by equilibrium dialysis or by fluorescence quenching, both technologies being routinely used in the art.

In the context of the invention, antigens or epitopes include: proteins, lipoproteins, polysaccharides, and glycoproteins. Said proteins include viral, bacterial, parasitic, animal, and fungal proteins such as albumins, tetanus toxoid, diphtheria toxoid, pertussis toxoid, bacterial outer membrane proteins (including meningococcal outer membrane protein), RSV-F protein, malarial derived peptide, B-lactoglobulin B, aprotinin, ovalbumin, lysozyme, linear peptides, oligopeptides etc. Said antigens can also be tumor associated antigens such as carcinoembryonic antigen (CEA), CA 15-3, CA 125, CA 19-9, prostate specific antigen (PSA), TAA complexes, SSX2 or NERCMSL. Said antigens can also be haptens, and other moieties comprising low molecular weight molecules, such as saccharides, oligosaccharides, polysaccharides, peptides, mucins, toxins, and allergens (pollen, egg white). Infectious toxins are well known in the art. One can cite, as examples, the botulinum neurotoxins, the Clostridium perfringens epsilon toxin, ricin, saxitoxin, shigatoxin, tetrodotoxin, staphylococcal enterotoxins, etc. Mucins are also well known in the art. MUC5AC, MUC5B and MUC2 are examples thereof. In particular, they can be

naturally-occurring polysaccharides such as Group B steptococcal and pneumococcal capsular polysaccharides (including type III), *Pseudomonas aeruginosa* mucoexopolysaccharide, and capsular polysaccharides (including fisher type I), and *Haemophilus influenzae* polysaccharides.

In another preferred embodiment, said antigen or epitope is expressed by a virus which is selected in the group consisting of: the influenza virus, the hepatitis A virus, the Hepatitis B virus, the Hepatitis C virus, the Hepatitis E virus, the Hepatitis G virus, the HIV virus, the yellow fever virus, the dengue virus, the Japanese encephalitis virus, the tick-borne encephalitis virus, the Usutu or West Nile viruses, the Rift Valley fever or Toscana viruses, the chikungunya virus, the respiratory synticial virus, the Rocio virus, the morbillivirus, the Murray encephalitis virus, the Wesselbron virus, the Zika virus, the lymphocytic choreomeningitis virus, the Ebola virus, the Marburg virus, the Crimean-Congo hemorrhagic fever virus, the Lassa virus, the Junin virus, the Machupo virus, the Sabia virus, the Guanarito virus, the mumps virus, the rabies virus, the rubella virus, the varicella zoster virus, the herpes simplex types 1 and 2, more generally an alphavirus, an adenovirus, an echovirus, a rotavirus, a flavivirus, a rhinovirus, an orthobunyavirus, a poliovirus, a human parvovirus, an enterovirus, a coronavirus, a human papillomavirus, the human cytomegalovirus, the Epstein-Barr virus, the parainfluenzae viruses from types 1, 2 and 3, or any identified virus.

In another preferred embodiment, said antigen or epitope is expressed by a virus belonging to a family which is selected from the group consisting of: the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) and the *Filoviridae* (Ebola, Marburg).

In another preferred embodiment, said antigen or epitope is expressed by a parasitic protozoa (such as those from the Leishmania genus, or *Toxoplasma Gondii*, *Entamoeba histolytica*, *Plasmodium falciparum*, *Pneumocystis carinii*, or *Giardia lamblia*), worms (such as nematodes, cestodes, or trematodes), or arthropods (such as crustaceans, insects, arachnids).

In another preferred embodiment, said antigen or epitope is expressed by an infectious bacterium, for example of the genera *Salmonella*, *Shigella*, *Streptococcus*, *Staphylococcus*, *Mycoplasma*, *Diphteriae*, *Leptospirosa, Rickettsia* or *Escherichia.* In a further preferred embodiment, the said bacterium belongs to one of the species selected from *H. influenzae*, *S. pneumoniae*, *Klebsiella pneumoniae*, *S. aureus*, *Bacillus anthracis*, *Listeria monocytogenes*, *Bordetella pertussis*, *Clostridium tetani*, *S. epidermidis*, *N. meningiditis*, *Pseudomonas aeruginosa, Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Coxiella burnetii, Leptospirosa interrogans* and *E.coli.*

In another preferred embodiment, said antigen or epitope is expressed by a fungus or yeast (e.g. from the species *Candida*, *Aspergillus, Cryptococcus*, *Histoplasma*, *Pneumocystis*, or *Stachybotrys*).

Antigens usually present several surface features that can act as points of interaction for specific antibodies. Any such distinct molecular feature constitutes an epitope. As used herein, the term "epitope" therefore designates a particular molecular surface feature of an antigen, for example a fragment of an antigen, which is capable of being bound by at least one antibody. On a molecular level, an epitope therefore corresponds to a particular molecular surface feature of an antigen (for example a fragment of an antigen) which is recognized and bound by a specific antibody. In the context of the present invention, the "fusion proteins" contain at least one epitope that is recognized by a target antibody. Preferably, said fusion proteins contain whole antigens, comprising several epitopes. These epitopes can be linear or conformational epitopes. As used herein, a linear (or sequential) epitope is an epitope that is recognized by antibodies by its linear sequence of amino acids, or primary structure. In contrast, a conformational epitope is recognized by its specific three-dimensional shape. Preferably, the fusion proteins of the invention contain conformational epitopes, as most polyclonal antibodies recognize same.

It is important however that such antigens do not present cross-reactive epitopes, i.e. epitopes that are recognized by non-specific antibodies that will bind thereto. If it was the case, the specificity of the method of the invention would be decreased.

In a more preferred embodiment, said epitope is present on a viral protein which is selected in the group consisting of: the EDIII protein of the dengue virus 1 encoded by SEQ ID NO:3, the EDIII protein of the dengue virus 2 encoded by SEQ ID NO:4, the

EDIII protein of the dengue virus 3 encoded by SEQ ID NO:5, the EDIII protein of the dengue virus 4 encoded by SEQ ID NO:6, the EDIII protein of the West Nile virus encoded by SEQ ID NO:7, the EDIII protein of the yellow fever virus encoded by SEQ ID NO:8, the EDIII protein of the Japanese encephalitis virus encoded by SEQ ID NO:9, the EDIII protein of the Zika virus encoded by SEQ ID NO:10, the EDIII protein of the Wesselbron virus encoded by SEQ ID NO:11, the EDIII protein of the Rocio virus encoded by SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus encoded by SEQ ID NO:13, the EDIII protein of the Saint-Louis encephalitis virus encoded by SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, and the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58 and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

In a preferred embodiment, the first and second epitopes (or antigens) that are fused with the hAGT enzyme in the fusion proteins used in the method of the invention belong to the same taxonomic level, i.e. they belong to the same family (e.g. the *Flaviviridae* family, the *Bunyaviridae* family, the *Arenaviridae* family or the *Filoviridae* family) or genus or species, but which have different serotypes. In other words, the said first and second epitopes can be expressed by closely related viruses, *e.g.* belong to the same family, genus or species but having different serotypes such as the dengue virus 1, 2, 3, or 4.

Alternatively, in another preferred embodiment, said first and second epitopes (or antigens) belong to unrelated biological families or genus or specie.

Importantly, the immunoassays of the invention rely on the detection of a large number of antibodies, which are known or unknown. By "large number", it is herein understood at least 5, more preferably at least 15, more preferably at least 50 and even more preferably at least 100 antibodies. Therefore, in a preferred embodiment, the assay method of the invention is used to detect at least 5, more preferably at least 15, and preferably at least 50

and even more preferably at least 100 target antibodies in a biological sample from a subject. It is of no relevance for the method of the invention whether the particular antibodies are properly characterized, since the procedure relies only on the detection of the presence of said antibodies, and not on their nature.

In a preferred embodiment of the invention, the said first and second fusion proteins that are coupled with the said first and second solid supports are selected in the group consisting of:

- SEQ ID NO:21 (corresponding to the fusion protein [SNAP-DEN1.EDIII])
- SEQ ID NO:42 (corresponding to the fusion protein [SNAP-SBV.N])
- SEQ ID NO:49 (corresponding to the fusion protein [SNAP-EV71.VP1])
- SEQ ID NO:51 (corresponding to the fusion protein [SNAP-JE.sE])
- SEQ ID NO:53 (corresponding to the fusion protein [SNAP-JE-1.EDIII])
- SEQ ID NO:60 (corresponding to the fusion protein [SNAP- JE-2.EDIII])
- SEQ ID NO:62 (corresponding to the fusion protein [SNAP- JE-4.EDIII])
- SEQ ID NO:64 (corresponding to the fusion protein [SNAP- JE-5.EDIII])
- SEQ ID NO:66 (corresponding to the fusion protein [SNAP- RabV.EDIII])
- SEQ ID NO:68 (corresponding to the fusion protein [SNAP-flavivirus.EDIII])
- SEQ ID NO:70 (corresponding to the fusion protein [SNAP- RR.sE2])
- SEQ ID NO:72 (corresponding to the fusion protein [SNAP- MAY.sE2])
- SEQ ID NO:74 (corresponding to the fusion protein [SNAP- WEE.sE2])
- SEQ ID NO:76 (corresponding to the fusion protein [SNAP- EEE.sE2])
- SEQ ID NO:78 (corresponding to the fusion protein [SNAP- VEE.sE2])
- SEQ ID NO:80 (corresponding to the fusion protein [SNAP- AKA.N])
- SEQ ID NO:82 (corresponding to the fusion protein [SNAP- AIN.N])
- SEQ ID NO:84 (corresponding to the fusion protein [SNAP- SHA.N])
- SEQ ID NO:86 (corresponding to the fusion protein [SNAP- huCOV.N])
- SEQ ID NO:88 (corresponding to the fusion protein [SNAP- huCOV.S])
- SEQ ID NO:90 (corresponding to the fusion protein [SNAP- HCV.C])
- SEQ ID NO:92 (corresponding to the fusion protein [SNAP- MSP+AMA])
- SEQ ID NO:94 (corresponding to the fusion protein [SNAP- HbpA1])
- SEQ ID NO:96 (corresponding to the fusion protein [SNAP- MUB40])

- SEQ ID NO:98 (corresponding to the fusion protein [SNAP- moCLEC5A])
- SEQ ID NO:100 (corresponding to the fusion protein [SNAP- huCLEC5A])
- SEQ ID NO:102 (corresponding to the fusion protein [SNAP- cxVAGO])
- SEQ ID NO:104 (corresponding to the fusion protein [SNAP- aaVAGO])
- SEQ ID NO:109 (corresponding to the fusion protein [SNAP- CCHF.N])
- SEQ ID NO:111 (corresponding to the fusion protein [SNAP- EBO.N])
- SEQ ID NO:113 (corresponding to the fusion protein [SNAP- MAR.N])
- SEQ ID NO:115 (corresponding to the fusion protein [SNAP- LAS.N])
- SEQ ID NO:117 (corresponding to the fusion protein [SNAP- JUN.N])
- SEQ ID NO:119 (corresponding to the fusion protein [SNAP- MAC.N])
- SEQ ID NO:121 (corresponding to the fusion protein [SNAP- GUA.N])
- SEQ ID NO:123 (corresponding to the fusion protein [SNAP- SAB.N])
- SEQ ID NO:125 (corresponding to the fusion protein [SNAP- OMSK.EDIII])
- SEQ ID NO:127 (corresponding to the fusion protein [SNAP- KYA.EDIII])
- SEQ ID NO:129 (corresponding to the fusion protein [SNAP- ALK.EDIII])
- SEQ ID NO:131 (corresponding to the fusion protein [SNAP- LAS.ectoGP1])
- SEQ ID NO:133 (corresponding to the fusion protein [SNAP- JUN.ectoGP1])
- SEQ ID NO:135 (corresponding to the fusion protein [SNAP-MAC.ectoGP1])
- SEQ ID NO:137 (corresponding to the fusion protein [SNAP-GUA.ectoGP1])
- SEQ ID NO:139 (corresponding to the fusion protein [SNAP- SAB.ectoGP1])
- SEQ ID NO:141 (corresponding to the fusion protein [SNAP- LAS.ectoGP2])
- SEQ ID NO:143 (corresponding to the fusion protein [SNAP- JUN.ectoGP2])
- SEQ ID NO:145 (corresponding to the fusion protein [SNAP-MAC.ectoGP2])
- SEQ ID NO:147 (corresponding to the fusion protein [SNAP-GUA.ectoGP2])
- SEQ ID NO:149 (corresponding to the fusion protein [SNAP- SAB.ectoGP2]), and
- SEQ ID NO:151 (corresponding to the fusion protein [SNAP- HEV.C]).

Consequently, the *in vitro* method of the invention enables to detect target disease(s) that is (are) viral, bacterial, yeast or fungi-mediated infection. Preferably said viral infection is caused by a Papillomavirus or RNA viruses from the families of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) and the *Filoviridae* (Ebola, Marburg). Preferably, said bacterial infection is caused by *Leptospirosa Interrogans*. Preferably, said infection is caused by *Plasmodium falciparum*.

As used herein, the term "biological sample" refers to any samples which have been obtained from a patient and which might contain antibodies. Preferably, said biological sample is a biological fluid, for example an unfiltered biological fluid such as urine, cerebrospinal fluid, pleural fluid, synovial fluid, peritoneal fluid, amniotic fluid, gastric fluid, blood, serum, plasma, lymph fluid, interstitial fluid, saliva, physiological secretions, tears, mucus, sweat, milk, semen, seminal fluid, vaginal secretions, fluid from ulcers and other surface eruptions, blisters, and abscesses. It also refers to an extract of tissues including biopsies of normal, malignant, and suspect tissues or any other constituents of the body which may contain antibodies. The said biological sample can be pre-treated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can involve filtration, distillation, concentration, inactivation of interfering compounds, and the addition of reagents. In a preferred embodiment, said biological sample is chosen from whole blood, serum, plasma, urine, seminal fluid, cerebrospinal fluid and saliva.

Any polypeptide having O^6-alkylguanine-DNA alkyltransferase activity can be used in the method of the present invention. For the purpose of the invention, these polypeptides will be referred to as "AGT polypeptides".

AGT irreversibly transfers the alkyl group from its substrate, O^6-alkylguanine-DNA, to one of its cysteine residues. A substrate analogue that rapidly reacts with AGT is O^6-benzyl-guanine, the second order rate constant being approximately 10^3 sec^{-1} M^{-1}.

In the context of the invention, a polypeptide is said to have "O^6-alkylguanine-DNA alkyltransferase activity" (or "AGT activity") if it is capable of irreversibly transferring an alkyl group from a O^6-alkylguanine-containing molecule to one of its own cysteine residues. The "O6-alkylguanine-DNA alkyltransferase activity" of the said polypeptide can be demonstrated by, for example, contacting known labeled O^6-benzyl-guanine derivatives and monitoring the transfer of said label on to the tested polypeptide. If the assay is performed *in cellulo* or in cell extracts, the reaction of the endogenous AGT of the host cells should be controlled, so that endogenous AGT does not interfere with the said polypeptide. Therefore, known AGT-deficient cell lines are preferably used. Assays for identifying AGT activity are now well described. Several O^6-benzyl-guanine derivatives are commercially available (O^6-benzyl-guanine is distributed for example by Santa Cruz biotechnology, and fluorescently-labeled O^6-benzyl-guanine derivatives can be obtained from New England Biolabs NEB). Some of these assays are disclosed in WO 2005/085470 and in WO 2004/031405.

In the context of the invention, the "catalytic domain" of the AGT polypeptide corresponds to the active site of said enzyme, or, in other words, to the part of the enzyme at which the transfer of the alkyl group from its substrate, O^6-alkylguanine-DNA, to a reactive cysteine residue, occurs. In the structure of hAGT bound with O^6-benzylguanine in its active site, four amino acids are in proximity of either the benzyl ring (Pro140, Ser159, Gly160), or could make contact with the N9 of the nucleobase (Asn157). Mutations at position Pro140 and Gly160 have previously been shown to affect the reaction of hAGT with O^6-benzylguanine (Xu-Welliver et al., Biochemical Pharmacology 1999): a proline at position 140 is believed to be essential for its interaction with the benzyl ring, and the mutation Gly160Trp has been shown to increase the reactivity of hAGT towards O^6-benzylguanine.

In a preferred embodiment, the AGT polypeptide having O^6-alkylguanine-DNA alkyltransferase activity is the human AGT polypeptide (referenced as NP_002403.2) of sequence SEQ ID NO: 1, the mouse AGT identified as NP_032624.1 (SEQ ID NO: 18), the rat MGMT identified as NP_036993.1 (SEQ ID NO: 19) or a homologous sequence thereof, said homologous sequence having O^6-alkylguanine-DNA alkyltransferase activity.

As used herein, the term "homologous" refers to sequences that have sequence similarity. The term "sequence similarity", in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences. In the context of the invention, two amino acid sequences are "homologous" when at least about 80 %, alternatively at least about 81 %, alternatively at least about 82 %, alternatively at least about 83 %, alternatively at least about 84 %, alternatively at least about 85 %, alternatively at least about 86 %, alternatively at least about 87 %, alternatively at least about 88 %, alternatively at least about 89 %, alternatively at least about 90 %, alternatively at least about 91 %, alternatively at least about 92 %, alternatively at least about 93 %, alternatively at least about 94 %, alternatively at least about 95 %, alternatively at least about 96 %, alternatively at least about 97 %, alternatively at least about 98 %, alternatively at least about 99 % of the amino acids are similar. Preferably the similar or homologous polypeptide sequences are identified by using the algorithm of Needleman and Wunsch.

Preferably, the homologous sequence to the AGT enzyme shares at least 64 % amino acid sequence identity, preferably at least about 65 % amino acid sequence identity, alternatively at least about 66 % amino acid sequence identity, alternatively at least about 67 % amino acid sequence identity, alternatively at least about 68 % amino acid sequence identity, alternatively at least about 69 % amino acid sequence identity, alternatively at least about 70 % amino acid sequence identity, alternatively at least about 71 % amino acid sequence identity, alternatively at least about 72 % amino acid sequence identity, alternatively at least about 73 % amino acid sequence identity, alternatively at least about 74 % amino acid sequence identity, alternatively at least about 75 % amino acid sequence identity, alternatively at least about 76 % amino acid sequence identity, alternatively at least about 77 % amino acid sequence identity, alternatively at least about 78 % amino acid sequence identity, alternatively at least about 79 % amino acid sequence identity, alternatively at least 80 % amino acid identity, alternatively at least about 81 % amino acid sequence identity, alternatively at least about 82 % amino acid sequence identity, alternatively at least about 83 % amino acid sequence identity, alternatively at least about 84 % amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86 % amino acid sequence identity, alternatively at least about 87 % amino acid sequence identity, alternatively at least about 88 % amino acid sequence identity, alternatively at least about 89 % amino acid sequence identity, alternatively at least about 90

% amino acid sequence identity, alternatively at least about 91 % amino acid sequence identity, alternatively at least about 92 % amino acid sequence identity, alternatively at least about 93 % amino acid sequence identity, alternatively at least about 94 % amino acid sequence identity, alternatively at least about 95 % amino acid sequence identity, alternatively at least about 96 % amino acid sequence identity, alternatively at least about 97 % amino acid sequence identity, alternatively at least about 98 % amino acid sequence identity and alternatively at least about 99 % amino acid sequence identity with SEQ ID NO: 1. In a preferred embodiment, an homologous sequence of SEQ ID NO: 1 is at least 64 %, preferably 70 %, and more preferably 80 % identical to SEQ ID NO: 1.

In a preferred embodiment, the said homologous polypeptide is a fragment or a mutant of the hAGT polypeptide of SEQ ID NO: 1, said fragment or mutant having a O^6-alkylguanine-DNA alkyltransferase activity.

Said fragments can have a size of at least 50, preferably 100, and more preferably 150 amino acids, and contain at least the "catalytic domain" of the AGT polypeptide as defined above, which is responsible of the O^6-alkylguanine-DNA alkyltransferase activity of the AGT enzyme. These fragments can be obtained using common techniques which are known by the skilled person.

Different mutant enzymes derived from native AGT have been described so far (Lim A. et al, 1996; Daniels D.S. et al, 2000; Juillerat A. et al, 2003, WO 2005/085470, WO 2004/031405). In particular, a mutant protein of 20 kDa containing the mutations Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Ser, Asn157Gly, Ser159Glu truncated at amino acid 182 has been obtained (the so-called "AGT26" mutant in WO 2005/085470, also called "SNAP 26" in WO 2006/114409). This particular mutant "SNAP26" has been shown to have enhanced labelling activity.

In the context of the present invention, the sequence of a more preferred AGT polypeptide contains the mutations described in WO 2005/085470, which positions can be easily transposed in view of SEQ ID NO: 1, the starting methionine residue of SNAP26 corresponding to the methionine residue in position 32 of SEQ ID NO: 1 (31 amino acids

should therefore be added to the positions disclosed in WO 2005/085470 so as to obtain the corresponding ones in SEQ ID NO: 1).

In a preferred embodiment, the AGT homologous sequence useful in the invention corresponds to the native AGT sequence of SEQ ID NO: 1, in which between 1 and 30, preferably between 6 and 25, and in particular 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 amino acids are substituted by other amino acids, and/or 1 to 40, preferably 1 to 20, in particular 10 to 20 amino acids, more preferably 15 amino acids at the C-terminus are deleted.

In a more preferred embodiment, the AGT homologous sequence contains the following mutations as compared with SEQ ID NO: 1:

(A) Lys31 replaced by Arg, or Met32 replaced by Ser, or Cys93 replaced by Ala, or Lys156 replaced by Ala, or Ala158 replaced by Thr, or Arg159 replaced by Ala, or Gly162 replaced by Lys, or Gly163 replaced by Thr, or Met165 replaced by Leu, or Arg166 replaced by Ser, or Cys181 replaced by Ser, or Asn188 replaced by Gly, or Ser190 replaced by Glu, or Gly214 replaced by Pro, or Ser215 replaced by Ala, or Ser216 replaced by Gly, or Gly217 replaced by Ile, or Leu218 replaced by Gly, or Gly220 replaced by Pro, or Ala221 replaced by Gly, or Trp222 replaced by Ser, or

(B) Lys31-Met32 replaced by Arg-Ser, or Ala158-Arg159 replaced by Thr-Ala, or Gly162-Gly163 replaced by Lys-Thr, or Met165-Arg166 replaced by Leu-Ser, or Gly162-Gly163/Met165-Arg166 replaced by Lys-Thr/Leu-Ser, or Asn188/Ser190 replaced by Gly/Glu, or Gly214-Ser215-Ser216-Gly217-Leu218 replaced by Pro-Ala-Gly-Ile-Gly, or Gly220-Ala221-Trp222 replaced by Pro-Gly-Ser, preferably in combination with any other amino acid replacements cited in (A), or

(C) Truncation after Leu223 (amino acids 224-238 are deleted), preferably in combination with any other amino acid replacement cited in (A) or (B).

Preferred AGT homologous sequences are those being truncated after Leu223.

Preferred AGT homologous sequences are those wherein two out of the modifications (B) are present, and optionally truncation after Leu223.

Preferred AGT homologous sequences are those wherein three out of the modifications (B) are present, and optionally truncation after Leu223.

Preferred AGT homologous sequences are those wherein four out of the modifications (B) are present, and optionally truncation after Leu223.

Preferred AGT homologous sequences are those wherein five out of the modifications (B) are present, and optionally truncation after Leu223.

Preferred AGT homologous sequences are those wherein six out of the modifications (B) are present, and optionally truncation after Leu223.

Other preferred AGT homologous sequences are those containing a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mutations chosen among the modifications disclosed in (A), and optionally truncated after Leu223.

In a far more preferred embodiment, the AGT polypeptide of the invention is the SNAP mutant of SEQ ID NO: 2, which is homologous to the hAGT enzyme and contains the mutations Lys31Arg, Met32Ser, Cys93Ala, Lys156Ala, Ala158Thr, Arg159Ala, Gly162Lys, Gly163Thr, Met165Leu, Arg166Ser, Cys181Ser, Asn188Gly, Ser190Glu, Gly214Pro, Ser215Ala, Ser216Gly, Gly217Ile, Leu218Gly, Gly220Pro, Ala221Gly, Trp222Ser and truncation after Leu223 as compared with SEQ ID NO: 1. The SNAP mutant of SEQ ID NO: 2 shares 77% homology with the amino acid sequence of the human 6-methylguanine-DNA-methyltransferase (NP_002403.2, SEQ ID NO: 1), and 70 % homology with the amino acid sequence of the mouse 6-methylguanine-DNA-methyltransferase (NP_032624.1, SEQ ID NO: 18).

In an even more preferred embodiment, the AGT enzyme is the SNAP mutant protein of SEQ ID NO: 2 or a homologous thereof, having O^6-alkylguanine-DNA alkyltransferase activity. Preferably, said homologous sequence to the SNAP mutant protein is at least identical at more than 80 %, preferably 81 %, more preferably 82 %, more preferably 83 %, more preferably 84 %, more preferably 85 %, preferably 86 %, more preferably 87 %, more preferably 88 %, more preferably 89 %, more preferably 90 %, more preferably 91 %, more preferably 92 %, more preferably 93 %, more preferably 94 %, more preferably 95 %, more preferably 96 % to the and even more preferably 97 % to the SNAP mutant protein of

sequence SEQ ID NO: 2, and has O^6-alkylguanine-DNA alkyltransferase activity as defined above.

Said homologous polypeptides having O^6-alkylguanine-DNA alkyltransferase activity can be produced using protein engineering techniques known to the skilled person and/or using molecular evolution to generate and select new O^6-alkylguanine-DNA alkyltransferases. Such techniques are e.g. targeted mutagenesis, phage display methods, saturation mutagenesis, error prone PCR to introduce variations anywhere in the sequence, DNA shuffling used after saturation mutagenesis and/or error prone PCR, or family shuffling using genes from several species.

In the most preferred embodiment, the AGT polypeptide used in the method of the invention is the SNAP mutant of SEQ ID NO: 2.

The AGT enzyme irreversibly transfers the alkyl group from its substrate, O^6-alkylguanine-DNA, to one of its cysteine residues. However, substitutions of O^6-benzylguanine at the C4 of the benzyl ring do not significantly affect the reactivity of AGT against O^6-benzylguanine derivatives. This property has been used to transfer a label attached to the C4 of the benzyl ring to AGT (see WO 2004/031404 and WO 2005/085470).

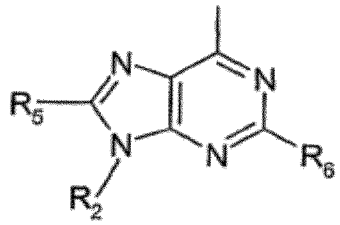
A number of O^6-benzylguanine derivatives have been shown to react with the AGT enzyme by transferring their benzyl group to the active site cysteine of the AGT enzyme (cf. Damoiseaux et al., *ChemBiochem.*, 2001, WO 2004/031404 and WO 2005/085470).

In a preferred embodiment, the AGT substrates used in the method of the invention are O^6 benzyl guanine derivatives having the formula I:

R1-X-CH_2-R3-R4-Y

wherein:

- R1 is a group recognized by said AGT polypeptide as a substrate, such as a heteroaromatic group containing 1 to 5 nitrogen atoms, and preferably a purine radical of the formula:



wherein R5 is hydrogen, halogen, e. g. chloro or bromo, trifluoromethyl, or hydroxy; R6 is hydrogen, hydroxy or unsubstituted or substituted amino; and R2 is hydrogen, an alkyl of 1 to 10 carbon atoms, or a saccharide moiety;

- X is an oxygen or sulfur atom; preferably an oxygen atom;

- R3 is an aromatic or a heteroaromatic group, or an optionally substituted unsaturated alkyl, cycloalkyl or heterocyclyl group with the double bond connected to CH_2; preferably a phenyl, e.g. a phenyl substituted by R4 in para or meta position,

- R4 is a linker moiety,

- Y is a reactive group, preferably an amino group.

In a preferred embodiment, said linker moiety R_4 is a flexible linker. Linker units are chosen in the context of the envisioned application, i.e. in the transfer of the substrate to a fusion protein comprising AGT. The linker does not interfere with the reaction with AGT nor with the target antibody.

For example, it can be a straight or branched chain alkylene group with 1 to 20 carbon atoms, preferably 5 to 15 carbon atoms, wherein :

(a) one or more carbon atoms are replaced by oxygen, in particular wherein every third carbon atom is replaced by oxygen, e.g. a poylethyleneoxy group with 1 to 5 ethyleneoxy units;

(b) one or more carbon atoms are replaced by nitrogen carrying a hydrogen atom, and the adjacent carbon atoms are substituted by oxo, representing an amide function -NH-CO-;

(c) one or more carbon atoms are replaced by oxygen, and the adjacent carbon atoms are substituted by oxo, representing an ester function -O-CO-;

(d) the bond between two adjacent carbon atoms is a double or a triple bond, representing a function -CH=CH- or -C≡C-;

(e) one or more carbon atoms are replaced by a phenylene, a saturated or unsaturated cycloalkylene, a saturated or unsaturated bicycloalkylene, a bridging heteroaromatic or a bridging saturated or unsaturated heterocyclyl group;

(f) two adjacent carbon atoms are replaced by a disulfide linkage -S-S-; or a combination of two or more, especially two or three, alkylene and/or modified alkylene groups as defined under (a) to (f) hereinbefore, optionally containing substituents.

Substituents considered are e.g. lower alkyl, e.g. methyl, lower alkoxy, e.g. methoxy, lower acyloxy, e.g. acetoxy, or halogenyl, e.g. chloro.

In a preferred embodiment, R4 is a polyethyleneoxy group with 1 to 8 ethyleneoxy units, further comprising one to four nitrogen atoms carrying a hydrogen atom, which adjacent carbon atoms are substituted by oxo, representing an amide function -NH-CO-.

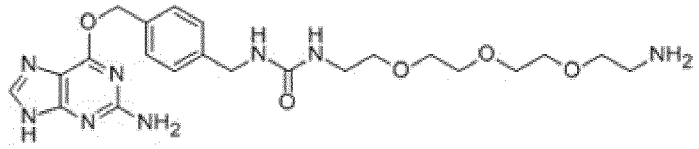
In a more preferred embodiment, R4 is $-CH_2-NH-CO-NH-[C_2H_4-O]_n-$, wherein n is comprised between 1 to 8, preferably 2 to 6, and is most preferably 3.

In a preferred embodiment, said reactive group is a functional group that facilitates the attachment and bonding of the substrate on the solid support. Such functional groups are well-known in the art. They include amine, activated esters, acrylamides, acyl azides, acyl halides, acyl nitriles, aldehydes, ketones, alkyl halides, anhydrides, aryl halides, aziridines, boronates, activated carnoxylic acids, carbodiimides, diazoalkanes, epoxides, haloacetamides, haloplatinate, halotriazines, imido esters, isocyanates, isothiocyanates, maleimides, phosphoramidites, solyl halides, sulfonate esters and sulfonyl halides. It is preferably the amine group $-NH_2$.

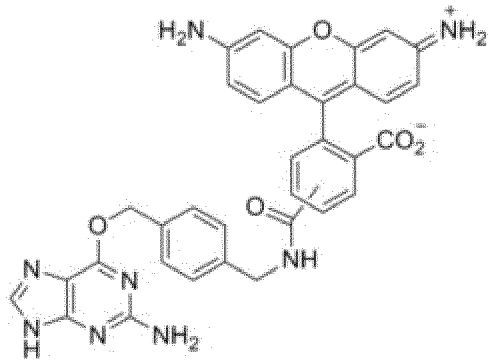
On the opposite side, the solid support should be functionalized by complementary groups corresponding to such reactive groups. The complementary groups corresponding to each

of these reactive groups are well-known in the art. They are given for example on the table I of WO 2010/107433.

In a preferred embodiment, the AGT substrate used in the method of the invention is:



In another preferred embodiment, the AGT substrate used in the method of the invention is the fluorescent linker designated "SNAP-cell® 505", having the following formula:



This benzylguanine derivative possesses one benzyl purine group (guanine) for the specific interaction with the SNAP domain, as well as one free amine group for the covalent coupling to the microsphere surface. It is commercialized by New England BioLaps and has been successfully coupled to the surface of the microparticles of the invention.

Substrates of the invention are generally prepared by standard methods known in the art. Particular methods are explained e.g. in patent application WO 2005/085470.

The methods of the invention require that the AGT substrates be covalently coupled to the solid supports. In the context of the present invention, an AGT substrate is "covalently coupled" to a solid support if it is permanently attached to the said solid support, and will not desorb or leach over time. According to the invention, an AGT substrate is permanently attached to the said solid support if it stays attached for a long period of storage, e.g., typically, at least 6 months of storage. A number of coupling proceedings have

been described so far. Any of these coupling proceedings can be used in the immunoassay of the invention, provided that the AGT substrate becomes permanently attached to the solid support.

In the immunoassay of the invention, the covalent coupling is preferably performed by contacting the AGT substrates (with contain a reactive group Y, as mentioned above) with solid supports which have been previously functionalized with a complementary group such as those disclosed in table I of WO 2010/107433, the disclosure of which is incorporated herein by reference.

Thus, in a preferred embodiment, the methods of the invention use solid supports that have been functionalized with a group which is complementary to the reactive group of the AGT substrate, before being contacted with the AGT substrate.

A preferred and conventional procedure for covalently coupling an AGT substrate to the surface of solid supports is based on the carbodiimide reaction and uses water-soluble carbodiimide. According to this procedure, solid supports have surface carboxyl groups available for attachment of the reactive amine- or sulfhydryl-containing AGT substrate. Thus, in this preferred embodiment, the methods of the invention use solid supports that have been functionalized with surface carboxyl groups prior to be contacted with the AGT substrate.

In this case, the first step of the method of the invention is to activate the carboxyl groups coating the solid supports. This activation is usually performed by adding a so-called "activation buffer", for example a 50 mg/mL EDAC solution or a 50 mg/mL S-NHS solution. These solutions are commercially available. Activation of the solid supports is typically performed by incubating said supports with the activation buffer at room temperature for a few minutes (e.g. 5 minutes to 30 minutes), according to the manufacturer's instructions.

Importantly, covalent coupling of the AGT substrate to the solid support has to be performed under particular conditions, so as to preserve the AGT substrate solubility and the integrity of the bead (internal fluorochrome). The inventors have observed that the AGT substrates should be suspended in a "covalent coupling" buffer containing between 0 and 20 % of dimethylsulfoxide (DMSO). In particular, the inventors have observed that

concentrations of DMSO above 20 % may affect the detection step of the methods of the invention. Preferably, said buffer is a PBS buffer containing between 0 and 20 % of DMSO, more preferably between 10 % and 20 % of DMSO.

Advantageously, the unspecific sites on the solid supports that have not been covalently attached to the AGT substrate can be further blocked by any conventional means, for example, by using a blocking buffer containing 1 % of bovine serum albumin (BSA) or any saturating protein (*e.g.* casein).

Once the solid supports of the invention have been covalently coupled with the AGT substrate (preferably through a carbodiimide covalent linkage), the solid supports are then contacted by the fusion proteins of the invention, so as to couple the epitopes that are specifically recognized by the target antibodies to said supports.

Again, this coupling step has to be performed under particular conditions. As a matter of fact, the catalytic site of the AGT enzyme and the conformational structure of the antigens / epitopes which are carried by the fusion proteins have to be conserved during the coupling proceedings. The inventors identified that the fusion protein should be suspended in a dithiothreitol (DTT)-containing buffer, preferably a PBS/DTT buffer, for the coupling to be efficient. Advantageously, the said coupling buffer contains tween 20; indeed, it has been observed by the present inventors that addition of tween 20 to the coupling medium helps avoiding bead aggregation. Preferably, the coupling buffer contains 0,02 % tween 20. More preferably, the covalent coupling buffer of the invention is a PBS buffer of pH 7,4, containing 0,02 % tween 20, and 1 mM DTT.

Other coupling conditions are usual ones. Preferably, the covalent coupling of the AGT substrate and the coupling of the fusion protein to the solid supports are performed at room temperature. If the solid supports are fluorescently labeled, said proceedings are more preferably performed in darkness.

In a second aspect, the present invention is thus drawn to a method for covalently coupling a AGT polypeptide having O^6-alkylguanine-DNA alkyltransferase activity, on a functionalized solid support, comprising the following steps:

a) activating the said functionalized solid support,

b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support,

c) contacting the said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer,

wherein unbound molecules are washed out after steps b) and c).

Washings can be performed by using any kind of appropriate washing buffers. Such buffers are routinely used by the person of skills in the art and need not be further detailed here. Preferably, a PBS buffer is used.

As used herein, "appropriate conditions" are usual ones. Preferably, the covalent coupling of the AGT substrate is performed at room temperature and, if the solid supports are fluorescently labeled, in darkness.

The functionalization of the solid support can be performed by any conventional means (as those reminded above). The activation of said functionalized solid support is performed accordingly. In a preferred embodiment, the said solid supports are functionalized with surface carboxyl groups and further activated with a classical activation buffer, for example a 50 mg/mL EDAC solution or a 50 mg/mL S-NHS solution.

In a preferred embodiment, DTT is at a concentration of 1 mM in the PBS/DTT buffer.

The present invention is also drawn to a solid support which has been obtained by the said method, and to the use of said solid support in the immunoassay of the invention.

Said solid supports can then be stored in conventional storage buffers, for example containing 0.5 g/L sodium azide, 0.1 % BSA, 0.02 % tween 20, and/or 1 mM DTT.

All these coupling steps are preferably performed *in vitro*, in buffers which are devoid of living cells, so that there is no need to take into account the reaction with endogenous AGT enzymes, and the reaction of the (exogenous) AGT fusion protein is therefore highly specific.

The solid supports that can be used in the methods of the invention can be of any kind, e.g. test tubes, microtiter wells, sheets, beads, chips, and/or microparticles, provided that

they can be specifically identified from each other. Such identification is possible for example when they are separately located in space (e.g. the wells in a microtiter plate, or different locations on a chip) or when they are differently labeled. A "solid support" has therefore to be understood in a broad meaning, that is, by designating either discrete small parts of a whole solid supports (in case of a plate or a biochip) or a large number of identical microparticles that share common detectable characteristics (hereafter referred to as microparticles "subset").

In a preferred embodiment, the solid supports used in this invention can be specifically identified by their specific location, size, diameter, weight, granulometry, and/or labeling. Such labeling is for example a fluorochrome, a fluorophore, a chromophore, a radioisotope, a mass tag, or any kind of detectable tag which is known in the art.

The solid supports used in the invention can be made of any material, for example in polystyrene, cellulose, nitrocellulose, glass, ceramic, resin, rubber, plastic, silica, silicone, metal, and/or polymer. Polymeric materials include brominated polystyrene, polyacrylic acid, polyacrylonitrile, polyamide, polyacrylamide, polyacrolein, polybutadiene, polycaprolactone, polycarbonate, polyester, polyethylene, polyethylene terephthalate, polydimethylsiloxane, polyisoprene, polyurethane, polyvinylacetate, polyvinylchloride, polyvinylpyridine, polyvinylbenzylchloride, polyvinyltoluene, polyvinylidene chloride, polydivinylbenzene, polymethylmethacrylate, polylactide, polyglycolide, poly(lactide-co-glycolide), polyanhydride, polyorthoester, polyphosphazene, polyphosophaze, polysulfone, or combinations thereof, that are acceptable as well. Most of these supports are commercially available. For example, beads from synthetic polymers such as polystyrene, polyacrylamide, polyacrylate, or latex are commercially available from numerous sources such as Bio-Rad Laboratories (Richmond, Calif.) and LKB Produkter (Stockholm, Sweden). Beads formed from natural macromolecules and particles such as agarose, cross-linked agarose, globulin, deoxyribose nucleic acid, and liposomes are commercially available from sources such as Bio-Rad Laboratories, Pharmacia (Piscataway, NJ), and IBF (France). Beads formed from copolymers of polyacrylamide and agarose are commercially available from sources such as IBF and Pharmacia.

When polymeric supports were used, carboxyl groups can be added to the surface of the solid support by incorporating monomers containing such groups into the polymers (for

example, acrylic acid, methacrylic acid, itaconic acid, and the like). Alternatively, they can be added to the support by further chemical reaction of a polymer having other precursor reactive groups which can be converted to carboxyl groups (for example, by hydrolysis of anhydrides, such as maleic anhydride, or by oxidation of surface methylol or aldehyde end groups), as already described.

In a preferred embodiment, the solid supports used in the invention are microparticles. Said microparticles have preferably a diameter of less than one millimeter, preferably a diameter ranging from about 0.1 to about 1,000 micrometers (μm). Even though the microparticles can be of any size, the preferred size is 1-100 μm, more preferably 2-50 μm, more preferably 3-25 μm, and even more preferably about 6-12 μm. Microparticles are made of any regularly shaped material. The preferred shape is spherical; however, particles of any other shape can be employed since this parameter is immaterial to the nature of the invention. The shape of the particle can serve as an additional distinction parameter, which is discriminated by flow cytometry, e.g., by a high-resolution slit-scanning method.

As used hereinafter the terms "microparticles", "microspheres", or "microbeads" are used interchangeably and bear equivalent meanings as they refer to small particles with overall diameter that falls essentially in the micrometer range. The terms "nanospheres", "nanoparticles", or "nanobeads" refer to smaller particles with overall size that falls essentially in the nanometer range. As used hereinafter the general term particles, spheres, or "beads" refers both to microparticles and nanoparticles, which can effectively serve as solid supports in the methods of the invention.

In the context of the present invention, a "subset" of microparticles corresponds to numerous identical microparticles having the same characteristics and that have been coated with the same epitope. Importantly, each subset of microparticles should be distinguishable from other subsets of the population by at least one characteristic (e.g. location, size, diameter, weight, granulometry, and/or labeling).

In a preferred embodiment, the different subsets of microparticles can be distinguished as they are differently labeled (e.g. with a fluorochrome, a fluorophore, a chromophore, a radioisotope, a mass tag, or any kind of detectable tag which is known in the art).

In a more preferred embodiment, the different subsets of microparticles can be distinguished as they are differently fluorescently labeled, as proposed in US 5,736,330, US 5,981,180, US 6,057,107, US 6,268,222, US 6,449,562, US 6,514,295 , US 6,524,793 and US 6,528,165. More precisely, these different subsets can be dyed with different fluorescent dyes, and/or different concentrations of one or more fluorescent dyes. As such, the different subsets can have different fluorescent signatures (e.g. different fluorescent wavelength(s), different fluorescent intensities, etc.) that can be measured and used by a measurement system to determine the subset that individual microparticles belong to (i.e., to classify the microparticles according to the subset).

In a preferred embodiment, the microparticles used in the invention are internally labeled with fluorescent dyes, as proposed in EP 1 204 869.

These microparticles may also incorporate magnet or magnetically responsive metal oxides selected from the group consisting of superparamagnetic, paramagnetic, and ferromagnetic metal oxide. Magnetic beads are for example commercially available from sources such as Dynal Inc. (Great Neck, NY) or can be prepared using known in the art methods as disclosed for example in U.S. 4,358,388; US 4,654,267; US 4,774,265; US 5,320,944; and US 5,356,713. In a preferred embodiment, the solid supports used in the invention are therefore magnetic.

In a more preferred embodiment, the solid supports used in the invention are microparticles internally labeled with fluorescent dyes with magnetite encapsulated in a functional polymer outer coat containing surface carboxyl groups for covalent coupling of ligands, such as those marketed by Luminex Corp under the trade name MagPlex.

It is also possible to use MicroPlex microspheres (sold by Luminex) that are carboxylated polystyrene micro-particles that have been color coded into spectrally distinct regions. These regions can be quickly distinguished by an xMAP Instrument allowing for the interrogation of up to 100 different analytes simultaneously from one single sample volume.

It is also possible to use SeroMAP microspheres (sold by Luminex) which are a special formulation of MicroPlex microspheres which have been optimized to reduce non-specific binding in serology assays.

The last step of the method of the invention consists in detecting the presence of the antibodies that are bound to the epitopes and therefore to the detectable solid support. By analyzing to which subset of microparticles antibodies are bound, it can be easily inferred which antibodies were present in the biological sample, and therefore by which pathogen the tested subject was infected.

Any known technology can be used to detect the presence of the antibodies that are bound to the solid supports. For example, labeled secondary antibodies recognizing specifically the constant part of the subject immunoglobulins can be used, as shown in the experimental part below. It is important to note that the labeling of the detecting-antibodies should be different from the one of the solid support, so as to distinguish between the solid supports that are coupled to antibodies, and those that are not.

Alternatively, immunoglobulins present in sera from infected animals or humans can be directly conjugated to R-phycoerythrin (R-PE), using a one-step antibody labeling protocol (Lightning-Link™ R-Phycoerythrin Conjugation Kit – Innova Biosciences). The hands-on time for the entire procedure is usually 20-30 seconds, and allows the labeling of small quantities of immunoglobulins with 100% recovery. This procedure eliminates the need for secondary reagents, such as conjugated anti-species antibodies and streptavidin-R-phycoerythrin, in multiplex-immunoassay experiments.

When microparticles internally labeled with fluorescent dyes are used, the fluorescent detection instrument should be equipped with a first laser for detecting the type of microsphere, and a second laser to ensure the quantification of captured IgM or IgG by exciting the fluorophore which is conjugated to the specific detection antibody.

With its extensive multiplexing capabilities and lower limit of detection, this approach offers substantial cost and sample savings over traditional ELISA measurements. Moreover, the selected sets of microspheres are adaptable to an affordable, compact, and robust fluorescent detection system such as the MagPix (Luminex Corporation).

In this embodiment, the method of the invention makes it possible to simultaneously analyze up to 100 types of coupled microspheres per well by using a flow analysis tool, and affords greatly enhanced sensitivity that is expected to be on the order of several orders of magnitude larger than that of currently used systems and methods.

Interestingly, the method of the invention enables to perform high throughput serological screening to diagnose multiple infections in an individual, either a human or an animal.

In a third aspect, the present invention provides a kit which is suitable for use in the detection of antibodies according to the method of the invention.

This kit comprises at least two solid supports as defined above, more precisely:

- a first solid support as obtained in step (c) of the method of the invention, said support being covalently coupled with a first epitope that is recognized by a first target antibody, and

- a second solid support as obtained in step (f) of the method of the invention, said support being covalently coupled with a second epitope that is recognized by a second target antibody, and not by said first target antibody,

wherein the at least two solid supports can be specifically identified from each other and enable to detect two different target antibodies.

In other terms, the present invention relates to a kit for the detection of at least two target antibodies in a biological sample comprising:

(a) a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody; and

b) a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said first target antibody.

In a preferred embodiment, said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis

virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

Preferably, this kit also contains the means to detect the at least two target antibodies which are bound to the solid supports. Said means are more preferably secondary antibodies recognizing the constant part of the target antibodies. Said secondary antibodies can be labeled, provided that the labeling is not the same as the ones that are present on the solid support. However, it is possible to use the same labeling for all the secondary antibodies that are used for detecting the antibodies bound to solid support(s), since the information concerning the infectious pathogen(s) are given only by the identification of the solid support which is bound to the antibodies.

The kit of the invention may contain other ingredients that are accepted as standard reagents such as a wash buffer, necessary plasticware, and the like.

In a preferred embodiment, the kit of the invention comprises at least 10, preferably at least 50, more preferably at least 100 differently coupled-solid supports, said solid supports being for example subsets of microparticles as defined above.

In a more preferred embodiment, the said solid supports are microspheres, for example those which are internally labeled with a fluorescent dye with magnetite encapsulated in a functional polymer outer coat containing surface carboxyl groups.

In another preferred embodiment, in the kit of the invention, the said solid supports are mixed together in at least one single compartment.

Advantageously, the kit of the invention contains conventional support(s), e.g., microtiter plates, containing the different antigen-coated microparticles subsets defined above. In a preferred embodiment, the said microparticles subsets are mixed together in at least one single compartment (e.g. a well or a tube). Such a device is disclosed on Figure 11.

The kit of the invention may also contain recipients (e.g., tubes) containing the said subsets of antigen-coated microparticles.

The present invention also targets the use of the kit of the invention for detecting at least two, preferably at least 10, more preferably at least 50 and even more preferably at least 100 target antibodies in a biological sample from a subject.

In a preferred embodiment, the kit of the invention is used for detecting at least two, preferably at least 10, and more preferably at least 20 target antibodies that are generated upon infection by endemic viruses or parasites of the same geographic region. For example, the kit of the invention could contain microparticles that are coated with antigens of viruses or parasites that are specific of Africa regions, such as the Dengue virus type 1, type 2, type 3, type 4, the Yellow fever virus, the West-Nile virus, the Usutu virus, the Zika virus, the Wesselsbron virus, the Shamonda virus, the Rift Valley fever virus, the Chikungunya virus, the Crimean-Congo hemorrhagic fever virus, the Ebola virus, the Marburg virus, the Lassa virus, the Hepatitis C virus, the Hepatitis E virus, the Enterovirus 71, *Plasmodium falciparum*, or *Leptospira interrogans*.

Table 1 below discloses examples of antigen-coupled microspheres combinations which can be included in the kit of the invention depending on the geographic region it is intended for (Asia, Europa, America, Oceania, or Africa).

The kit of the invention may alternatively contain antigen-coupled microspheres that enable the diagnosis of viruses or parasites inducing specific symptoms (flu-like, encephalitis, or hemorrhagic fever) or infecting specific animals, so that it can be adapted to each patient / animal.

Table 1 below discloses examples of antigen-coupled microspheres combinations which can be included in the kit of the invention depending on the symptoms of the patient or of the animal.

Finally, kits containing antigen combinations that are proposed by national sanitary agencies are obviously also encompassed in the present invention.

In particular, the kit of the invention comprises at least two solid supports coated with at least two fusion proteins that are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

In a preferred embodiment, the kit of the invention contains a combination of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen or at least twenty solid supports coated with said fusion proteins.

In a more preferred embodiment, the kit of the invention contains a combination of at least five solid supports (e.g., microsphere subsets) that are coated with at least five different fusion proteins containing antigens as recommended by the Food and Drug Administration, namely, antigens from the HBV, HCV, HIV1, HIV2 and West Nile viruses.

Table 1: Advantageous combinations of antigen-coupled microspheres to be included in the kit of the invention

Agent		Antigen-coupled microspheres		Microsphere panels								
				Geographical					Syndromic		Veterinary	
Genus	Species	Abbreviation	Description	Africa	Asia	Europe	Americas	Oceania	Flu-like	Encephalitis	Hemorrhagic fever	Bovine disease	Equine disease
Flavivirus	Dengue virus type 1	SNAP+ DEN1.EDIII	Domain III of envelope E protein	x	x	x	x	x	x		x		
	Dengue virus type 2	SNAP+ DEN2.EDIII	Domain III of envelope E protein	x	x	x	x	x	x		x		
	Dengue virus type 3	SNAP+ DEN3.EDIII	Domain III of envelope E protein	x	x	x	x	x	x		x		
	Dengue virus type 4	SNAP+ DEN4.EDIII	Domain III of envelope E protein	x	x	x	x	x	x		x		
	Yellow fever virus	SNAP+ YF.EDIII	Domain III of envelope E protein	x			x		x		x		
	West-Nile virus	SNAP+ WNV.EDIII	Domain III of envelope E protein	x	x	x	x	x	x	x			x
		WNV.prM-sE+SNAP	secreted soluble form of envelope E protein	x	x	x	x	x	x	x			x
	Usutu virus	SNAP+ USU.EDIII	Domain III of envelope E protein	x		x				x			x
	Japanese encephalitis virus genotype 3	JE.prM-sE+SNAP	secreted soluble form of envelope E protein		x			x	x	x			
	Japanese encephalitis virus genotype 1	SNAP+JE-1. EDIII	Domain III of envelope E protein		x			x	x	x			
	Japanese encephalitis virus genotype 2	SNAP+JE-2. EDIII	Domain III of envelope E protein		x			x	x	x			
	Japanese encephalitis virus genotype 3	SNAP+JE-2. EDIII	Domain III of envelope E protein		x			x	x	x			
	Japanese encephalitis virus genotype 4	SNAP+JE-4. EDIII	Domain III of envelope E protein		x			x	x	x			
	Japanese encephalitis virus genotype 5	SNAP+JE-5. EDIII	Domain III of envelope E protein		x			x	x	x			
	Murray Valley encephalitis virus	SNAP+MVE. EDIII	Domain III of envelope E protein		x			x	x	x			
	Saint-Louis encephalitis virus	SNAP+SLE. EDIII	Domain III of envelope E protein				x		x	x			x
	Zika virus	SNAP+ZIKV. EDIII	Domain III of envelope E protein	x	x			x	x				
	Wesselsbron virus	SNAP+WSL. EDIII	Domain III of envelope E protein	x					x			x	
	Rocio virus	SNAP+ROCV. EDIII	Domain III of envelope E protein				x		x				
	Rabensburg virus	SNAP+RabV.EDIII	Domain III of envelope E protein			x							
	Insectivore flavivirus (coll.La Timone)	SNAP+Insectflavi.EDIII	Domain III of envelope E protein			x							
	Tickborne encephalitis virus	SNAP+ TBE.EDIII	Domain III of envelope E protein			x						x	x
	Omsk Hemorrhagic Fever virus	SNAP+ OMSK.EDIII	Domain III of envelope E protein		x						x		
	Kyasanur Forest Disease virus	SNAP+ KAS.EDIII	Domain III of envelope E protein		x						x		
	Alkhumra virus	SNAP+ALK.EDIII	Domain III of envelope E protein		x						x		
Orthobunyavirus	Schmallenberg virus	SNAP+AKA.N	Nucleoprotein N			x						x	
	Akabane virus	SNAP+AKA.N	Nucleoprotein N			x		x				x	
	Aino virus	SNAP+AIN.N	Nucleoprotein N			x		x				x	
	Shamonda virus	SNAP+SHA.N	Nucleoprotein N	x		x						x	
Bunyavirus	Rift Valley fever virus	SNAP+ RVF.N	Nucleoprotein N	x		x			x		x	x	
Alphavirus	Chikungunya virus	CHIK.sE2+SNAP	secreted soluble form of envelope E2 protein	x	x	x	x	x	x				
	Ross River virus	RR.sE2+SNAP	secreted soluble form of envelope E2 protein					x	x				x
	Mayaro virus	MAY.sE2+SNAP	secreted soluble form of envelope E2				x		x				

			protein											
	Eastern equine encephalitis virus	EEE.sE2+SNAP	secreted soluble form of envelope E2 protein				x		x			x		
	Western equine encephalitis virus	WEE.sE2+SNAP	secreted soluble form of envelope E2 protein				x		x			x		
	Venezuelan equine encephalitis	VEE.sE2+SNAP	secreted soluble form of envelope E2 protein				x		x			x		
Nairovirus	Crimean-Congo hemorrhagic fever virus	SNAP+ CCHF.N	Nucleoprotein N	x	x	x					x		x	
Ebolavirus	Ebola virus (Zaire)	SNAP+ EBO.N	Nucleoprotein N	x							x			
Marburgvirus	Marburg virus	SNAP+ MAR.N	Nucleoprotein N	x							x			
Arenavirus	Lassa virus	LAS.ectoGP1+SNAP	Ectodomain of Glycoprotein1	x							x			
		LAS.ectoGP2+SNAP	Ectodomain of Glycoprotein2	x							x			
		SNAP+ LAS.N	Nucleoprotein N	x							x			
	Junin virus	JUN .ectoGP1+SNAP	Ectodomain of Glycoprotein1				x				x			
		JUN .ectoGP2+SNAP	Ectodomain of Glycoprotein2				x				x			
		SNAP+ JUN.N	Nucleoprotein N				x				x			
	Machupo virus	MAC .ectoGP1+SNAP	Ectodomain of Glycoprotein1				x				x			
		MAC .ectoGP2+SNAP	Ectodomain of Glycoprotein2				x				x			
		SNAP+ MAC.N	Nucleoprotein N				x				x			
	Sabia virus	SAB .ectoGP1+SNAP	Ectodomain of Glycoprotein1				x				x			
		SAB .ectoGP2+SNAP	Ectodomain of Glycoprotein2				x				x			
		SNAP+ SAB.N	Nucleoprotein N				x				x			
	Guanarito virus	GUA .ectoGP1+SNAP	Ectodomain of Glycoprotein1				x				x			
		GUA .ectoGP2+SNAP	Ectodomain of Glycoprotein2				x				x			
		SNAP+ GUA.N	Nucleoprotein N				x				x			
Betacoronavirus	Human betacoronavirus (2cEMC/2012)	SNAP+ huCOV.N	Nucleoprotein N		x									
		huCOV.S+SNAP	Soluble form of spike S protein		x									
Hepacivirus	Hepatitis C virus genotype 1b (strain TCHM-R2/03)	SNAP+ HCV.C	Capsid protein C	x	x	x	x	x						
Hepevirus	Hepatitis E virus	SNAP+ HEV.C	Capsid protein C	x	x	x	x	x						
Enterovirus	Enterovirus 71 (strain JL-AFP-EV71-07-03)	SNAP+ EV71.VP1	Capsid protein VP1	x	x	x	x	x			x			
Plasmodium	*Plasmodium falciparum*	SNAP+MSP-1(19)+AMA-1 (III)	Proteins MSP-1(19)+ AMA-1(III) in tandem	x	x	x	x	x	x	x				
Leptospira	*Leptospira interrogans* serovar Lai str.56601	SNAP+ HbpA	The 55 kDa-form of protein HbpA	x	x	x	x	x			x	x	x	

In another aspect, the present invention relates to a method for manufacturing the kit of the invention as defined above, said method comprising the steps of:

(a) providing a least a first fusion protein comprising :

- a polypeptide comprising a first epitope that is recognized by a first target antibody and

- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,

(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,

(d) providing at least a second fusion protein comprising :

- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and

- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,

(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody,

wherein said at least first and at least second solid supports can be specifically identified from each other,

the kit of the invention comprising at least said first and second supports.

In another aspect, the present invention relates to a multiplex immuno screening assay comprising at least 2, 25, 50, 96 solid supports as defined above and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

In another aspect, the present invention relates to a multiplex immuno screening assay method comprising:

a) contacting one or several biological sample(s) with at least 2, 25, 50, 96 solid supports as defined above and wherein each of the solid supports emits a different and distinguishable wave length after excitation, and

b) detecting the presence or absence of target antibodies.

In a preferred embodiment, said target antibodies are specific to antigen from viruses to be detected in blood bank according to WHO or FDA guidelines, such as for example viruses selected from HBV, HCV, HIV1, HIV2, and WNV.

In another preferred embodiment, said target antibodies are specific to oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

In another preferred embodiment, each of said target antibodies are labeled with a detectable label.

In another aspect, the present invention relates to an apparatus for carrying out the method for manufacturing the kit of the invention as defined above, comprising a technical device for detecting the light sources emitted from the solid supports and the light source emitted from the target antibodies or labeled antibodies binding to the target antibodies, and a calculating or computer device for identifying which solid supports are bound with target antibodies, thereby indicating the presence or absence of antigens, bacteria, virus, or parasites in the analyzed sample.

In another aspect, the present invention relates to an *in vitro* method for diagnosing at least one target disease in a subject, said target disease being known to induce the synthesis of at least one target antibody in said subject, comprising performing the immunoassay of the invention, wherein said subject is diagnosed to be suffering from said at least one target disease if the amount of said at least one target antibody is higher than a control value.

This diagnosing method preferably enables to diagnose two, preferably three, and more preferably four target diseases in a subject in need thereof. This number is however not

limiting: it is indeed possible to diagnose until 100 target diseases in so far as it is possible to detect until 100 different antibodies with the detecting method of the invention.

In a preferred embodiment, said at least one target disease is a viral, a bacterial, a yeast or a fungi-mediated infection, preferably a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum*.

In a preferred embodiment, said *in vitro* method is used to diagnose at least 5, more preferably at least 15, more preferably at least 50, and even more preferably at least 100 viral and/or bacterial and/or parasite infections in said subject.

In a preferred embodiment, the control value used in said method represents the amount of said target antibody in a sample from a subject which is not suffering from said target disease, preferably, a healthy subject.

The methods of the invention can be used to diagnose infections in animals.

In particular, they can be used for the diagnosis of animal diseases, as well as a DIVA (Differentiating Infected from Vaccinated Animals) approach to differentiate naturally infected animals from vaccinated animals. The use of a DIVA strategy complementing novel vaccines would allow the implementation of vaccination as targeted control strategy alongside conventional strategies (test, slaughter and meat inspection). Moreover, increased test specificity would have a major economic benefit by reducing the numbers of false-positive animals that may be slaughtered needlessly. Lastly, improved sensitivity, particularly when novel diagnostic assays are used, would have a further benefit in reducing the economic burden of disease control even in the absence of vaccination

In a preferred embodiment, the methods of the invention are applied to human individuals.

The present invention finally relates to the use of the kit of the invention for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum*.

A new emerging arbovirus has been recently sequenced and affects cattle in Germany, Benelux and France. This virus is called Schmallenberg virus (SBV), and is related to the Akabane virus belonging to the Simbu serogroup of the *Orthobunyavirus* genus of the *Bunyaviridae* family. The viral genome of the Schmallenberg virus comprises three single-stranded RNA segments known as S, L and M. The S segment encodes the N nucleoprotein and the NSs non-structural protein. The N nucleoprotein shares antigenic determinants with different Bunyaviruses. The three RNA viral sequences of the BH80/11-4 strain of the Schmallenberg virus are available under the numbers HE649913.1, HE649914.1, and HE649912.1.

The present inventors observed that the fusion as a chimeric protein of the 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) with the SBV N protein greatly improves the production of recombinant N protein, in particular in invertebrate cells such as S2 cells.

The present inventors propose here for the first time to use the AGT enzyme (EC 2.1.1.63), a mutant thereof, a catalytic domain thereof or sub-fragments thereof, for enhancing the production of the N nucleoprotein from SBV in host cells, in particular in non-vertebrate cells. The enhancing effect is observed when the host cells express a fusion polypeptide comprising at least i) a secretion signal peptide which is functional in said host cells, ii) the AGT enzyme, mutant, catalytic domain or sub-fragments thereof, and iii) the N nucleoprotein of SBV. For the enhancing effect to occur, the AGT enzyme has to be physically linked, directly or indirectly (spacers and other amino acids might be introduced), to the protein of interest. Without being bound by theory, it is contemplated that the AGT enzyme acts as a chaperone protein, for example by facilitating the secretion from the host

cell and stabilising the synthesised fusion polypeptide in the supernatant of the host cells, or for preventing it to be metabolised during and after its synthesis and secretion from the host cells. In addition, it has been observed that AGT has a 3D globular structure comprising α helix (Wibley J.E.A. et al, 2000), which is compatible with a scaffolding role of AGT.

In the context of the present invention, "host" cells are any cells which can be used for producing recombinant proteins, such as "non-vertebrate" (or invertebrate) cells, vertebrate cells, plant cells, yeast cells, or prokaryote cells. They are preferably non-vertebrate and vertebrate cells.

Non-vertebrate (also known as invertebrate) comprises different phyla, the most famous being the Insect, Arachnida, Crustacea, Mollusca, Annelida, Cirripedia, Radiata, Coelenterata and Infusoria. They are now classified into over 30 phyla, from simple organisms such as sea sponges and flatworms to complex animals such as arthropods and molluscs. In the context of the invention, non-vertebrate cells are preferably insect cells, such as Drosophila or Mosquito cells, more preferably Drosophila S2 cells.

Examples of cells derived from vertebrate organisms that are useful as host cell lines include non-human embryonic stem cells or derivative thereof, for example avian EBX cells; monkey kidney CVI line transformed by SV40 sequences (COS-7, ATCC CRL 1651); a human embryonic kidney line (293); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells (CHO); mouse sertoli cells [TM4]; monkey kidney cells (CVI, ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor cells (MMT 060562, ATCC CCL51); rat hepatoma cells [HTC, Ml.5]; YB2/O (ATCC n° CRL1662); NIH3T3; HEK and TRI cells. In the context of the invention, vertebrate cells are preferably EBX, CHO, YB2/O, COS, HEK, NIH3T3 cells or derivatives thereof.

Plant cells which can be used in the context of the invention are the tobacco cultivars Bright Yellow 2 (BY2) and *Nicotiana tabaccum* 1 (NT-1).

Yeast cells which can be used in the context of the invention are: *Saccharomyces cerevisiae, Schizosaccharomyces pombe*, and *Hansenula polymorpha*, as well as methylotropic yeasts like *Pichia pastoris* and *Pichia methanolica*.

Prokaryote cells which can be used in the context of the invention are typically *E.coli* bacteria or *Bacillus subtilis* bacteria.

In another aspect, the present invention is thus drawn to a vector for expressing the N nucleoprotein from SBV in an host cell (SBV.N), comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the N nucleoprotein of SBV of SEQ ID NO: 16.

The N nucleoprotein from SBV will be referred to hereafter as the "heterologous protein", the "protein of interest", "chimeric protein", or the "recombinant protein".

The term "vector" herein means the vehicle by which a DNA or RNA sequence of a foreign gene can be introduced into a host cell so as to transform it and promote expression of the introduced sequence. As understood herein, a vector is a nucleic acid molecule, such as, for example, plasmids, phages, and viruses. They are discussed in greater detail below. Any type of plasmid, cosmid, YAC or viral vector may be used to prepare a recombinant nucleic acid construct which can be introduced to a host cell where expression of the protein of interest is desired. When expression of the protein of interest in a particular type of host cell is desired, viral vectors that selectively infect the desired cell type or tissue type can be used. Also important in the context of the invention are vectors for use in gene therapy (i.e. which are capable of delivering the nucleic acid molecule to a host organism).

For example, viral vectors, such as lentiviruses, retroviruses, herpes viruses, adenoviruses, adeno-associated viruses, vaccinia virus, baculovirus, and other recombinant viruses with desirable cellular tropism. Methods for constructing and using viral vectors are known in the art (see, Miller and Rosman, *BioTechniques*, 7:980-990, 1992).

Viral vectors that are actually preferred in the present invention are those that are well suited for use in vertebrate and non-vertebrate cells.

For non-vertebrate cells, preferred vectors are the arboviruses, the West Nile virus being particularly preferred, which are arthropod vectors. Other vectors that are known to efficiently be expressed in non-vertebrate cells are the baculoviruses.

For vertebrate cells, lentiviral, AAV, baculoviral and adenoviral vectors are preferred. The vectors suited for expression in mammalian host cells can also be of non-viral (e.g. plasmid DNA) origin. Suitable plasmid vectors include, without limitation, pREP4, pCEP4 (Invitrogen), pCI (Promega), pCDM8 and pMT2PC, pVAX and pgWiz.

For prokaryotic cells, plasmid, bacteriophage and cosmid vectors are preferred. Suitable vectors for use in prokaryotic systems include without limitation pBR322 (Gibco BRL), pUC (Gibco BRL), pBluescript (Stratagene), p Poly, pTrc; pET 11d; pIN; and pGEX vectors.

For plant cells, plasmid expression vectors such as Ti plasmids, and virus expression vectors such as Cauliflower mosaic virus (CaMV) and tobacco mosaic virus TMV are preferred.

Expression of recombinant proteins in yeast cells can be performed using three types of vectors: integration vectors (YIp), episomal plasmids (YEp), and centromeric plasmids (YCp): Suitable vectors for expression in yeast (e.g. *S. cerevisiae*) include, but are not limited to pYepSec1, pMFa, pJRY88, pYES2 (Invitrogen Corporation, San Diego, Calif.) and pTEF-MF (Dualsystems Biotech Product code: P03303).

Vectors which can be used for gene therapy are well-known in the art. They are for example lentivirus, retrovirus, adenovirus, poxvirus, herpes virus, measles virus, foamy virus or adeno-associated virus (AAV). Viral vectors can be replication-competent, or can be genetically disabled so as to be replication-defective or replication-impaired. Preferred gene therapy vector are the DNA Flap vectors as described in WO 99/055892, US 6,682,507 and WO 01/27300.

A sequence "encoding" an expression product, such as a RNA, polypeptide, protein or enzyme, is a nucleotide sequence that, when expressed, results in the production of that RNA, polypeptide, protein or enzyme; i.e., the nucleotide sequence "encodes" that RNA or it encodes the amino acid sequence for that polypeptide, protein or enzyme.

The vector of the invention contains a nucleotide sequence encoding a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof. These polypeptides have been defined above. Preferably, said AGT mutant is the SNAP enzyme of SEQ ID NO: 2, and is encoded for example by SEQ ID NO:15 or SEQ ID NO: 31, the latter having a G/C content of 51 %.

Preferably, the nucleotide expression vector of the invention further comprises cloning sites enabling the in-frame insertion of a heterologous DNA sequence encoding the protein of interest.

As meant in the present invention, the term "secretion signal peptide" designates a short (3-60 amino acids long) peptide chain that directs the transport of the N nucleoprotein outside the host cells.

Examples of secretion signals appropriate for the present invention include, but are not limited to, the signal peptide sequences of the mating factor (MF) alpha (US 5,879,926); invertase (WO 84/01153); PHO5 (DK 3614/83); YAP3 (yeast aspartic protease 3; WO 95/02059); and BAR1 (WO 87/02670).

In the context of the invention, this secretion signal peptide is preferably functional either in non-vertebrate cells or in vertebrate cells, or both.

Examples of secretion signal peptides which are functional in insect cells are: the insect ssBiP (SEQ ID NO: 37, for example encoded by the DNA sequence SEQ ID NO: 22), the BiP-like peptide signal of SEQ ID NO: 24 (for example encoded by the DNA sequence SEQ ID NO: 23), the BiP-like peptide signal of SEQ ID NO:153 (for example encoded by the DNA sequence SEQ ID NO:152) and any peptide signal present in an arbovirus, for example the envelop E protein of the West-Nile virus (SEQ ID NO: 38).

Interestingly, the above-mentioned BiP-like peptide signal of SEQ ID NO:24 is functional in both non-vertebrate and vertebrate cells. This BiP-like signal corresponds to the BiP peptide signal of SEQ ID NO: 37 in which the last Glycine amino acid has been replaced by the amino acid sequence Pro Thr Ala Leu Ala (SEQ ID NO: 39) which corresponds to the cleavage site of the E protein of the Dengue virus. Accordingly, the BiP-like signal will

be advantageously cleaved once the protein will be translated and secreted in the supernatant of the host cells.

A variety of secretion signals is also available for expression in yeast host cells, e.g. in *S. cerevisiae*. These include the prepro-alpha factor, HSp150, PHO1, SUC2, KILM1 (killer toxin type 1), and GGP1.

A cloning site is a sequence which facilitates cloning of a gene encoding a protein of interest into the expression system. It contains restriction sites, or restriction recognition sites, i.e. locations on a DNA molecule containing specific sequences of nucleotides, which are recognized by restriction enzymes (see for example in the figures). These are generally palindromic sequences (because restriction enzymes usually bind as homodimers), and a particular restriction enzyme may cut the sequence between two nucleotides within its recognition site, or somewhere nearby. The cloning sites are well known for the man skilled in the art.

In a preferred embodiment of the invention, the DNA sequence encoding said AGT enzyme is located in 5' or in 3' of the DNA sequence encoding said heterologous protein of interest, preferably in 5'. Therefore, the AGT enzyme is directly or indirectly linked to the heterologous protein/polypeptide of interest, and preferably located at the N-terminal end of the heterologous protein/polypeptide of interest. The DNA sequence encoding the fusion polypeptide comprising said peptide signal, said AGT enzyme, mutant or catalytic domain, and said recombinant protein of interest, can be operatively associated with an inducible promoter which is functional in the same host cells as the peptide signal is.

More preferably, in the vector of the invention, said open reading frame is operatively associated with an inducible promoter which is functional in the same host cell as the peptide signal is.

A coding sequence is "operatively associated with" an expression control sequence (i.e. transcriptional and translational control sequences) in a cell, when RNA polymerase transcribes the coding sequence into RNA, which is then trans-RNA spliced (if it contains introns) and, if the sequence encodes a protein, is translated into that protein.

A "promoter" is a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA). Within the promoter sequence will be found a transcription initiation site (conveniently found, for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

Promoters which may be used to control gene expression in the context of the present invention are for example the one that are functional in non-vertebrate cells or in vertebrate cells. For example, for non-vertebrate cells, the regulatory sequences of the metallothionein gene can be used (Brinster et al., *Nature,* 296:39-42, 1982).

Preferably, the inducible promoter which is present in the vector of the invention has a promoter activity in an insect cell, and more preferably in a *Drosophila* cell. It is for example the *Drosophila metallothionein* promoter pMT (Lastowski-Perry et al, *J.Biol. Chem.* 260:1527 (1985)), which directs high level transcription of the gene in the presence of metals, e.g. $CuSO_4$. Alternatively, the Drosophila actin 5C gene promoter, which is a constitutive promoter and does not require addition of a metal, can be used (B.J.Bond et al, *Mol. Cell. Biol.* 6:2080 (1986)). Examples of other known Drosophila promoters include, e.g. the inducible heatshock (Hsp70) and COPIA LTR promoters. The SV40 early promoter gives lower level of expression than the *Drosophila* metallothionein promoter.

Preferably, the inducible promoter which is present in the vector of the invention has a promoter activity in a *Drosophila melanogaster* cell, preferably in Drosophila S2 cells. It is for example the metallothionein promoter which is thoroughly described in Lastowski-Perry et al, *J.Biol. Chem.* 260: 1527 (1985).

Promoters suitable for constitutive expression in mammalian cells include the cytomegalovirus (CMV) immediate early promoter, the adenovirus major late promoter, the phosphoglycero kinase (PGK) promoter, and the thymidine kinase (TK) promoter of herpes simplex virus (HSV)-1. Inducible eukaryotic promoters regulated by exogenously supplied compounds, include without limitation, the zinc-inducible metallothionein (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088), the ecdysone insect

promoter, the tetracycline-repressible promoter, the tetracycline-inducible promoter, the RU486-inducible promoter and the rapamycin-inducible promoter.

Preferably, the promoter which is present in the vector of the invention has a promoter activity in a mammal cell, preferably in HeLa cells. It is for example the SV 40 promoter.

A range of yeast promoters is available for protein expression in yeast host cells. Some like ADH2, SUC2 are inducible and others like GAPDH are constitutive in expression. Other promoters suitable for expression in yeast include the TEF, PGK, MF alpha, CYC-1, GAL-1, GAL4, GAL10, PHO5, glyceraldehyde-3-phosphate dehydrogenase (GAP or GAPDH), and alcohol dehydrogenase (ADH) promoters.

For use in plant cells, the most commonly used promoter is the cauliflower mosaic virus (CaMV) 35S promoter or its enhanced version, but a number of alternative promoter can be used, such as the hybrid *(ocs)3mas* promoter or the ubiquitin promoter from maize and *Arabidospsis thaliana*. In contrast to these constitutive promoters, the rice α-amylase RAmy3D promoter is induced by sugar deprivation (Hellwig S et al., *Nat. Biotechnol.*2004; 22(11):1415-22).

Promoters suitable for expression in *E. coli* host cell include, but are not limited to, the bacteriophage lamba pL promoter, the lac, TRP and IPTG-inducible pTAC promoters.

It is preferred that the secretion signal peptide and the inducible promoter are functional in the same host cell.

More preferably, the secretion signal peptide and the inducible promoter are functional in both Drosophila S2 cells and vertebrate cells.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus.

Once an appropriate vector has been constructed and transfected into the selected host cell, preferably a Drosophila cell line, the expression of a heterologous protein is induced by the addition of an appropriate inducing agent for the inducible promoter. For example cadmium or copper are inducing agents for the Hsp70 promoter. For constitutive promoters, such as the actin 5C promoter, no inducing agent is required for expression.

In another embodiment of the invention, the nucleotide expression vector encodes at least one peptide cleavage site, which is preferably located between the AGT enzyme or its catalytic domain and the recombinant protein of interest.

A peptide cleavage site (also called "peptide cleavage site") is an amino acid sequence which is recognized by at least one protease enzyme (for example serine protease, cysteine protease, among others). An example of a peptide cleavage site is the enterokinase cleavage site of SEQ ID NO: 40 (AspAspAspAspLys/Asp). The enterokinase is a serine protease enzyme (EC 3.4.21.9) which is known to convert inactive trypsinogen into active trypsin by cleavage at the C-terminal end of the sequence: Val--$(Asp)_4$--Lys--Ile--Val~ (trypsinogen) → Val--$(Asp)_4$--Lys (hexapeptide) + Ile--Val~ (trypsin). Enterokinase cleaves after lysine if the Lys is preceded by four Asp and not followed by a proline residue.

Another useful peptide cleavage site is the cleavage site of the so-called "TEV protease", having the amino acid sequence SEQ ID NO: 32 (pro-TEV1) or SEQ ID NO: 33 (pro-TEV2) (Glu Asn Leu Tyr Phe Gln Ser or Gly respectively). Such cleavage sites can be encoded for example by SEQ ID NO:29 and 30. TEV protease is the common name for the 27 kDa catalytic domain of the nuclear inclusion protein encoded by the tobacco etch virus. It is commercially available (Invitrogen).

The cleavage site from the membrane precursor prM from Dengue virus serotype 1 (SEQ ID NO: 39) may also be used in the vector of the invention.

In another embodiment, the nucleotide expression vector of the invention further encodes a label, preferably located at the C-terminal end of the recombinant protein in the fusion polypeptide of the invention (comprising the peptide signal, the AGT protein or homologous thereof, and the recombinant protein). In the context of the invention, a "label" is dedicated to facilitate the recovery of the polypeptide from the crude lysate of the host cell, and is preferably selected from the group comprising: fluorescent proteins, poly-

histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; flu HA tags; c-myc tag *Herpes simplex* virus glycoprotein D (gD) tags, Flag-peptides, alpha-tubulin epitopes, or T7 gene 10 protein peptide tags. However, any other label might be used. In a preferred embodiment of the invention, the vectors comprise the DNA of SEQ ID NO: 28 encoding a hexa-histidine tag which has the SEQ ID NO: 27.

In another embodiment, the nucleotide expression vector of the invention further encodes spacer sequence(s), located preferably between the AGT enzyme (or its catalytic domain) and the recombinant protein of interest and/or between the recombinant protein of interest and the label. In the context of the invention, a spacer sequence is an amino acid sequence comprising at least three amino acids, dedicated to spatially separate two linked polypeptides (these polypeptides being then indirectly linked). Such spacer can be for example the amino acid sequence Glycine-Glycine-Glycine-Serine (GGGS, SEQ ID NO: 25) and the DNA spacer sequence encoding it can be SEQ ID NO: 26. In the context of this invention, this DNA sequence is hereafter designated as "DNA spacer sequence" and is located between the DNA encoding AGT or its catalytic domain, and the recombinant DNA sequence, preferably upstream from the DNA sequence encoding the peptide cleavage site.

As used herein, the term "pDeSNAPUniv" designates a DNA cassette encoding, in a single open reading frame, from 5' to 3':

a) a secretion signal peptide,

b) an AGT protein of SEQ ID NO:1, a mutant, a fragment or a catalytic domain thereof, in particular the SNAP mutant of SEQ ID NO:2,

c) at least one peptide cleavage site,

d) at least one label, and

e) at least one spacer sequence.

This pDeSNAPUniv DNA cassette encodes a secretion signal peptide which is advantageously the BiP-like peptide signal of SEQ ID NO:24 or the ssBiP peptide signal of SEQ ID NO:37, the SNAP mutant of SEQ ID NO:2, a label which is advantageously a

His-tag of SEQ ID NO:27, a peptide cleavage site which is advantageously either the pro-TEV of SEQ ID NO:32 or the pro-TEV of SEQ ID NO:33, and/or a spacer sequence which has advantageously the amino acid sequence SEQ ID NO:25.

More preferably, the pDeSNAPUniv DNA cassette comprises, from 5' to 3', the sequence SEQ ID NO:23 encoding the BiP-like secretion signal, the SEQ ID NO:15 or 31 encoding the SNAP mutant, the spacer sequence of SEQ ID NO:26, the peptide cleavage site pro-TEV of SEQ ID NO:29, the peptide cleavage site pro-TEV of SEQ ID NO:30, the spacer sequence of SEQ ID NO:26 and the sequence SEQ ID NO:28 encoding the His-tag label (see figure 8, showing the structure of the pDeSNAPUniv cassette). Such a pDeSNAPUniv DNA cassette is for example SEQ ID NO:34.

This "pDeSNAPUniv" cassette is held as "universal" since it can be inserted in any kind of vectors dedicated to transfect host cells in order to produce heterologous proteins, namely vertebrate vectors (such as pcDNA3 or pCI-neo vectors) as well as non-vertebrate vectors (such as pMT/BiP/V5-HisA which is useful in the DES system from Invitrogen). Examples of plasmid comprising said universal sequence is SEQ ID NO:43 (pMT/BiP/V5-HisA from Invitrogen comprising the pDeSNAP Univ cassette), SEQ ID NO:44 (pUC57 from Invitrogen comprising the pDeSNAP Univ cassette) or SEQ ID NO:45 (pcDNA3 from Invitrogen comprising the pDeSNAP Univ cassette).

Another example of plasmid comprising said universal sequence is SEQ ID NO:105 which is a pUC57 plasmid comprising, from 5' to 3', the constitutive promoter of *Orgyia pseudotsugata* multicapsid nucleoprotein virus-immediate-early 2 promoter (OpIE2SP) the BiPlike signal peptide of SEQ ID NO:152, the SNAP-like sequence of SEQ ID NO:31, the spacer sequence of SEQ ID NO:26, the pro-TEV1 sequence SEQ ID NO:29, and the C-term peptide tag of SEQ ID NO:106.

Once the heterologous sequence of a protein of interest such as SBV.N is cloned herein, such a vector can be advantageously transfected in either vertebrate or non-vertebrate host cells, so as to produce the protein of interest in high amounts.

In a preferred embodiment, the vector of the invention comprises a so-called "pDeSNAP Univ/SBV.N cassette" i.e., a pDeSNAPUniv DNA cassette in which the sequence of the

N nucleoprotein of SBV has been inserted, said pDeSNAP Univ/SBV.N cassette comprising a nucleotide sequence encoding, in a single open reading frame, from 5' to 3':

a) a secretion signal peptide,

b) an AGT protein of SEQ ID NO:1, a mutant, a fragment or a catalytic domain thereof, in particular the SNAP mutant of SEQ ID NO:2,

c) at least one peptide cleavage site,

d) the N nucleoprotein of SBV of SEQ ID NO: 16,

e) at least one label, and

f) at least one spacer sequence.

This pDeSNAP Univ/SBV.N DNA cassette encodes a secretion signal peptide which is advantageously the BiP-like peptide signal of SEQ ID NO:24 or the ssBiP peptide signal of SEQ ID NO:37, the SNAP mutant of SEQ ID NO:2, the N nucleoprotein of SBV of SEQ ID NO:16, a label which is advantageously a His-tag of SEQ ID NO:27, a peptide cleavage site which is advantageously either the pro-TEV of SEQ ID NO:32 or the pro-TEV of SEQ ID NO:33, and/or a spacer sequence which has advantageously the amino acid sequence SEQ ID NO:25.

More preferably, the pDeSNAP Univ/SBV.N DNA cassette comprises, from 5' to 3', the sequence SEQ ID NO:23 encoding the BiP-like secretion signal or the SEQ ID NO:22 encoding the ssBiP secretion signal, the SEQ ID NO:15 or 31 encoding the SNAP mutant, the spacer sequence of SEQ ID NO:26, the peptide cleavage site pro-TEV1 of SEQ ID NO:29, the sequence SEQ ID NO: 17 encoding the N nucleoprotein of SBV, the peptide cleavage site pro-TEV2 of SEQ ID NO:30, the spacer sequence of SEQ ID NO:26 and the sequence SEQ ID NO:28 encoding the His-tag label.

Even more preferably, the pDeSNAP Univ/SBV.N DNA cassette comprises, from 5' to 3', the sequence SEQ ID NO:22 encoding the ssBiP secretion signal, the SEQ ID NO:31 encoding the SNAP mutant, the spacer sequence of SEQ ID NO:26, the peptide cleavage site pro-TEV1 of SEQ ID NO:29, the sequence SEQ ID NO: 17 encoding the N

nucleoprotein of SBV, the peptide cleavage site pro-TEV2 of SEQ ID NO:30, the spacer sequence of SEQ ID NO:26 and the sequence SEQ ID NO:28 encoding the His-tag label. Such a pDeSNAP Univ/SBV.N cassette is for example SEQ ID NO:35.

Alternatively, the pDeSNAP Univ/SBV.N DNA cassette can comprise, from 5' to 3', the sequence SEQ ID NO:23 encoding the BiP-like secretion signal, the SEQ ID NO:31 encoding the SNAP mutant, the spacer sequence of SEQ ID NO:26, the peptide cleavage site pro-TEV1 of SEQ ID NO:29, the sequence SEQ ID NO: 17 encoding the N nucleoprotein of SBV, the peptide cleavage site pro-TEV2 of SEQ ID NO:30, the spacer sequence of SEQ ID NO:26 and the sequence SEQ ID NO:28 encoding the His-tag label. Such a pDeSNAP Univ/SBV.N cassette is for example SEQ ID NO:36 (whose structure is shown on figure 9).

Thus, in a preferred embodiment, the vector of the invention comprises the pDeSNAP Univ/SBV.N cassette having the nucleotide sequence SEQ ID NO: 35 or the nucleotide sequence SEQ ID NO:36.

More precisely, the pDeSNAP Univ/SBV.N cassette nucleotide sequence of SEQ ID NO:35 comprises:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the SBV.N DNA sequence SEQ ID NO: 17 (which corresponds to the natural SBV.N sequence, in which the internal *EcoRV* site has been deleted and two *EcoRV* and *XmaI* sites have been added at the extremities),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

And the pDeSNAP Univ/SBV.N cassette nucleotide sequence SEQ ID NO:36 comprises (see also figure 9):

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the SBV.N DNA sequence SEQ ID NO: 17 (which corresponds to the natural SBV.N sequence, in which the internal *EcoRV* site has been deleted and two *EcoRV* and *XmaI* sites have been added at the extremities),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

Interestingly, the pDeSNAP Univ/SBV.N cassette nucleotide sequence of SEQ ID NO:36 cassette also comprises in addition an *NheI* site upstream of the ATG, a *BglII* site between the BiP-like sequence and the SNAP-like sequence, and an *AgeI* site and a *HindIII* site which are both located downstream of the stop codon.

Vectors of the invention are for example SEQ ID NO:43 (which is the pMT/BiP/V5-HisA plasmid from Invitrogen comprising the pDeSNAP Univ cassette) in which the SBV.N DNA sequence SEQ ID NO: 17 has been inserted, SEQ ID NO:44 (which is the pUC57 plasmid from Invitrogen comprising the pDeSNAP Univ cassette) in which the SBV.N DNA sequence SEQ ID NO: 17 has been inserted or SEQ ID NO:45 (which is the pcDNA3 plasmid from Invitrogen comprising the pDeSNAP Univ cassette) in which the SBV.N DNA sequence SEQ ID NO: 17 has been inserted.

Vectors of the invention are also provided in the S2 cells which have been deposited at the Centre National de Culture et de Microorganismes (CNCM), Institut Pasteur (25 rue du Docteur Roux, 75724 Paris cedex 15, France) on April 24, 2012, under the number CNCM I-4616.

In another aspect, the present invention targets a recombinant cell which is stably transfected by a vector of the invention, preferably a vector comprising the nucleotide sequence SEQ ID NO: 35 or SEQ ID NO: 36.

Preferably, in this aspect of the invention, said recombinant cell is a non-vertebrate cell, preferably an insect cell, and more preferably a S2 cell.

Non-vertebrate cells can be any cells from the Insect, Arachnida, Crustacea, Mollusca, Annelida, Cirripedia, Radiata, Coelenterata and Infusoria. In the context of the invention, non-vertebrate cells are preferably insect cells, such as Drosophila or Mosquito cells. They are more preferably a Drosophila S2 cells. In this case, the expression vector of the invention comprises for example SEQ ID NO: 35.

Drosophila S2 cells have been widely described. They are especially suited to high-yield production of protein, because they can be maintained in suspension cultures at room temperature (24 ± 1°C). Culture medium is M_3 supplemented with between 5 and 10 % (v/v) heat-inactivated fetal bovine serum (FBS). In the preferred embodiment of the invention, the culture medium contains 5% FBS. After induction, the cells are cultured in serum-free media. In this media, the S2 cells can be grown in suspension cultures, for example in 250 mL to 2000 mL spinner flasks, with stirring at 50-60 rpm. Cells densities are typically maintained between 10^6 and 10^7 cells per mL.

In a preferred embodiment, the recombinant cell of the invention is the S2 cell which has been deposited at the Centre National de Culture et de Microorganismes (CNCM), Institut Pasteur (25 rue du Docteur Roux, 75724 Paris cedex 15, France) on April 24, 2012, under the number CNCM I-4616.

In another preferred embodiment, said recombinant cell is a vertebrate cell.

Preferably, said vertebrate recombinant cell is a mammal cell, a preferably CHO, YB2/O, COS, HEK, NIH3T3, HeLa cell or derivatives thereof. More preferably, in this case, the expression vector of the invention comprises SEQ ID NO: 36.

In another aspect of the present invention, the said recombinant cell is used to amplify and purify the expression vectors of the invention, preferably those comprising SEQ ID NO: 35 or 36.

In this aim, the nucleotide expression vectors of the invention may also comprise a gene encoding a selection marker, and/or a terminator sequence. Selection markers genes that can be included in the construct are typically those that confer selectable phenotypes such as resistance to antibiotics (e.g. blasticidin, ampicillin, kanamycin, hygromycin, puromycin, chloramphenicol).

Methods for producing expression vectors are well-known in the art.

In another aspect, the recombinant cell of the invention is used so as to produce the N nucleoprotein of the Schmallenberg virus in high amounts.

Thus, in a particular embodiment, the present invention is also drawn to a method for the production of the N nucleoprotein of the Schmallenberg virus, the method comprising the steps of:

(a) obtaining the vector of the invention, said vector comprising for example the DNA sequence SEQ ID NO:35 or SEQ ID NO:36,

(b) transfecting an host cell (preferably an insect cell or a mammal cell) with the polynucleotide obtained under step (a);

(c) allowing for the expression of said polynucleotide obtained under step (b) to produce the N nucleoprotein of the Schmallenberg virus;

(d) optionally, cleaving the AGT polypeptide,

(e) recovering the N nucleoprotein of the Schmallenberg virus,

(f) optionally, purifying the N nucleoprotein of the Schmallenberg virus.

For performing the different steps of the method of the present invention, there may be employed conventional molecular biology, microbiology and recombinant DNA techniques within the skills of the person of the art. Such techniques are fully explained in the literature. See, for example, Sambrook, Fitsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (referred to herein as "Sambrook et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization(B. D. Hames & S. J. Higgins, eds. 1984); Animal Cell Culture (R. I. Freshney, ed. 1986); Immobilized Cells and Enzymes (IRL Press,

1986); B. E. Perbal, A Practical Guide to Molecular Cloning (1984); F. M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

The term "transfection" means the introduction of a foreign nucleic acid into a eukaryotic host cell so that the host cell will express the introduced gene or sequence to produce the N nucleoprotein of Schmallenberg virus. A host cell that receives and expresses introduced DNA or RNA has been "transfected" and is a "transfectant" or a "clone". The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell or cells of a different genus or species.

In the context of the invention, the transfection of the host cells with the polynucleotides can be performed by a classical method in the art, for example by transfection, infection, or electroporation. In another embodiment, the vector of the invention can be introduced *in vivo* by lipofection (as naked DNA), or with other transfection facilitating agents (peptides, polymers, etc.). Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 84:7413-7417, 1987). Useful lipid compounds and compositions for transfer of nucleic acids are described in WO 95/18863 and WO 96/17823, and in U.S. 5,459,127. Lipids may be chemically coupled to other molecules for the purpose of targeting (see, Mackey et al., *Proc. Natl. Acad. Sci. U.S.A.*, 85:8027-8031, 1988). Targeted peptides, such as hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically. Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptides (see WO 95/21931), peptides derived from DNA binding proteins (see WO 96/25508), or a cationic polymer (see WO 95/21931). It is also possible to introduce the vector *in vivo* as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, such as electroporation, microinjection, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, Wu et al., *J. Biol. Chem.*, 267:963-967, 1992; Wu and Wu, J. *Biol. Chem.*, 263:14621-14624, 1988; Williams et al., *Proc. Natl. Acad. Sci. U.S.A.*, 88:2726-2730, 1991).

The term “allowing for the expression” of a polynucleotide herein means that the stimulus of the regulatory sequences that are present in the vector (e.g. the stimulus activating the

inducible promoter), and all the required components are present in a sufficient amount for the translation of the polynucleotide to occur.

If need be, the AGT/SNAP polypeptide can be cleaved off the produced fusion protein by adding a protease having a defined cleavage site to the supernatant of or into the recombinant cells. For example, when a vector comprising the pDeSNAP Univ cassette of SEQ ID NO: 35 or 36 is used, the cleavage of the pro-TEV cleavage site ENLKYFQ/G(S) is obtained by adding the TEV protease to the supernatant of the recombinant cells. Alternatively, the AGT/SNAP polypeptide can be maintained so as to enhance the life-span of the N nucleoprotein from SBV.

Moreover, the skilled artisan will appreciate that an expressed or secreted protein or polypeptide can be detected in the culture medium used to maintain or grow the present host cells. The culture medium can be separated from the host cells by known procedures, such as centrifugation or filtration. The protein or polypeptide can then be detected in the cell-free culture medium by taking advantage of known properties characteristic of the protein or polypeptide. Such properties can include the distinct immunological, enzymatic or physical properties of the protein or polypeptide. For example, if a protein or polypeptide has a unique enzyme activity an assay for that activity can be performed on the culture medium used by the host cells. Moreover, when antibodies reactive against a given protein or polypeptide are available, such antibodies can be used to detect the protein or polypeptide in any known immunological assay (for example as in Harlowe, et al., 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press).

Recovery of the nucleoprotein N from SBV is mediated by the means well-known in the art, including, but not limited to, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, precipitation and salting-out chromatography, extraction, and countercurrent distribution, and the like. As it is preferable to produce the protein of interest in the recombinant system of the invention linked with a label, said label will facilitate the recovery of the polypeptide from the crude lysate of the host cell by chromatography on an appropriate solid-phase matrix. Alternatively, antibodies produced against the protein or against peptides derived therefrom can be used as recovery reagents.

The present Inventors discovered that the fusion proteins generated with the method of the invention generally do not need to be further purified. However, a further step (g) of purification may be performed, if required.

A purified material may contain less than about 50 %, preferably less than about 75 %, and most preferably less than about 90 %, of the cellular components with which it was originally associated. The term "substantially pure" indicates the highest degree of purity which can be achieved using conventional purification techniques known in the art.

In an embodiment of the invention, the methods of the invention enable to obtain at least 40 mg/L, preferably at least 50 mg/L, more preferably at least 60 mg/L of the substantially pure N nucleoprotein of the Schmallenberg virus in the recovered cell culture supernatant.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the N nucleoprotein of Schmallenberg virus of SEQ ID NO: 16.

In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above).

This fusion polypeptide preferably further comprises a label, as defined above. This label is preferably a poly-histidine label, and is preferably located at the C terminal end of the N nucleoprotein of the Schmallenberg virus.

The fusion polypeptide of the invention is for example the amino acid sequence of SEQ ID NO: 41 (corresponding to the BiPlike/SNAP/SBV.N/Histag fusion protein) or SEQ ID NO: 46 (corresponding to the ssBiP/SNAP/SBV.N/Histag fusion protein) or SEQ ID NO:42 (corresponding to the SNAP/SBV.N fusion protein).

Finally, the chimeric protein SNAP-SBV. N may be useful as a diagnostic agent for the detection of the viral infection by the Schmallenberg virus, or for the detection of antibodies specific of the said virus in biological fluids, such as blood, serum, saliva, and the like.

Thus, in another aspect, the present invention is also drawn to the use of the fusion protein [SNAP- SBV. N] obtained by any method of the invention for identifying the presence of

said pathogenic or non-pathogenic microorganisms in a biological sample, for example thanks to the immunoassay of the present invention.

In other aspects, the present invention also relates to vectors expressing fusion proteins of particular interest, said fusion proteins comprising a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, that is fused in frame with interesting antigens, such as viral or bacterial antigens, microbial peptides and/or polypeptides of interest. These vectors are detailed below.

Echovirus antigen

In another aspect, the present invention relates to a vector for expressing an echovirus antigen, for example the VP1 protein of the enterovirus 71 (*Picornaviridae*), in a host cell. In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the VP1 protein of the enterovirus 71 (EV71, see for example Kolpe A.B. et al, *Virus Research* 2012).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/EV71.VP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence SEQ ID NO:47 encoding the VP1 protein from the EV71 virus strain JL-AFP-EV71-07-03 (Genebank#JQ715713) has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/EV71.VP1 cassette having the nucleotide sequence SEQ ID NO: 48 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),

- the DNA sequence SEQ ID NO:47 encoding the VP1 protein from the EV71 virus strain JL-AFP-EV71-07-03 (Genebank#JQ715713),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the VP1 protein from the EV71 virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 49 (corresponding to the SNAP-like/proTEV1/EV71.VP1/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- EV71.VP1] for identifying the presence of the enterovirus 71 in a biological sample, for example thanks to the immunoassay of the present invention.

Flavivirus Antigens

In another aspect, the present invention relates to vectors for expressing particular Flavivirus antigens in a host cell.

In a preferred embodiment, said Flavivirus antigen is the soluble E protein (sE) from the Japanese Encephalitis virus (JEV.sE). More particularly, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT),

a mutant, a fragment or a catalytic domain thereof, and c) the sE protein from the Japanese Encephalitis virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JEV.sE cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequences of gene encoding the soluble E protein (sE) from the Japanese Encephalitis virus (JEV) have been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/JEV.sE cassette having the nucleotide sequence SEQ ID NO: 50 comprising, from 5' to 3':

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the prM/M sequence from JEV strain SA-14 (Genbank#M55506),
- the DNA sequence encoding the E[1-395] sequence from JEV strain SA-14 (Genbank#M55506),
- a DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the soluble E protein (sE) from the Japanese Encephalitis virus (JEV.sE). In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 51 (corresponding to the JEV.sE/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-JEV.sE] for identifying the presence of the Japanese Encephalitis virus

(JEV) in a biological sample, for example thanks to the immunoassay of the present invention.

In a preferred embodiment, said Flavivirus antigen is the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 1 (JE-1.EDIII), of genotype 2 (JE-3.EDIII), of genotype 4 (JE-4.EDIII), or of genotype 5 (JE-5.EDIII).

In another aspect, the present invention therefore relates to a vector for expressing the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype I (JE-I.EDIII), of genotype 2 (JE-2.EDIII), of genotype 4 (JE-4.EDIII), or of genotype 5 (JE-5.EDIII) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the EDIII protein from the Japanese encephalitis virus of genotype 1 (JE-I.EDIII), of genotype 2 (JE-2.EDIII), of genotype 4 (JE-4.EDIII), or of genotype 5 (JE-5.EDIII).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JE-I.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of gene encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 1 (JE-1.EDIII) has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ JE-1.EDIII cassette having the nucleotide sequence SEQ ID NO: 52 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence SEQ ID NO:54 encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 1 (Genebank#AY377577),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JE-2.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the

sequence of the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 2 (JE-2.EDIII) has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ JE-2.EDIII cassette having the nucleotide sequence SEQ ID NO: 59 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence SEQ ID NO:55 encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 2 (Genebank#L-43566),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JE-4.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 4 (JE-4.EDIII) has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ JE-4.EDIII cassette having the nucleotide sequence SEQ ID NO: 61 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence SEQ ID NO:56 encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 4 (Genebank#U70408),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JE-5.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 5 (JE-5.EDIII) has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ JE-5.EDIII cassette having the nucleotide sequence SEQ ID NO: 63 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence SEQ ID NO:57 encoding the domain III of the envelope E protein (EDIII protein) from the Japanese encephalitis virus of genotype 5 (Genebank#JN587258),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to recombinant cells which are stably transfected by said vectors.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the EDIII protein from the JE-1, JE-2, JE-4, or JE-5 virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 53 (corresponding to the SNAP-like/JE-1.EDIII/Histag fusion protein), SEQ ID NO: 60 (corresponding to the SNAP-like/JE-2.EDIII/Histag fusion protein) SEQ ID NO: 62 (corresponding to the SNAP-like/JE-4.EDIII/Histag fusion protein) or SEQ ID NO: 64 (corresponding to the SNAP-like/JE-5.EDIII/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of any of these fusion proteins [SNAP- JE-1.EDIII], [SNAP- JE-2.EDIII], [SNAP- JE-4.EDIII] or [SNAP- JE-5.EDIII] for identifying the presence of the Japanese encephalitis virus of genotype 1, 2, 4 or 5 respectively in a biological sample, for example thanks to the immunoassay of the present invention.

In another aspect, the present invention is drawn to a vector for expressing the domain III of the envelope E protein (EDIII protein) from the Rabensburg virus (RabV) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the domain III of the envelope E protein (EDIII protein) from the Rabensburg virus (RabV).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/RabV.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the EDIII protein from the Rabensburg virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ RabV.EDIII cassette having the nucleotide sequence SEQ ID NO: 65 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence SEQ ID NO:58 encoding the domain III of the envelope E protein (EDIII protein) from the Rabensburg virus (Genebank#AY65264),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the EDIII protein from the Rabensburg virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 66 (corresponding to the SNAP-like/RabV.EDIII/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- RabV.EDIII] for identifying the presence of the Rabensburg virus in a biological sample, for example thanks to the immunoassay of the present invention.

Alphavirus Antigens

In another aspect, the present invention is relates to vectors for expressing particular alphavirus antigens, for example the soluble E2 protein from the Ross River virus (RR.sE2) or from the Mayaro virus (MAY.sE2), in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the sE2 protein from the Ross River virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/RR.sE2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the sE2 gene from the Ross River virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ RR.sE2 cassette having the nucleotide sequence SEQ ID NO: 69 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the sE2 protein of the Ross River virus strain QML1 (Genbank#GQ433354),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the sE2 protein from the Ross River virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 70 (corresponding to the RR.sE2/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- RR.sE2] for identifying the presence of the Ross River virus in a biological sample, for example thanks to the immunoassay of the present invention.

The present invention is also drawn to a vector for expressing the soluble E2 protein from the Mayaro virus (MAY.sE2) in an host cell, comprising the nucleotide sequence encoding

a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the sE2 protein from the Mayaro virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MAY.sE2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the sE2 gene from the Ross River virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAY.sE2 cassette having the nucleotide sequence SEQ ID NO: 71 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the corrected sE2 protein (E2-S203C) of the Mayaro virus strain IQD2668 (Genbank#DQ487429.1),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the sE2 protein from the Mayaro virus (MAY.sE2). In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 72 (corresponding to the MAY.sE2/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- MAY.sE2] for identifying the presence of the Mayaro virus in a biological sample, for example thanks to the immunoassay of the present invention.

Equine Encephalitis virus antigens

In another aspect, the present invention relates to vectors for expressing particular Equine Encephalitis virus antigens, for example the soluble E2 protein from the Western Equine Encephalitis virus (WEE.sE2), the Eastern Equine Encephalitis virus (EEE.sE2) or the Venezuelan Equine Encephalitis virus (VEE.sE2) in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the soluble E2 protein from the Western Equine Encephalitis virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/ WEE.sE2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the sE2 gene from the Western Equine Encephalitis virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ WEE.sE2 cassette having the nucleotide sequence SEQ ID NO: 73 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the sE2 protein from Western Equine Encephalitis virus strain (Genbank#NC00390808),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the sE2 protein from the WEE virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 74 (corresponding to the WEE.sE2/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- WEE.sE2] for identifying the presence of the Western Equine Encephalitis virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is also drawn to a vector for expressing the soluble E2 protein from the Eastern Equine Encephalitis virus (EEE.sE2) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the soluble E2 protein from the Eastern Equine Encephalitis virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/ EEE.sE2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the sE2 gene from the Eastern Equine Encephalitis virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ EEE.sE2 cassette having the nucleotide sequence SEQ ID NO: 75 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the sE2 protein from Eastern Equine Encephalitis virus strain (Genbank#EF151502),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the sE2 protein from the EEE virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino

acid sequence of SEQ ID NO: 76 (corresponding to the EEE.sE2/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- EEE.sE2] for identifying the presence of the Eastern Equine Encephalitis virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is also drawn to a vector for expressing the soluble E2 protein from the Venezuelan Equine Encephalitis virus (VEE.sE2) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the soluble E2 protein from the Venezuelan Equine Encephalitis virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/ VEE.sE2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the sE2 gene from the Venezuelan Equine Encephalitis virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ VEE.sE2 cassette having the nucleotide sequence SEQ ID NO: 77 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the sE2 protein from Venezuelan Equine Encephalitis virus strain (Genbank#AY973944),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the sE2 protein from the VEE virus. In this fusion polypeptide, said

AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 78 (corresponding to the VEE.sE2/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- VEE.sE2] for identifying the presence of the Venezuelan Equine Encephalitis virus in a biological sample, for example thanks to the immunoassay of the present invention.

Orthobunyavirus antigens

In another aspect, the present invention relates to vectors for expressing particular orthobunyavirus antigens, for example the Nucleoprotein N from the Akabane virus (AKA.N), from the Aino virus (AIN.N) or from the Shamonda virus (SHA.N), in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Akabane virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/AKA.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene encoding the Nucleoprotein N from the Akabane virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ AKA.N cassette having the nucleotide sequence SEQ ID NO: 79 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),

- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the natural N nucleoprotein of the Akabane virus,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the N nucleoprotein from the Akabane virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 80 (corresponding to the SNAP-like/proTEV1/AKA.N/pro-TEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- AKA.N] for identifying the presence of the Akabane virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector for expressing the Nucleoprotein N from the Aino virus (AIN.N) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Aino virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/AIN.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene encoding the Nucleoprotein N from the Aino virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/AIN.N cassette having the nucleotide sequence SEQ ID NO: 81 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the natural N nucleoprotein of the Aino virus,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the N nucleoprotein from the Aino virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 82 (corresponding to the SNAP-like/proTEV1/AIN.N/pro-TEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- AIN.N] for identifying the presence of the Aino virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector for expressing the Nucleoprotein N from the Shamonda virus (SHA.N) in an host cell, comprising the

nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Shamonda virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/SHA.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene encoding the Nucleoprotein N from the Shamonda virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/SHA.N cassette having the nucleotide sequence SEQ ID NO: 83 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the natural N nucleoprotein of the Shamonda virus,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the N nucleoprotein from the Shamonda virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 84 (corresponding to the SNAP-like/proTEV1/SHA.N/pro-TEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- SHA.N] for identifying the presence of the Shamonda virus in a biological sample, for example thanks to the immunoassay of the present invention.

Betacoronavirus antigens

In another aspect, the present invention relates to vectors for expressing particular betacoronavirus antigens, for example the Nucleoprotein N from human betacoronavirus (huCOV.N) or the protein S of the human betacoronavirus (huCOV.S), in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from human betacoronavirus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/huCOV.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene encoding the Nucleoprotein N from human betacoronavirus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/huCOV.N cassette having the nucleotide sequence SEQ ID NO: 85 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the gene N from human betacoronavirus 2cEMC/2012 (Genbank#JX869059),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from human betacoronavirus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 86 (corresponding to the SNAP-like/proTEV1/huCOV.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- huCOV.N] for identifying the presence of the human betacoronavirus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector for expressing the soluble form of the spike S protein from human betacoronavirus (huCOV.S) in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the soluble form of the spike S protein from human betacoronavirus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/huCOV.S cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene S from human betacoronavirus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/huCOV.S cassette having the nucleotide sequence SEQ ID NO: 87 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the gene S from human betacoronavirus 2cEMC/2012 (Genbank#JX869059),
- a DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the soluble form of the spike S protein from human betacoronavirus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 88 (corresponding to the huCOV.S/SNAP-like/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- huCOV.S] for identifying the presence of the human betacoronavirus in a biological sample, for example thanks to the immunoassay of the present invention.

Hepacivirus antigen

In another aspect, the present invention relates to vectors for expressing particular hepacivirus antigens, for example the protein C from Hepatitis C virus (HCV.C) or from Hepatitis E virus (HEV.C), in a host cell.

In one embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the protein C from Hepatitis C virus.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/HCV.C cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene of the protein C from Hepatitis C virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/HCV.C cassette having the nucleotide sequence SEQ ID NO: 89 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,

- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the C protein from hepatitis C virus genotype 1b (strain TCHM-R2/03),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the protein C from Hepatitis C virus (HCV.C). In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 90 (corresponding to the SNAP-like/proTEV1/HCV.C/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- HCV.C] for identifying the presence of the Hepatitis C virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the protein C from Hepatitis E virus (HEV.C).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/HEV.C cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence of the gene of the protein C from Hepatitis E virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/HEV.C cassette having the nucleotide sequence SEQ ID NO: 150 comprising:

- an insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the C protein from hepatitis E virus (Genbank#AB29196),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the protein C from Hepatitis E virus (HEV.C). In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 151 (corresponding to the SNAP-like/proTEV1/HEV.C/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- HEV.C] for identifying the presence of the Hepatitis E virus in a biological sample, for example thanks to the immunoassay of the present invention.

Malaria antigens

In another aspect, the present invention is drawn to a vector for expressing particular Malaria antigens, for example, the MSP-1 and the AMA-1 proteins from *Plasmodium falciparum* (MSP-1+AMA-1) (see Pan W. et al, *The Journal of Immunology*, 2004), in an host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the MSP-1 and the AMA-1 proteins from the parasite *Plasmodium falciparum*.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MSP-1+AMA-1cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the MSP-1 and the AMA-1 proteins from the parasite *Plasmodium falciparum* has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MSP-1+AMA-1cassette having the nucleotide sequence SEQ ID NO: 91 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- the DNA sequence encoding the MSP-1 (19) sequence (50% G+C) from *Plasmodium falciparum*,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- the DNA sequence encoding the AMA-1 (III) sequence (50% G+C) from *Plasmodium falciparum*,
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the MSP-1+AMA-1 protein from *Plasmodium falciparum*. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 92 (corresponding to the SNAP-like/MSP-1/proTEV2/AMA-1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- MSP-1+AMA-1] for identifying the presence of the parasite *Plasmodium falciparum* in a biological sample, for example thanks to the immunoassay of the present invention.

Leptospirosis antigens

In another aspect, the present invention is drawn to a vector for expressing a particular leptospirosis antigen, such as the HbpA protein of *Leptospira* bacteria (see Sivakolundu S. et al, *Journal of Medical Microbiology*, 2012), in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the HbpA protein from *Leptospira interrogans* bacteria.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/HbpA cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the HbpA protein from *Leptospira* bacteria has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/HbpA cassette having the nucleotide sequence SEQ ID NO: 93 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the modified short form of HbpA (TonB-dependent outer membrane receptor or LB191) from *Leptospira interrogans* serovar Lai str.56601 (Genbank#AA51750.1),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the HbpA protein from *Leptospira interrogans* bacteria. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 94 (corresponding to the SNAP-like/proTEV1/HbpA/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-HbpA] for identifying the presence of the *Leptospira* bacteria in a biological sample, for example thanks to the immunoassay of the present invention.

Microbial peptides

In another aspect, the present invention is drawn to a vector for expressing a microbial peptide, for example the microbial peptide MUB-40, in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the MUB-40 peptide.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MUB40 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the MUB40 peptide has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MUB40 cassette having the nucleotide sequence SEQ ID NO: 95 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the MUB-40 peptide,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33), and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the MUB 40 peptide. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous

being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 96 (corresponding to the SNAP-like/proTEV1/MUB40/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-MUB40] for identifying the presence of a ligand in a biological sample, for example thanks to the immunoassay of the present invention.

Lectins involved in Flavivirus pathogenesis

In another aspect, the present invention is drawn to vectors for expressing particular lectins involved in Flavivirus pathogenesis, for example the mouse or the human soluble form of C-type like lectin (CLEC5A), in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the mouse CLEC5A (mo-CLEC5A) or the human CLEC5A (hu-CLEC5A).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/mo-CLEC5A cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the mouse soluble form of C-type like lectin has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/mo-CLEC5A cassette having the nucleotide sequence SEQ ID NO: 97 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),

- the DNA sequence encoding the mouse soluble form of C-type like lectin (CLEC5A),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25), and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/hu-CLEC5A cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the human soluble form of C-type like lectin has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/hu-CLEC5A cassette having the nucleotide sequence SEQ ID NO: 99 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the human soluble form of C-type like lectin (CLEC5A),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25), and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the mouse or the human soluble form of C-type like lectin (CLEC5A). In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 98 (corresponding to the SNAP-like/proTEV1/mo-CLEC5A/proTEV2/Histag fusion protein) or the amino acid sequence of SEQ ID NO: 100 (corresponding to the SNAP-like/proTEV1/hu-CLEC5A/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-mo-CLEC5A] or [SNAP-hu-CLEC5A] for detection of presence of flaviviruses in a biological sample, for example thanks to the immunoassay of the present invention.

Anti-flaviviral mosquito proteins

In another aspect, the present invention is drawn to vectors for expressing particular antiviral mosquito proteins, for example the VAGO protein from the *Culex* species (cxVAGO) or from the *Aedes* species (aaVAGO) in a host cell.

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the VAGO protein from the *Aedes albopictus* mosquito.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/aaVAGO cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the VAGO protein from the *Aedes albopictus* mosquito has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/aaVAGO cassette having the nucleotide sequence SEQ ID NO: 103 comprising:

- an insect BiP-like sequence of SEQ ID NO: 152,

- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- the DNA sequence encoding the VAGO protein from the *Aedes albopictus* mosquito, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the VAGO protein from the *Aedes albopictus* mosquito. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 104 (corresponding to the SNAP-like/aaVAGO/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-aaVAGO] for identifying the presence of a ligand in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the VAGO protein from the *Culex quinquefasciatus* mosquito.

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/cxVAGO cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the VAGO protein from the *Culex quinquefasciatus* mosquito has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/cxVAGO cassette having the nucleotide sequence SEQ ID NO: 101 comprising:

- an insect BiP-like sequence of SEQ ID NO: 152,
- the SNAP-like sequence of SEQ ID NO: 31,
- a DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- the DNA sequence encoding the VAGO protein from the *Culex quinquefasciatus* mosquito, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the VAGO protein from the *Culex quinquefasciatus* mosquito. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 102 (corresponding to the SNAP-like/cxVAGO/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-cxVAGO] for identifying the presence of a ligand in a biological sample, for example thanks to the immunoassay of the present invention.

Viral Hemorragic fever antigens

In another aspect, the present invention is drawn to vectors for expressing particular viral hemorragic fever antigens such as:

- the Nucleoprotein N from the Crimean-Congo virus (CCHF.N), from the Ebola virus (EBO.N), from the Marburg virus (MAR.N), from the Lassa virus (LAS.N), from the Junin virus (JUN.N), from the Machupo virus (MAC.N), from the Sabia virus (SAB.N), or from the Guanarito virus (GUA.N),

- the Ectodomain of GP1 from the Lassa virus (LAS.ectoGP1), from the Junin virus (JUN.ectoGP1), from the Machupo virus (MAC.ectoGP1), from the Sabia virus (SAB.ectoGP1), or from the Guanarito virus (GUA.ectoGP1),
- the Ectodomain of GP2 from the Lassa virus (LAS.ectoGP2), from the Junin virus (JUN.ectoGP2), from the Machupo virus (MAC.ectoGP2), from the Sabia virus (SAB.ectoGP2), or from the Guanarito virus (GUA.ectoGP2),
- the domain III of the envelope E protein from the Omsk virus (OMSK.EDIII), from the Kasyanur virus (KAS.EDIII), or from the Alkhurma virus (ALK.EDIII).

In particular, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Crimean-Congo virus (CCHF.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/CCHF.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Crimean-Congo virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/CCHF.N cassette having the nucleotide sequence SEQ ID NO: 108 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Crimean-Congo virus,
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),

- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Crimean-Congo virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 109 (corresponding to the SNAP-like/proTEV1/CCHF.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- CCHF.N] for identifying the presence of the Crimean-Congo virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Ebola virus (EBO.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/EBO.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Ebola virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/EBO.N cassette having the nucleotide sequence SEQ ID NO: 110 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),

- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Ebola virus (Genbank#NC_002549),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Ebola virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 111 (corresponding to the SNAP-like/proTEV1/EBO.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- EBO.N] for identifying the presence of the Ebola virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Marburg virus (MAR.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MAR.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Marburg virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAR.N cassette having the nucleotide sequence SEQ ID NO: 112 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Marburg virus (Genbank#NC_001608),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Marburg virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 113 (corresponding to the SNAP-like/proTEV1/MAR.N /proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-MAR.N] for identifying the presence of the Marburg virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Lassa virus (LAS.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/LAS.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Lassa virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/LAS.N cassette having the nucleotide sequence SEQ ID NO: 114 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Lassa virus (Genbank#NC_004296),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Lassa virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is

for example the amino acid sequence of SEQ ID NO: 115 (corresponding to the SNAP-like/proTEV1/LAS.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-LAS.N] for identifying the presence of the Lassa virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Junin virus (JUN.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JUN.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Junin virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/JUN.N cassette having the nucleotide sequence SEQ ID NO: 116 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Junin virus (Genbank#NC_005081),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Junin virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 117 (corresponding to the SNAP-like/proTEV1/JUN.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-JUN.N] for identifying the presence of the Junin virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Machupo virus (MAC.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MAC.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Machupo virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAC.N cassette having the nucleotide sequence SEQ ID NO: 118 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),

- the DNA sequence encoding the Nucleoprotein N from the Machupo virus (Genbank#NC_005078),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Machupo virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 119 (corresponding to the SNAP-like/proTEV1/MAC.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-MAC.N] for identifying the presence of the Machupo virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Guanarito virus (GUA.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/GUA.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Guanarito virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/GUA.N cassette having the nucleotide sequence SEQ ID NO: 120 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Guanarito virus (Genbank#NC_005077),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Guanarito virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 121 (corresponding to the SNAP-like/proTEV1/GUA.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-GUA.N] for identifying the presence of the Guanarito virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention relates to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Nucleoprotein N from the Sabia virus (SAB.N).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/SAB.N cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Nucleoprotein N from the Sabia virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/SAB.N cassette having the nucleotide sequence SEQ ID NO: 122 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the DNA sequence encoding the Nucleoprotein N from the Sabia virus (Genbank#NC_006317),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a second DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Nucleoprotein N from the Sabia virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is

for example the amino acid sequence of SEQ ID NO: 123 (corresponding to the SNAP-like/proTEV1/SAB.N/proTEV2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-SAB.N] for identifying the presence of the Sabia virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the domain III of the Envelop protein E from the Omsk virus (OMSK.EDIII).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/OMSK.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the EDIII protein from the Omsk virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/OMSK.EDIII cassette having the nucleotide sequence SEQ ID NO: 124 comprising:

- an insect BiP-like sequence of SEQ ID NO: 152,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence encoding the EDIII protein of the Omsk virus (Genbank#NC_005062),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the EDIII protein from the Omsk virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the

amino acid sequence of SEQ ID NO: 125 (corresponding to the SNAP-like/OMSK.EDIII/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-OMSK.EDIII] for identifying the presence of the Omsk virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the domain III of the Envelop protein E from the Kyasanur Forest Disease virus (KYA.EDIII).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/KYA.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the EDIII protein from the Kyasanur Forest Disease virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/KYA.EDIII cassette having the nucleotide sequence SEQ ID NO: 126 comprising:

- an insect BiP-like sequence of SEQ ID NO: 152,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence encoding the EDIII protein of the Kyasanur Forest Disease virus (Genbank#JF416958),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the EDIII protein from the Kyasanur Forest Disease virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a

homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 127 (corresponding to the SNAP-like/KYA.EDIII/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-KYA.EDIII] for identifying the presence of the Kyasanur Forest Disease virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment, the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the domain III of the Envelop protein E from the Alkhurma virus (ALK.EDIII).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/ALK.EDIII cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the EDIII protein from the Alkhurma virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/ALK.EDIII cassette having the nucleotide sequence SEQ ID NO: 128 comprising:

- an insect BiP-like sequence of SEQ ID NO: 152,
- the SNAP-like sequence of SEQ ID NO: 31,
- the DNA sequence encoding the EDIII protein of the Alkhurma virus (Genbank#NC_004355),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic

domain thereof and b) the EDIII protein from the Alkhurma virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 129 (corresponding to the SNAP-like/ALK.EDIII/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-ALK.EDIII] for identifying the presence of the Alkhurma virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP1 ectodomain from the Lassa virus (LAS.ectoGP1).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/LAS.ectoGP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP1 ectodomain from the Lassa virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/LAS.ectoGP1 cassette having the nucleotide sequence SEQ ID NO: 130 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP1 ectodomain from the Lassa virus (Genbank#NC_004296),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP1 ectodomain from the Lassa virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 131 (corresponding to the SNAP-like/ LAS.ectoGP1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- LAS.ectoGP1] for identifying the presence of the Lassa virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP1 ectodomain from the Junin virus (JUN.ectoGP1).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JUN.ectoGP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP1 ectodomain from the Junin virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/JUN.ectoGP1 cassette having the nucleotide sequence SEQ ID NO: 132 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP1 ectodomain from the Junin virus (Genbank#NC_005081),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP1 ectodomain from the Junin virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 133 (corresponding to the SNAP-like/ JUN.ectoGP1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP- JUN.ectoGP1] for identifying the presence of the Junin virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP1 ectodomain from the Machupo virus (MAC.ectoGP1).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MAC.ectoGP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP1 ectodomain from the Machupo virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAC.ectoGP1 cassette having the nucleotide sequence SEQ ID NO: 134 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP1 ectodomain from the Machupo virus (Genbank#NC_005078),
- the SNAP-like sequence of SEQ ID NO: 31, and

- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP1 ectodomain from the Machupo virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 135 (corresponding to the SNAP-like/ MAC.ectoGP1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-MAC.ectoGP1] for identifying the presence of the Machupo virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP1 ectodomain from the Guanarito virus (GUA.ectoGP1).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/GUA.ectoGP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP1 ectodomain from the Guanarito virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/GUA.ectoGP1 cassette having the nucleotide sequence SEQ ID NO: 136 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP1 ectodomain from the Guanarito virus (Genbank#NC_005077),

- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP1 ectodomain from the Guanarito virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 137 (corresponding to the SNAP-like/ GUA.ectoGP1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-GUA.ectoGP1] for identifying the presence of the Guanarito virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP1 ectodomain from the Sabia virus (SAB.ectoGP1).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/SAB.ectoGP1 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP1 ectodomain from the Sabia virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/SAB.ectoGP1 cassette having the nucleotide sequence SEQ ID NO: 138 comprising:

- an insect BiP sequence of SEQ ID NO: 22,

- the DNA sequence encoding the Glycoprotein GP1 ectodomain from the Guanarito virus (Genbank#NC_006317),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP1 ectodomain from the Sabia virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 139 (corresponding to the SNAP-like/ SAB.ectoGP1/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-SAB.ectoGP1] for identifying the presence of the Sabia virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP2 ectodomain from the Lassa virus (LAS.ectoGP2).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/LAS.ectoGP2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP2 ectodomain from the Lassa virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/LAS.ectoGP2 cassette having the nucleotide sequence SEQ ID NO: 140 comprising:

- an insect BiP sequence of SEQ ID NO: 22,

- the DNA sequence encoding the Glycoprotein GP2 ectodomain from the Lassa virus (Genbank#NC_004296),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP2 ectodomain from the Lassa virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 141 (corresponding to the SNAP-like/ LAS.ectoGP2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-LAS.ectoGP2] for identifying the presence of the Lassa virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP2 ectodomain from the Junin virus (JUN.ectoGP2).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/JUN.ectoGP2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP2 ectodomain from the Junin virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/JUN.ectoGP2 cassette having the nucleotide sequence SEQ ID NO: 142 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP2 ectodomain from the Junin virus (Genbank#NC_005081),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP2 ectodomain from the Junin virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 143 (corresponding to the SNAP-like/ JUN.ectoGP2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-JUN.ectoGP2] for identifying the presence of the Junin virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP2 ectodomain from the Machupo virus (MAC.ectoGP2).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/MAC.ectoGP2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP2 ectodomain from the Machupo virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAC.ectoGP2 cassette having the nucleotide sequence SEQ ID NO: 144 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP2 ectodomain from the Machupo virus (Genbank#NC_005078),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP2 ectodomain from the Machupo virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 145 (corresponding to the SNAP-like/ MAC.ectoGP2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-MAC.ectoGP2] for identifying the presence of the Machupo virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP2 ectodomain from the Guanarito virus (GUA.ectoGP2).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/GUA.ectoGP2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in

which the sequence encoding the Glycoprotein GP2 ectodomain from the Guanarito virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/GUA.ectoGP2 cassette having the nucleotide sequence SEQ ID NO: 146 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP2 ectodomain from the Guanarito virus (Genbank#NC_005077),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP2 ectodomain from the Guanarito virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 147 (corresponding to the SNAP-like/ GUA.ectoGP2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-GUA.ectoGP2] for identifying the presence of the Guanarito virus in a biological sample, for example thanks to the immunoassay of the present invention.

In another embodiment the present invention is drawn to a vector comprising the nucleotide sequence encoding a) a secretion signal peptide which is functional in said host cells, and b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the Glycoprotein GP2 ectodomain from the Sabia virus (SAB.ectoGP2).

In a preferred embodiment, this vector comprises a so-called "pDeSNAP Univ/SAB.ectoGP2 cassette" i.e., a pDeSNAPUniv DNA cassette as defined above, in which the sequence encoding the Glycoprotein GP2 ectodomain from the Sabia virus has been inserted.

In a preferred embodiment, this vector comprises the pDeSNAP Univ/MAC.ectoGP2 cassette having the nucleotide sequence SEQ ID NO: 148 comprising:

- an insect BiP sequence of SEQ ID NO: 22,
- the DNA sequence encoding the Glycoprotein GP2 ectodomain from the Sabia virus (Genbank#NC_006317),
- the SNAP-like sequence of SEQ ID NO: 31, and
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

In another aspect, the present invention relates to a recombinant cell which is stably transfected by said vector.

In another aspect, the present invention is drawn to a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the Glycoprotein GP2 ectodomain from the Sabia virus. In this fusion polypeptide, said AGT enzyme is preferably the protein of SEQ ID NO: 2, or a homologue thereof (said homologous being as defined above). This fusion polypeptide is for example the amino acid sequence of SEQ ID NO: 149 (corresponding to the SNAP-like/ SAB.ectoGP2/Histag fusion protein).

Thus, in another aspect, the present invention is also drawn to the use of this fusion protein [SNAP-SAB.ectoGP2] for identifying the presence of the Sabia virus in a biological sample, for example thanks to the immunoassay of the present invention.

Examples

In the context of the invention, a multiplex bead-based immunoassay was developed for rapid and simultaneous detection of antibodies to arboviruses in biological fluids.

The system is based on the xMAP technology (Luminex corporation) and uses a mixture of antigen-coated microspheres as capture reagents for specific human immunoglobulins. Distinct sets of microspheres (Magplex, Luminex corporation) were coupled with purified AGT fusion proteins, namely the SNAP-tagged viral recombinant proteins: sSNAP-DV1.EDIII, sSNAP-DV2.EDIII, sSNAP-DV3.EDIII, sSNAP-DV4.EDIII, sSNAP-WN.EDIII, sSNAP-JE.EDIII, sSNAP-USU.EDIII, sSNAP-TBE.EDIII, sSNAP-YF.EDIII, sSNAP-MVE.EDIII, sSNAP-Rocio.EDIII, sSNAP-WSL.EDIII, sSNAP-ZIKA.EDIII, SNAP-DV1ectoM, sSNAP-N.RVF, sSNAP-N.TOS, and CHIK.sE2-SNAP. Recombinant antigens were covalently coupled to the carboxyl microsphere surface using a substrate of the AGT protein as linker (BG-PEG-NH2, New England Biolabs), thereby enhancing antibody capture efficiency as compared to standard amine coupling procedures.

Technical validation using anti-SNAP-tag antibodies and specific mouse monoclonal antibodies confirmed coupling efficiency and demonstrated long-term antigen stability (up to six month). This application is not limited to viral antigens as any peptide or polypeptide can be used for bead coating and subsequent antibody capture.

I. <u>Material and methods</u>

1. The following buffers and solutions are used:

a) **PBS buffer**: 100 mL of 10X PBS, pH 7.4 in 1 L H2O sterile

b) **SNAP coupling buffer** (PBS-DTT) : 100 mL of 10 X PBS, pH 7.4, 0.5 mL 10 % tween 20, 1 mL of 1.0 M DTT, in 1 L H_2O sterile

c) **blocking / assay buffer** (PBS-B): PBS, 1 % BSA, pH 7.4 in 1 L H_2O sterile

d) **storage buffer** (PBS-TBN): 100 mL of 10X PBS, 1 g of BSA, 2 mL of 10 % tween 20, 500 mg of sodium azide, 1 mL of 1.0M DTT, in 1 L H_2O sterile

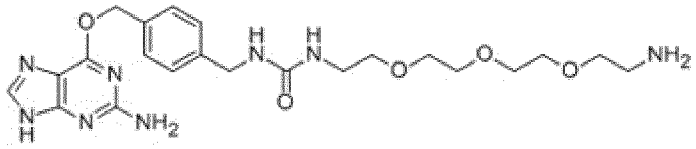
e) **Substrate solution** (4 mg/mL): 2 mg of BG-PEG-NH_2, DMSO 500 µL.

f) **Activation solution** (EDAC /SNHS): 50 mg/mL of EDAC solution or 50 mg/mL of SNSHS in distilled water

2. The following materials were used:

2.1. MagPlex Luminex microspheres : MC 100XX-ID (where XX is the fluorescence region), XX can be *e.g.* 26, 27, 28, 29, 34, 35, 36, 37, 45, 52, 53, 63, 64, as mentioned on figure 7B

2.2. **hAGT substrate**: PEG-BG-NH_2 (NEB S9150S)



2.3. **Fusion proteins SNAP-viral EDIII** :

The generation of a fusion protein comprising AGT and viral EDIII moieties is well-known to the skilled person. Every known synthesis process can be used for this purpose, provided that the AGT enzyme remains active in the fusion protein.

In the present case, the AGT mutant SNAP of SEQ ID NO: 2 has been used and SNAP-viral EDIII fusion proteins have been generated.

The Drosophila S2 inducible expression system (DES, Invitrogen), has been chosen for the mass production of individual EDIII from flaviviruses in non-vertebrate cells and the plasmid pMT/BiP/V5-HisA from Invitrogen has been used.

This plasmid contains:

- The metallothionein promoter pMT,
- An insect ssBiP sequence of SEQ ID NO: 22,
- *Bgl II* and *Age I* restriction sites,

- the DNA of SEQ ID NO: 28 encoding a His_6tag located downstream of the *AgeI* restriction site, and

- the DNA spacer sequence of SEQ ID NO: 26 located between the *AgeI* restriction site and the DNA encoding a His_6tag.

The synthetic genes coding for the full-length domain III of the E proteins from flaviviruses WN, USU, JE, TBE, DEN-1 to DEN-4, YF, Rocio, MVE, Zika, SLE, and WSL are listed in SEQ ID NO: 3 to SEQ ID NO: 14. The ED III amino acid sequences were fused in frame to the C-terminus of the SNAP protein, with both moieties being separated by a linker GGGS (SEQ ID NO: 25). The DNA sequences encoding SNAP-EDIII were inserted in the plasmid pMT/BiP/V5-Histag (Invitrogen) to generate the plasmids pMT/BiP/SNAP/EDIII/Histag.

The resulting plasmids pMT/BiP/SNAP-EDIII-Histag, which can drive the expression of secreted SNAP-EDIII-His_6 fusion proteins, were co-transfected with selection marker pCo-Blast into S2 cells to generate the stable S2/sSNAP-ED III- Histag cell line showing resistance to blasticidine.

Stable S2 cell lines grown in spinner (1000 ml) were stimulated 10 days with heavy metal cadmium (Cd^{2+}) and proteins from extracellular medium were concentrated and purified.

Accumulation of secreted SNAP-tagged EDIII protein was observed in the supernatants of stable S2/sSNAP-EDIII-Histag cells after 10days of induction with heavy metal cadmium.

The proteins SNAP-DEN1.EDIII of SEQ ID NO: 21, SNAP-DEN2.EDIII of SEQ ID NO:X, SNAP-DEN3.EDIII of SEQ ID NO:X, SNAP-DEN4.EDIII of SEQ ID NO:X, SNAP-WN.EDIII of SEQ ID NO:X, SNAP-JE.EDIII of SEQ ID NO:X, SNAP-YF.EDIII of SEQ ID NO:X, SNAP-MVE.EDIII of SEQ ID NO:X, SNAP-Rocio.EDIII of SEQ ID NO:X, SNAP-WSL.EDIII of SEQ ID NO:X, SNAP-ZIKA.EDIII of SEQ ID NO:X, SNAP-SLE.EDIII of SEQ ID NO:X have been produced accordingly.

3. Preparation of the antigen-coupled beads

The production of antigen-coupled beads comprised two steps: functionalization of microsphere surfaces with an O^6-benzylguanine derivative (BG-PEG-amino), and covalent

immobilization of the chimeric SNAP-viral Ags proteins using the BG-PEG-amino as an anchor (Figure 1). The carboxyl microsphere surfaces were covalently coated with BG-PEG-amino substrate using an optimized two-step carbodiimide process (Wong et al *Journal of Clinical Microbiology* **42**(1): 65–72, 2004). Subsequently, coupled BG-PEG-amino compounds were irreversibly linked to the chimeric SNAP-viral Ags proteins by transfer of the benzyl group to the active site cysteine of the SNAP protein. Due to the high specificity of this reaction, the fusion protein is exclusively coupled via the SNAP domain, leaving the viral antigen accessible for interactions with antibodies.

3.1. First, the commercial beads were activated as per the manufacturer instructions (by using the EDAC and SNHS activation solutions), and washed in a PBS buffer. All the steps were performed in darkness so as to prevent the fluorescent quenching of the beads, according to the manufacturer instructions.

About 1.25 x 10^6 beads were used for each coupling process.

3.2. The AGT substrate PEG-BG-NH_2 in the DMSO solution was then added for 6 hours at room temperature or overnight at 4° C on the activated beads, and subsequently washed with PBS buffer.

3.3. The unbound carboxylic sites were then blocked with the blocking buffer for 30 minutes at room temperature, and the beads subsequently washed with the SNAP coupling buffer.

3.4. SNAP-EDIII proteins resuspended in the SNAP coupling buffer (1 mg/mL) were incubated with the thus obtained beads for two hours at room temperature, and then washed once with the SNAP coupling buffer, and three times with the storage buffer (PBS-TBN).

4. Microsphere fluorescence immunoassays

The bead sets, conjugated with different SNAP-viral Ags, were mixed by vortex to ensure total bead dispersal. After adjusting the bead density to 100 beads/μL, 25 μl of each of the bead sets (containing 2500 microspheres) were transferred to a 96-well microtiter plate (Bio-Plex Pro flat bottom plate, BioRad) in separate wells for singleplex assays, or mixed in the same wells for multiplex assays. The microspheres were washed 3 times with 100 μL washing buffer (BioPlex Wash buffer, BioRad) using a microplate wash station for magnetic beads (BioPlex Pro Wash Station, BioRad). The samples (antibodies or sera) were diluted in assay buffer (PBS-BSA) and 50 μL of the resulting solutions were added to the test wells containing the conjugated beads. After incubation in darkness on a plate shaker for 30 min, the plate was washed as previously. Subsequently, a fluorochrome-labeled secondary antibody was diluted in assay buffer (PBS-BSA) at 2 μg/mL, and 50 μL of the resulting solutions were added to the test wells containing the conjugated beads. After incubation in darkness on a plate shaker for 30 min, the plate was washed as previously. Finally, streptavidin-phycoerythrin (SAPE, Invitrogen Molecular Probes) was diluted in assay buffer (PBS-BSA) at 2 μg/ml, and 50 μL of the resulting solution was added to the microplate wells. The plate was incubated in darkness on a plate shaker for 10 min and washed as previously, before resuspending the contents of the wells in 125 μl of assay buffer. The median fluorescence intensity (MFI) of the detection antibody bound to the individual microspheres was evaluated from flow analysis of 50 microspheres per well using a dual-laser flow analyzer (BioPlex 200 instrument, BioRad). The fluorescent detection instrument is equipped with a first laser for detecting the type of bead, and a second to ensure the quantification of captured IgM or IgG by exciting the fluorophore (red-phycoerythrin) conjugated to the specific detection antibody.

4.1 Confirmation of antigen coupling

Antigen coupling was confirmed by testing the antigen-coupled microspheres with dilutions of rabbit anti-SNAP-tag polyclonal antibody (GenScript). The fluorescence immunoassays were performed in singleplex format, as described above. A two-fold dilution series of anti-SNAP antibody starting at 4000 ng/mL and ending at 3.9 ng/mL was performed in PBS-BSA, and volumes of each dilution were added to the test wells

containing the beads. A biotin-conjugated goat anti-rabbit IgG (2 µg/mL in 50 µL PBS-BSA) was used as secondary antibody to detect bound anti-SNAP antibodies.

Figure 2 shows the fluorescence results observed for the detection of anti-SNAP antibody on 8 different sets of microspheres coupled to chimeric SNAP-viral antigens proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE).

4.2 Detection of specific antibodies

The capture and detection of specific antibodies by the antigen-conjugated microspheres was assessed using purified monoclonal mouse antibodies (anti-WNV, anti-DV1 and anti-DV2) and polyclonal mouse sera (anti-DV3, anti-DV4, anti-YF and anti-JE) or human sera (anti-DV1). The fluorescence immunoassays were performed in singleplex and multiplex format, as described above. A four-fold dilution series of purified mouse monoclonal antibodies starting at 400 ng/mL and ending at 0.1 ng/mL, and of mouse and human sera starting at 1:25 and ending at 1:102400, was performed in PBS-BSA, and volumes of each dilution were added to the test wells containing the beads. A biotin-conjugated goat anti-mouse IgG (2 µg/mL in 50 µL PBS-BSA), was used as secondary antibody to detect bound monoclonal and polyclonal mouse antibodies. A biotin-conjugated goat anti-human IgM (2 µg/mL in 50 µL PBS-BSA) or a biotin-conjugated goat anti-human IgG (2 µg/mL in 50 µL PBS-BSA), was used to detect bound IgM or IgG antibodies in human serum, respectively.

Figure 3 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV2 antibody on chimeric SNAP-DV2.EDIII protein conjugated to microspheres via the substrate of the hAGT protein (coupling of the invention) or coupled through Bio-Plex Amine Coupling Kit, BIORAD.

Figure 4 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV1 antibody on chimeric SNAP-DV1.EDIII protein conjugated to microspheres, either in a singleplex or in a multiplex format with other chimeric SNAP-viral Ags proteins (SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE) coupled to microspheres.

Figure 5 shows the reactivity and specificity of the multiplex immunoassay experiment for the detection of dilutions of purified monoclonal anti-WNV antibody on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE) coupled to microspheres.

Figure 6 shows the reactivity and specificity of anti-DV3 IgG detection in mouse polyclonal serum against DV3 (A) and anti-YF IgG detection in mouse polyclonal serum against YF (B) in multiplex immunoassays on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-WSL, SNAP-ROCIO, SNAP-MVE, SNAP-SLE, SNAP-ZIKA) coupled to microspheres.

Figure 7 shows the reactivity and specificity of anti-DV1 IgM detection (A) and anti-DV1 IgG detection (B) in DV1-infected serum of a human patient in multiplex immunoassays on chimeric SNAP-viral Ags proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-WSL, SNAP-ROCIO, SNAP-MVE, SNAP-SLE, SNAP-ZIKA, SNAP-TBE) coupled to microspheres.

II. Results

The system of the invention uses a mixture of antigen-coated Magplex microspheres (Luminex Corporation) as capture reagents for specific human immunoglobulins. Each set of internally color-coded microspheres have been coupled to a specific recombinant antigen and mixed with other types of microspheres in a small sample volume. The power of this system lies in the fact that it is possible to simultaneously analyze up to 100 types of coupled microspheres per well using a flow analysis tool. The fluorescent detection instrument is equipped with a first laser for detecting the type of bead, and a second to ensure the quantification of captured IgM or IgG by exciting the fluorophore (phycoerythrin) conjugated to the specific detection antibody. With its extensive multiplexing capabilities and lower limit of detection, this approach offers substantial cost and sample savings over traditional ELISA measurements.

Presently, 16 distinct sets of microspheres have been coupled with purified chimeric SNAP-viral Ags proteins, allowing titration of serum antibodies specific to dengue serotypes 1 to 4, West Nile, Yellow fever, Japanese encephalitis, tick-borne encephalitis, Saint-Louis encephalitis, Murray Valley encephalitis, Wesselsbron, Zika, Rocio, Usutu, Rift Valley fever, and Chikungunya virus. The production of the system is highly time- and cost-effective, as only a very small amount of recombinant antigen (< 50µg) is required to produce one set of antigen-coupled microspheres (~1.25 x 10^6 microspheres), sufficient to perform 500 individual assays. Moreover, the selected sets of microspheres are adaptable to an affordable, compact, and robust fluorescent detection system such as the MagPix (Luminex Corporation).

The evaluation of antigen coupling using an anti-SNAP antibody (Figure 2) confirmed the coupling efficiency and demonstrated that the relative quantities of bound antigens are comparable between the different coupled microsphere sets. The assessment of antibody capture and detection using purified mouse antibodies showed enhanced capture of specific antibodies by the produced antigen-coupled microspheres as compared to antigen-coupled microspheres obtained by standard amine coupling procedures (Figure 3). In addition, it demonstrated the low detection limit of the method and confirmed that multiplexing does not affect antibody detection (Figure 4). Additionally, the antigen-conjugated microspheres exhibited long-term stability when stored at 4° C (> 6 months). Finally, the specificity of each set of coupled microspheres in multiplex immunoassays was demonstrated for purified mouse monoclonal antibodies (Figure 5), for IgG antibodies in polyclonal mouse sera (Figure 6A-B) and for both IgM and IgG antibodies in polyclonal sera from infected humans (Figure 7).

With its extensive multiplexing capabilities (up to 100 types of coupled microspheres per well) and lower limit of detection, this approach offers substantial cost and sample savings over traditional ELISA measurements.

III. **Generation of a fusion protein comprising SNAP and the N nucleoprotein of the Schmallenberg virus**

1. Construction of the vectors encoding the fusion protein SNAP-SBV.N

The chimeric fusion protein comprising SNAP and the N nucleoprotein of the Schmallenberg virus has been obtained as follows:

In a first step, the sequence of the open reading frame of the S segment encoding the N nucleoprotein and the NSs protein of the BH80/11-4 strain was mutated by inserting an *EcoRV* restriction site at its 5' terminus and an *XmaI* restriction site at its 3' terminus. In addition, the internal *EcoRV* restriction site was removed by mutating the 294T nucleotide into 294A. This mutated sequence is shown on SEQ ID NO: 17.

This mutated sequence was then inserted into the *EcoRV* and *XmaI* restriction sites of the pDeSNAP Univ cassette of SEQ ID NO: 34, generating the "pDeSNAP Univ/SBV.N" DNA cassette of SEQ ID NO: 36.

The so-called "pDeSNAP Univ/SBV.N" DNA cassette comprises (see figure 9 and SEQ ID NO: 36):

- the insect BiP-like sequence of SEQ ID NO: 23,
- the SNAP-like sequence of SEQ ID NO: 31,
- a first DNA sequence SEQ ID NO: 26 encoding the spacer sequence GGGS (SEQ ID NO: 25),
- a DNA sequence SEQ ID NO: 29 encoding a pro-TEV1 cleavage site of sequence ENLYFQS (SEQ ID NO: 32),
- the SBV.N DNA sequence SEQ ID NO: 17 (which corresponds to the natural SBV.N sequence, in which the internal *EcoRV* site has been deleted and two *EcoRV* and *XmaI* sites have been added at the extremities),
- a DNA sequence SEQ ID NO: 30 encoding a pro-TEV2 cleavage site of sequence ENLYFQG (SEQ ID NO: 33),
- a DNA sequence SEQ ID NO: 28 encoding a HisTag sequence.

Note that this cassette comprises in addition an *NheI* site upstream of the ATG, a *BglII* site between the BiP-like sequence and the SNAP-like sequence, and an *AgeI* site and a *HindIII* site which are both located downstream of the stop codon.

The sequence comprised between the *BglII* and *AgeI* restriction sites of the pDeSNAPUniv/SBV.N cassette (see figure 9) was excised by enzymatic digestion, then cloned into the pMT/BiP/V5-A plasmid (Invitrogen) to generate the pMT/BiP/SNAP-SBV.N vector. This vector has been used to generate stable S2 cells secreting the SNAP-SBV.N fusion protein.

The sequence comprised between the *NheI* and *NotI* restriction sites of the pDeSNAPUniv/SBV.N cassette is then cloned into the pcDNA3 plasmid (Invitrogen) to generate the pcDNA3/SNAP-SBV.N vector. This vector is then used to generate stable mammalian cells secreting the SNAP-SBV.N fusion protein.

2. Production of the fusion protein SNAP-SBV.N

The resulting plasmids pMT/BiP/SNAP-SBV.N that allow the production of SNAP-tagged SBV.N proteins as secreted fusion proteins, were co-transfected with selection marker pCo-Blast into S2 cells to generate the stable S2/SNAP-SBV.N cell line showing resistance to blasticidine.

This cell line has been deposited to the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institut Pasteur, 25, rue du Docteur Roux, 75724 PARIS CEDEX 15, under the number CNCM I-4616.

Stable S2 cell lines grown in spinner (1000 ml) were stimulated 10 days with heavy metal cadmium (Cd^{2+}).

Accumulation of secreted SNAP-SBV.N protein was observed in the supernatants of the S2/SNAP-SBV.N cells after 10days of induction with heavy metal cadmium.

0.01mL from 4mL of supernatant of S2/SNAP-SBV.N cells induced 10 days with Cd^{2+} were tested by immunoblot assay using anti-Histag antibody (dilution 1:1,000) (see figure 10).

The chimeric protein SNAP-SBV.N was compared with defined amounts of the SNAP-TOS.N chimeric protein (corresponding to the fusion protein comprising SNAP and the N nucleoprotein from the Toscana virus, which is a phlebovirus).

The production of purified SNAP-SBV.N from induced S2/SNAP+SBV.N cells for 10 days is 18 mg per liter of cell culture (Fig. 10B).

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In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

CLAIMS

1. An *in vitro* assay method for detecting at least two target antibodies in a biological sample comprising:
(a) contacting a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody with the biological sample, wherein said AGT substrate is further covalently coupled to said first solid support;
(b) contacting a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said first target antibody with the biological sample, wherein said AGT substrate is further covalently coupled to said second solid support; and
(c) detecting the presence or absence of the two target antibodies.

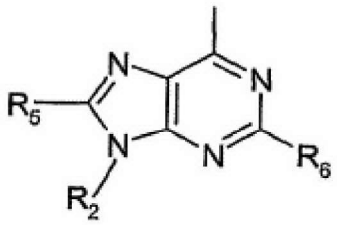
2. The assay method of claim 1, wherein said method is used to detect at least 5, more preferably at least 15 and even more preferably at least 50 target antibodies in a biological sample from a subject.

3. The assay method of any one of claims 1 or 2, wherein said AGT polypeptide is the SNAP mutant of SEQ ID NO:2.

4. The assay method of any one of claims 1 to 3, wherein said substrate of said AGT polypeptide is a O6 benzyl guanine derivative having the formula I:

R1-X-CH_2-R3-R4-Y

wherein:
- R1 is a heteroaromatic group containing 1 to 5 nitrogen atoms, preferably a purine radical of the formula:



wherein R5 is hydrogen, halogen, e. g. chloro or bromo, trifluoromethyl, or hydroxy; R6 is hydrogen, hydroxy or unsubstituted or substituted amino; and R2 is hydrogen, an alkyl of 1 to 10 carbon atoms, or a saccharide moiety;

- X is an oxygen or sulfur atom; preferably an oxygen atom;
- R3 is an aromatic or a heteroaromatic group, or an optionally substituted unsaturated alkyl, cycloalkyl or heterocyclyl group with the double bond connected to CH_2; preferably a phenyl, e.g. a phenyl substituted by R4 in para or meta position,
- R4 is a linker moiety, preferably $-CH_2-NH-CO-NH-[C_2H_4-O]_n-$, wherein n is comprised between 1 to 8, preferably 2 to 6;
- Y is a reactive group.

5. The assay method of any one of claims 1 to 4, wherein said solid supports can be specifically identify by their specific location, size, diameter, weight, granulometry, and/or labeling.

6. The assay method of any one of claims 1 to 5, wherein said solid supports are labeled with a fluorochrome, a chromophore, a radioisotope, and/or a mass tag.

7. The assay method of any one of claims 1 to 5, wherein said solid supports are microparticles internally labeled with fluorescent dyes.

8. The assay method of any one of claims 1 to 7, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII

protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

9. The assay method of any one of claims 1 to 8, wherein said first and second fusion proteins that are coupled with said first and second solid supports are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

10. A kit for the detection of at least two target antibodies in a biological sample comprising:
(a) a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a first epitope that is recognized by a first target antibody, wherein said AGT substrate is further covalently coupled to said first solid support; and
(b) a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity and a second epitope that is recognized by a second target antibody, but not by said

first target antibody, wherein said AGT substrate is further covalently coupled to said first solid support,

and wherein the said solid supports are mixed together in at least one single compartment.

11. The kit according to claim 10, comprising at least 10, preferably at least 50, more preferably at least 100 differently coupled-solid supports.

12. The kit according to any one of claims 10 to 11, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO:14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

13. The kit according to any one of claims 10-12, comprising at least two solid supports coated with at least two fusion proteins that are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID

NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

14. Use of the kit as defined in any one of the claims 10 to 13, for detecting at least two target antibodies in a biological sample from a subject**.**

15. Use as defined in claim 14, for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum*.

16. An *in vitro* method for diagnosing at least one target disease in a subject, said target disease being known to induce the synthesis of at least one target antibody in said subject, comprising performing the assay method as defined in any of the claims 1 to 9, wherein said subject is diagnosed to be suffering from said at least one target disease if the amount of said at least one target antibody is higher than a control value.

17. The *in vitro* diagnosis method according to claim 16, wherein said method is used to diagnose at least 5, more preferably at least 16 and even more preferably at least 50 viral infections in said subject.

18. The assay method according to any one of claims 1 to 9, wherein the first and the second solid supports are obtained by covalently coupling the AGT polypeptide comprised in the fusion protein as defined in claim 1 to a first and a second functionalized solid supports, by performing a method comprising the following steps:
a) activating the said functionalized solid support,

b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support,
c) contacting the said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer, DTT being preferably at a concentration of 1mM in the PBS/DTT buffer, wherein unbound molecules are washed out after steps b) and c).

19. A method for manufacturing a kit as defined in any one of claims 10 to 13, said method comprising the steps of:
(a) providing a least a first fusion protein comprising :
- a polypeptide comprising a first epitope that is recognized by a first target antibody and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,
(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,
(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,
(d) providing at least a second fusion protein comprising :
- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and
- a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity,
(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,
(f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody,
wherein said at least first and at least second solid supports can be specifically identified from each other.

20. A multiplex immuno screening assay method comprising at least 2, 25, 50, or 96 solid supports as defined in claim 1 or 19 and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

21. A multiplex immuno screening assay method comprising:

a) contacting one or several biological sample(s) with at least 2, 25, 50, or 96 solid supports as defined in claim 1 or 19 and wherein each of the solid supports emits a different and distinguishable wave length after excitation, and

b) detecting the presence or absence of target antibodies.

Figure 1

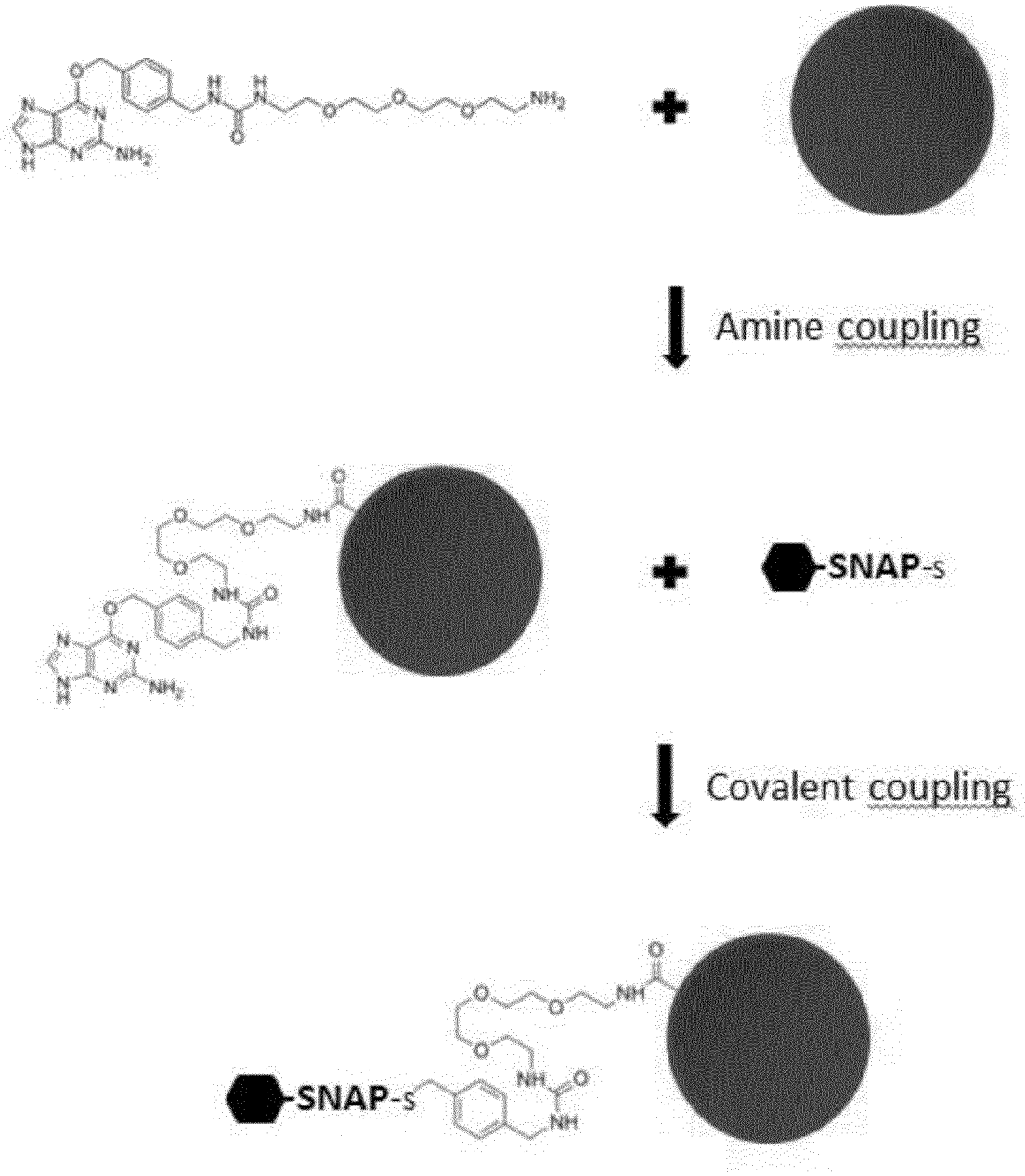


Figure 2

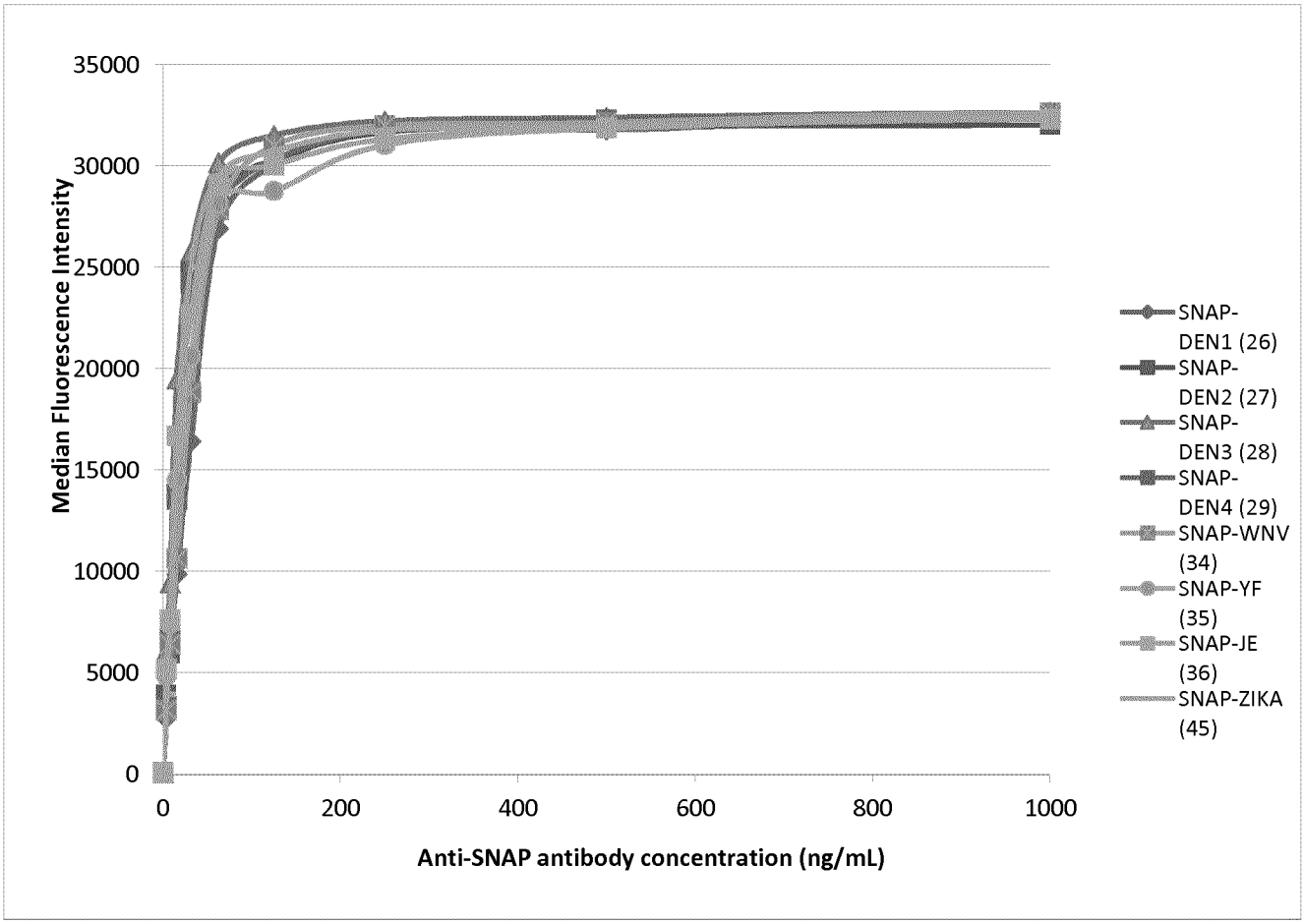

35000
30000
25000
20000
15000
10000
5000
0
Median Fluorescence Intensity
0
200
400
600
800
1000
Anti-SNAP antibody concentration (ng/mL)
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-ZIKA (45)

Figure 3

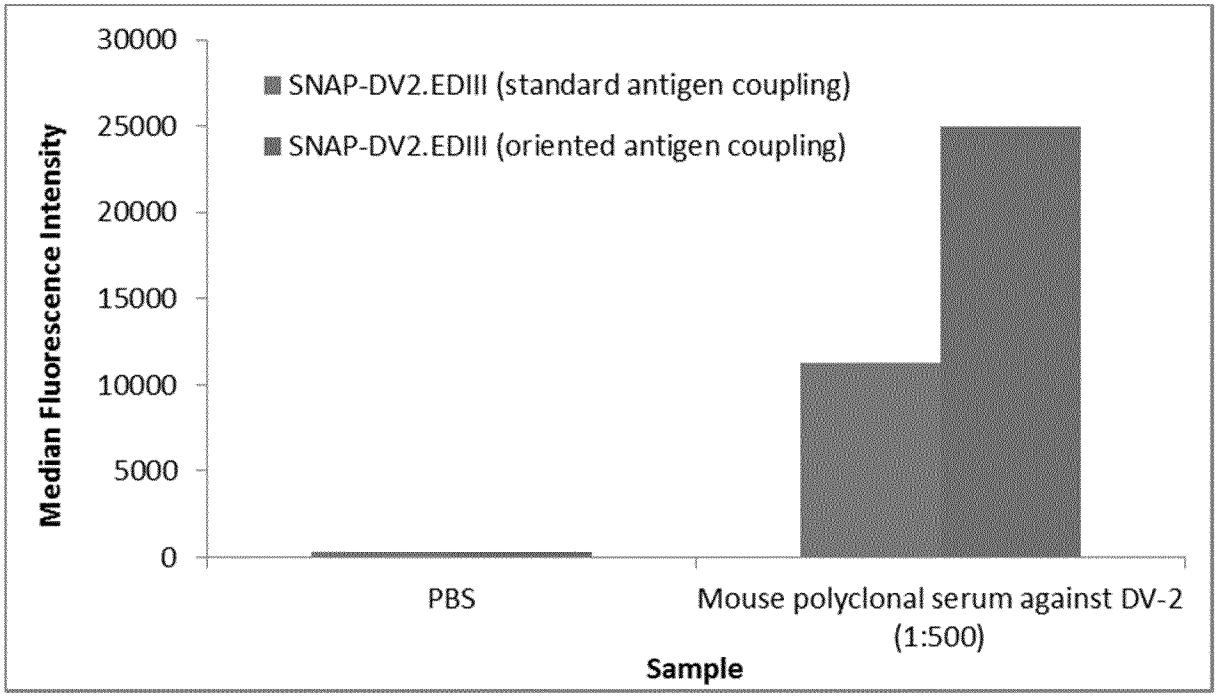

30000
25000
20000
15000
10000
5000
0
Median Fluorescence Intensity
SNAP-DV2.EDIII (standard antigen coupling)
SNAP-DV2.EDIII (oriented antigen coupling)
PBS
Mouse polyclonal serum against DV-2 (1:500)
Sample

Figure 4

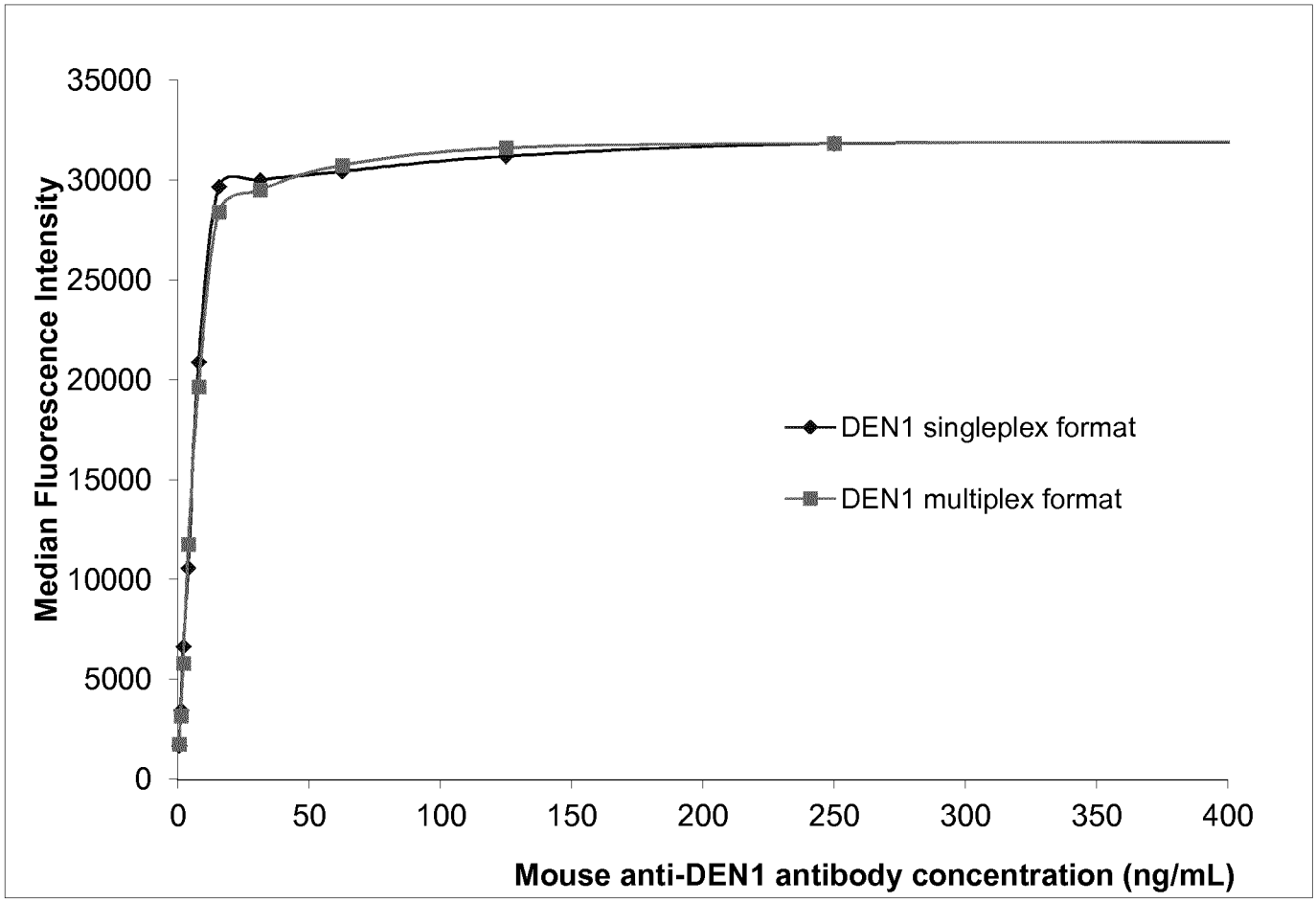

35000
30000
25000
20000
15000
10000
5000
0
Median Fluorescence Intensity
0
50
100
150
200
250
300
350
400
Mouse anti-DEN1 antibody concentration (ng/mL)
DEN1 singleplex format
DEN1 multiplex format

Figure 5

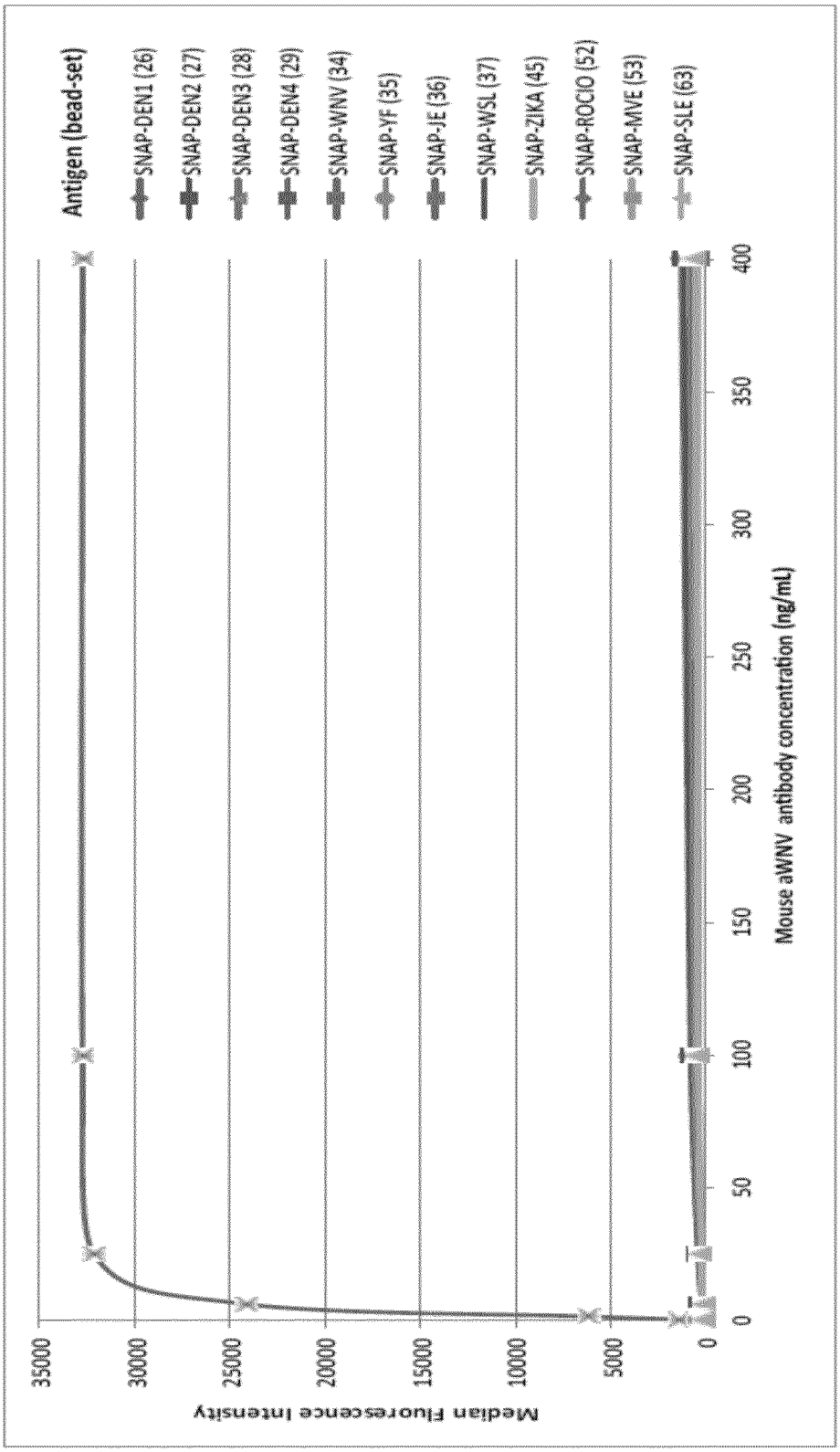
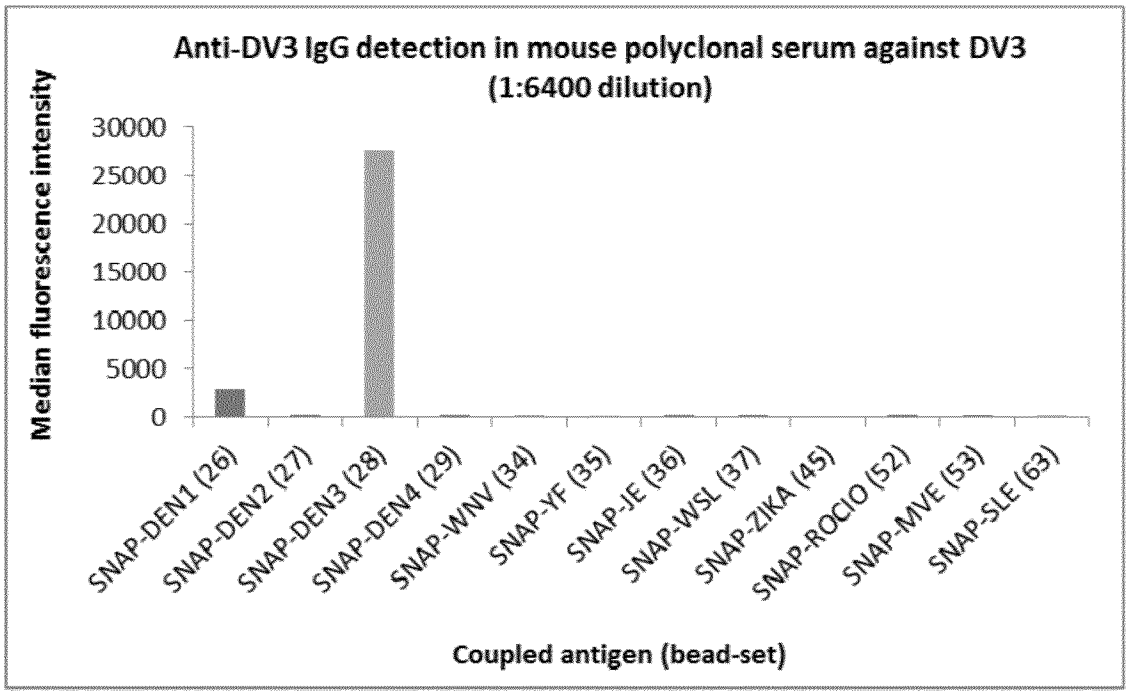

Antigen (bead-set)
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-WSL (37)
SNAP-ZIKA (45)
SNAP-ROCIO (52)
SNAP-MVE (53)
SNAP-SLE (63)
Median Fluorescence Intensity
35000
30000
25000
20000
15000
10000
5000
0
0
50
100
150
200
250
300
350
400
Mouse aWNV antibody concentration (ng/mL)

Figure 6

A


Anti-DV3 IgG detection in mouse polyclonal serum against DV3
(1:6400 dilution)
Median fluorescence intensity
30000
25000
20000
15000
10000
5000
0
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-WSL (37)
SNAP-ZIKA (45)
SNAP-ROCIO (52)
SNAP-MVE (53)
SNAP-SLE (63)
Coupled antigen (bead-set)

B

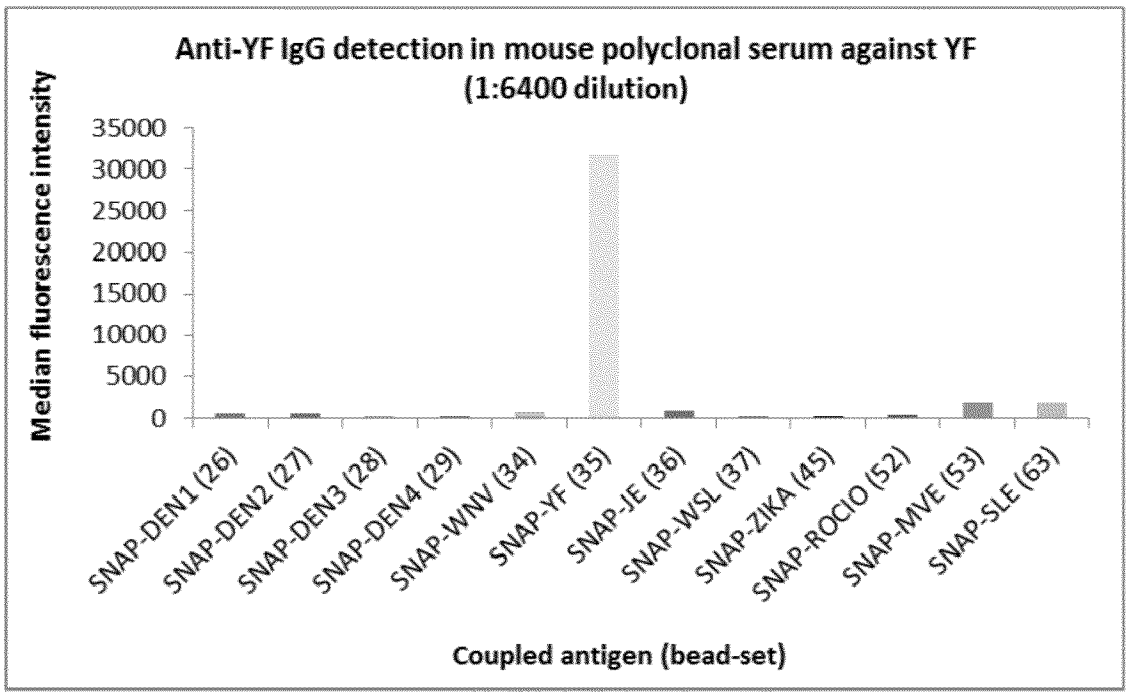
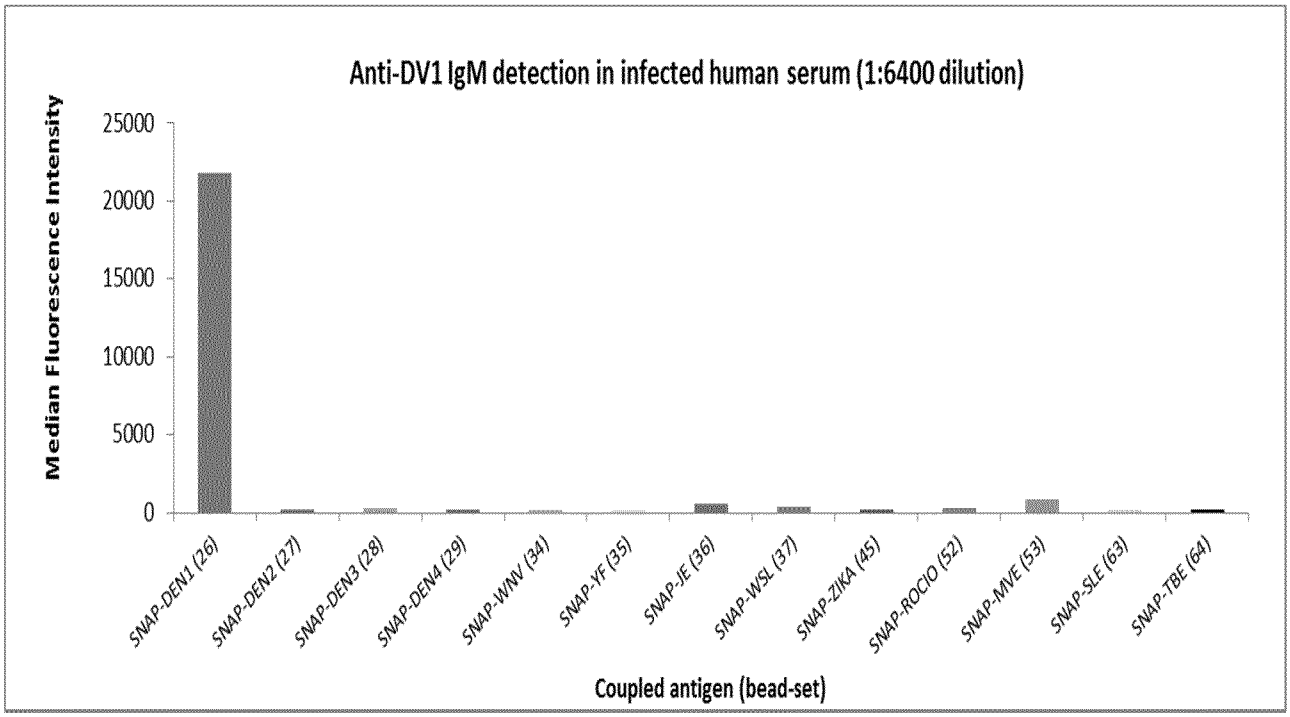

Anti-YF IgG detection in mouse polyclonal serum against YF
(1:6400 dilution)
Median fluorescence intensity
35000
30000
25000
20000
15000
10000
5000
0
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-WSL (37)
SNAP-ZIKA (45)
SNAP-ROCIO (52)
SNAP-MVE (53)
SNAP-SLE (63)
Coupled antigen (bead-set)

Figure 7

A


Anti-DV1 IgM detection in infected human serum (1:6400 dilution)
Median Fluorescence Intensity
25000
20000
15000
10000
5000
0
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-WSL (37)
SNAP-ZIKA (45)
SNAP-ROCIO (52)
SNAP-MVE (53)
SNAP-SLE (63)
SNAP-TBE (64)
Coupled antigen (bead-set)

B

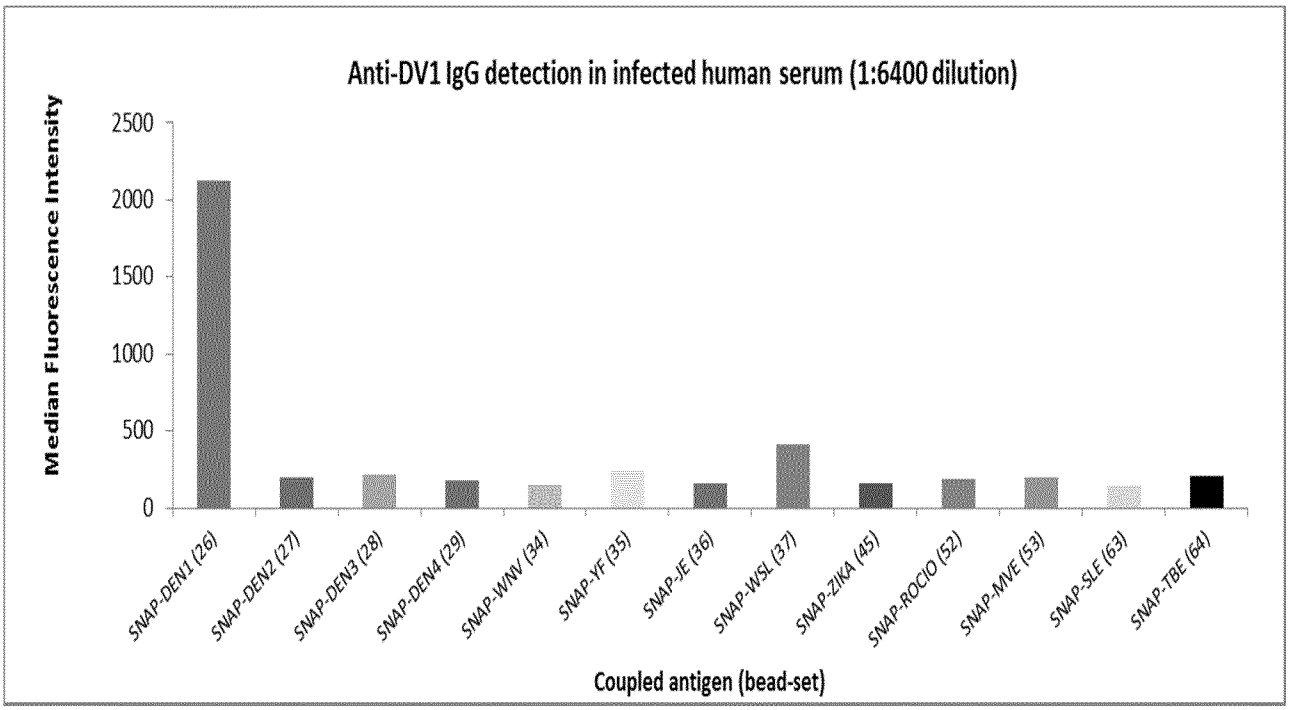

Anti-DV1 IgG detection in infected human serum (1:6400 dilution)
Median Fluorescence Intensity
2500
2000
1500
1000
500
0
SNAP-DEN1 (26)
SNAP-DEN2 (27)
SNAP-DEN3 (28)
SNAP-DEN4 (29)
SNAP-WNV (34)
SNAP-YF (35)
SNAP-JE (36)
SNAP-WSL (37)
SNAP-ZIKA (45)
SNAP-ROCIO (52)
SNAP-MVE (53)
SNAP-SLE (63)
SNAP-TBE (64)
Coupled antigen (bead-set)

Figure 8

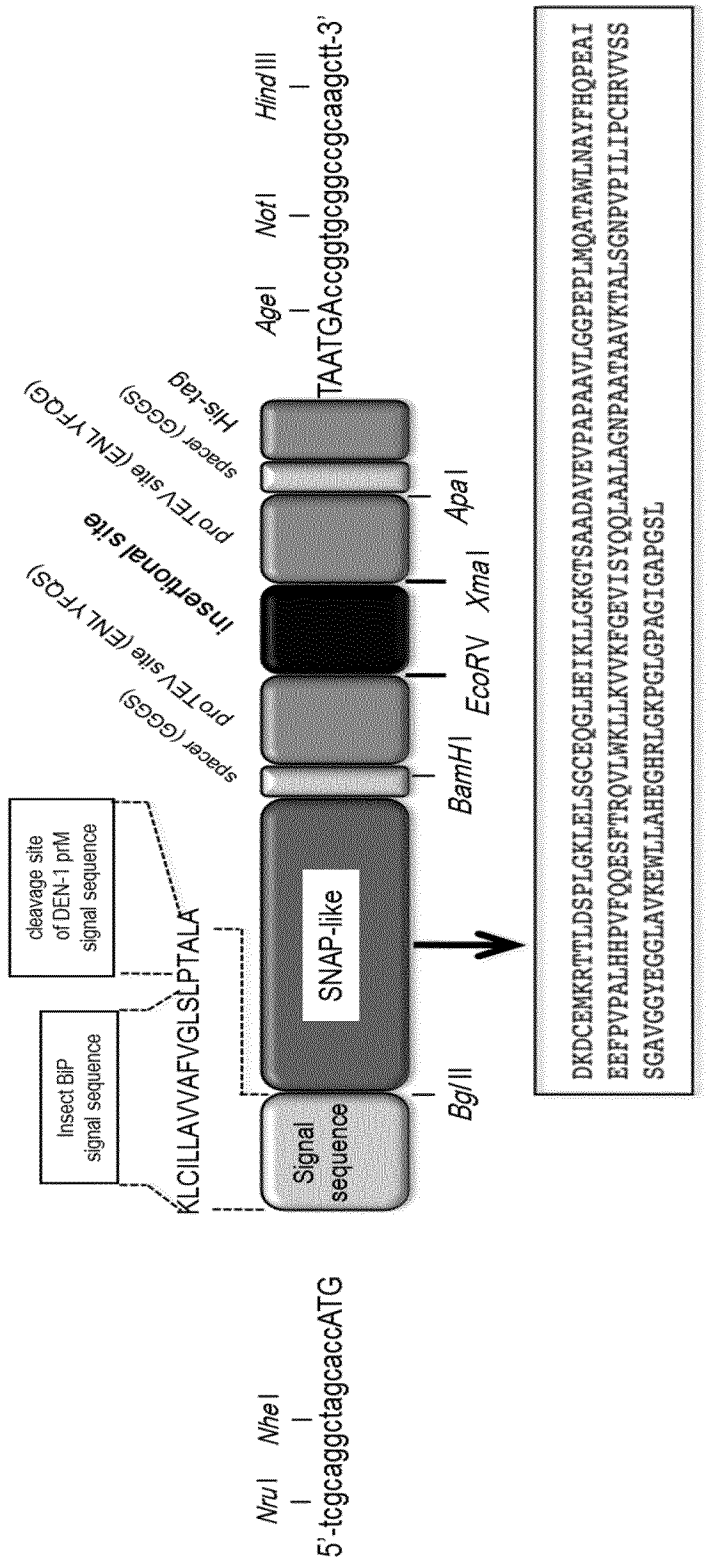

5'-tcgcaggctagcaccATG
NruI NheI
KLCILLAVVAFVGLSLPTALA
Insect BiP signal sequence
cleavage site of DEN-1 prM signal sequence
Signal sequence
SNAP-like
BglII
BamHI
EcoRV
XmaI
ApaI
spacer (GGGS)
proTEV site (ENL YFQG)
insertional site
spacer (GGGS)
proTEV site (ENL YFQG)
His-tag
AgeI
NotI
HindIII
TAATGAccggtgcggccgcaagctt-3'
DKDCEMKRTTLDSPLGKLELSGCEQGLHEIKLLGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNAYFHQPEAI
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Figure 9

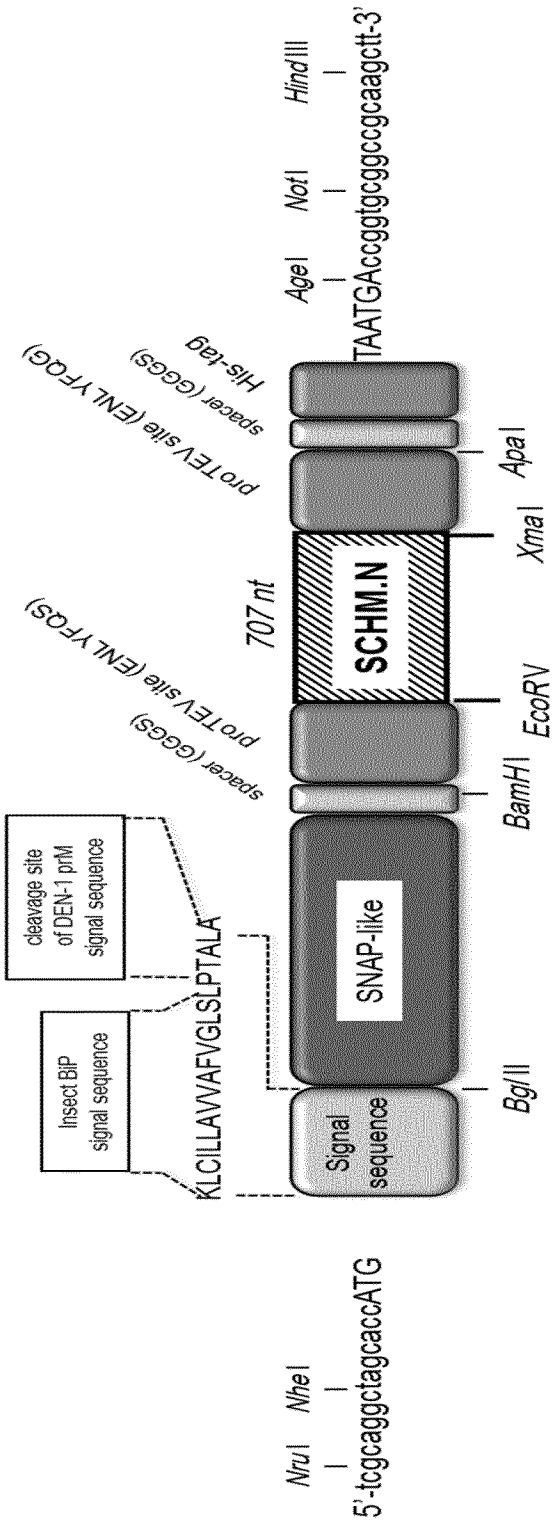

5'-tcgcaggctagcaccATG
NruI
NheI
KLCILLAVVAFVGLSLPTALA
Insect BiP signal sequence
cleavage site of DEN-1 prM signal sequence
Signal sequence
SNAP-like
BglII
spacer (GGGS)
proTEV site (ENL YFQS)
BamHI
707 nt
SCHM.N
EcoRV
proTEV site (ENL YFQG)
spacer (GGGS)
His-tag
XmaI
ApaI
AgeI
NotI
HindIII
TAATGAccggtgcggccgcaagctt-3'

Figure 10 A

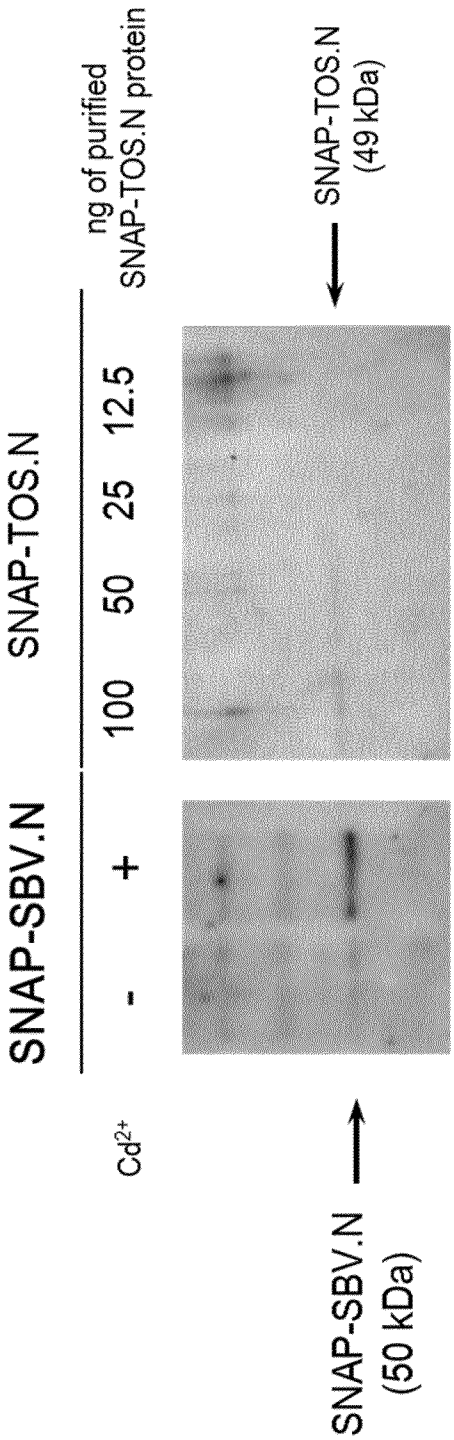

SNAP-SBV.N
SNAP-TOS.N
ng of purified SNAP-TOS.N protein
Cd2+
-
+
100
50
25
12.5
SNAP-TOS.N (49 kDa)
SNAP-SBV.N (50 kDa)

Figure 10 B

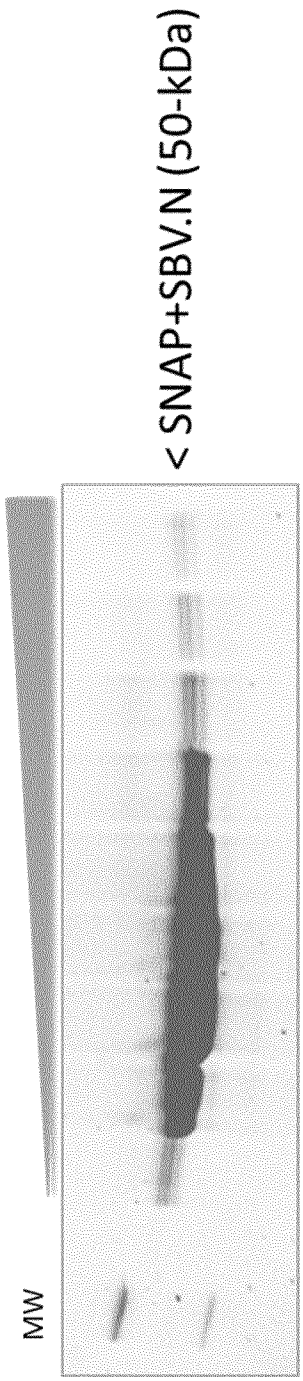
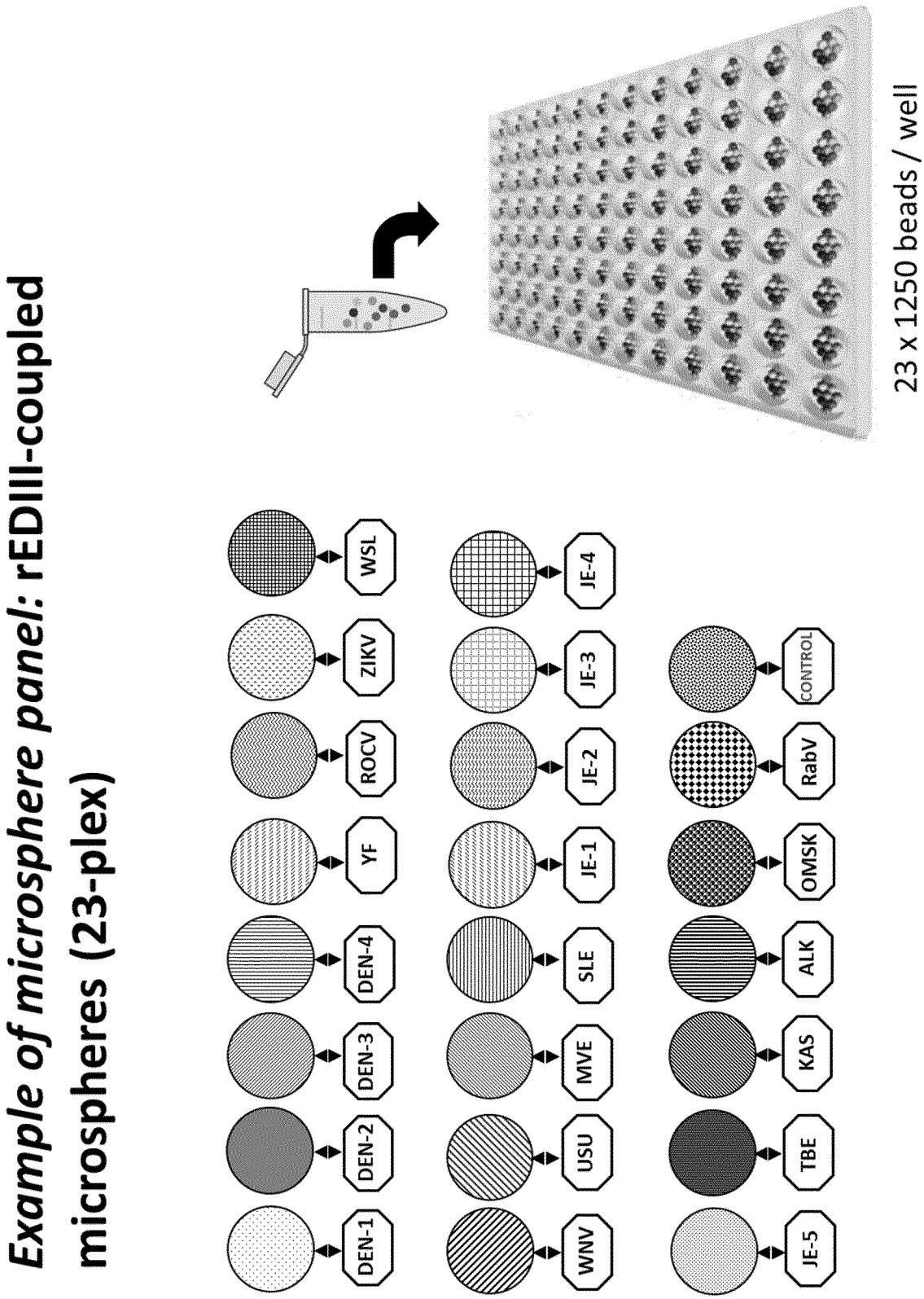


Figure 11


Example of microsphere panel: rEDIII-coupled microspheres (23-plex)
DEN-1
DEN-2
DEN-3
DEN-4
YF
ROCV
ZIKV
WSL
WNV
USU
MVE
SLE
JE-1
JE-2
JE-3
JE-4
JE-5
TBE
KAS
ALK
OMSK
RabV
CONTROL
23 x 1250 beads / well

SEQUENCE LISTING

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<130> 362007D30751

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<151> 2011-12-09

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<151> 2012-05-04

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<400> 1

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1               5                  10                  15

Gln Val Leu Ala Val Arg Thr Val Cys Asp Leu Val Leu Gly Lys Met
            20                  25                  30

Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
        35                  40                  45

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
    50                  55                  60

Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
65                  70                  75                  80

Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Cys Thr Ala Trp
                85                  90                  95

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
            100                 105                 110

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
        115                 120                 125

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
    130                 135                 140

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Lys Ala Ala Arg Ala
145                 150                 155                 160

Val Gly Gly Ala Met Arg Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
                165                 170                 175
```

```
His Arg Val Val Cys Ser Ser Gly Ala Val Gly Asn Tyr Ser Gly Gly
            180                 185                 190

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
        195                 200                 205

Lys Pro Gly Leu Gly Gly Ser Ser Gly Leu Ala Gly Ala Trp Leu Lys
    210                 215                 220

Gly Ala Gly Ala Thr Ser Gly Ser Pro Pro Ala Gly Arg Asn
225                 230                 235
```

<210> 2
<211> 193
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of SNAP

<400> 2

```
Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1               5                   10                  15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
```

165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu

<210> 3
<211> 404
<212> DNA
<213> Dengue virus 1

<400> 3
taggcgcgcc agggtccctg gagggaggtg gcgggtctct gactttaaaa gggatgtcat 60

atgtgatgtg cacaggctca tttaagctag agaaggaagt ggctgagacc agcatggaa 120

ctgtcctagt gcaggttaaa tacgaaggaa cagatgcgcc atgcaagatc ccctttcga 180

cccaagatga gaaaggagtg acccagaatg ggagattgat aacagccaat cccatagtta 240

ctgacaaaga aaaaccaatc aacattgaga cagaaccacc ttttggtgag agctacatca 300

tagtaggggc aggtgaaaaa gctttgaaac taagctggtt caagaaagga agcagcatag 360

ggaaaggagg tggccatcac catcaccatc actgatgacc ggtt 404

<210> 4
<211> 404
<212> DNA
<213> Dengue virus 2

<400> 4
taggcgcgcc agggtccctg gagggaggtg gcgggtctct acagctcaaa ggaatgtcat 60

attctatgtg tacaggaaag tttaaagttg tgaaggaaat agcagaaaca caacatggaa 120

caatagttct cagagtacaa tatgaagggg acggttctcc gtgcaagatc ccttttgaaa 180

taatggattt ggaaaaaaga catgtcttag gtcgcttgat cacagtcaac ccaattgtta 240

cagaaataga cagcccagtc aacatagaag cagaacctcc attcggagac agctacatca 300

ttataggagt agaaccggga caactgaagc tcagctggtt taagaaagga agttccattg 360

gccaaggagg tggccatcac catcaccatc actgatgacc ggtt 404

<210> 5
<211> 401
<212> DNA
<213> Dengue virus 3

<400> 5
taggcgcgcc agggtccctg gagggaggtg gcgggtctga actcaagggg atgagctatg 60

caatgtgctt gaataccttt gtgttgaaga aagaagtctc cgaaacgcag catgggacaa 120

tactcattaa ggttgagtac aaaggggaag atgcaccttg caagattcct ttctccacag 180

aggatggaca agggaaagcc cacaatggta gactgatcac agccaaccca gtggttacta 240

agaaggagga gcctgtcaac attgaggctg aacctccttt tggggaaagc aacatagtga 300

ttggagttgg agacaaagcc ttgaaaatta actggtacaa gaagggaagc tcgattggga 360

agggaggtgg ccatcaccat caccatcact gatgaccggt t 401

<210> 6
<211> 401
<212> DNA
<213> Dengue virus 4

<400> 6
taggcgcgcc agggtccctg gagggaggtg gcgggtctag aatcaaggga atgtcataca 60

cgatgtgctc aggaaagttc tcaattgaca aagagatggc agaaacacag catgggacaa 120

cagtggtgaa agtcaagtat gaaggtgctg gagctccgtg taaagtcccc atagagatac 180

gagatgtaaa taaggaaaaa gtggttgggc gtgtcatctc atccacccct ctagctgaga 240

ataccaacag tgtgaccaac atagaactgg aacccccctt tggggacagt tacatagtca 300

taggtgttgg gaacagtgca ttgacactcc attggttcag gaaaggaagt tctattggca 360

agggaggtgg ccatcaccat caccatcact gatgaccggt t 401

<210> 7
<211> 398
<212> DNA
<213> West Nile Virus

<400> 7
taggcgcgcc aggaggtggc gggtctcagt tgaagggaac aacctatggc gtctgttcaa 60

aggctttcaa gtttcttggg actcccgcag acacaggtca cggcactgtg gtgttggaat 120

tgcagtacac tggcacggat ggaccttgca aagttcctat ctcgtcagtg gcttcattga 180

acgacctaac gccagtgggc agattggtca ctgtcaaccc ttttgtttca gtggccacgg 240

ccaacgctaa ggtcctgatt gaattggaac cacccttttgg agactcatac atagtggtgg 300

gcagaggaga acaacagatc aatcaccatt ggcacaagtc tggaagcagc attggcaaag 360

gaggtggcca tcaccatcac catcactgat gaccggtt 398

<210> 8
<211> 410
<212> DNA
<213> Yellow fever virus

<400> 8
taggcgcgcc agggtccctg gagggaggtg gcgggtcttc agctttgaca ctcaagggga 60

catcctacaa aatgtgcact gacaaaatgt cttttgtcaa gaacccaact gacactggcc 120

atggcactgt tgtgatgcag gtgaaagtgc caaaaggagc cccctgcaag attccagtga 180

tagtagctga tgatcttaca gcggcaatca ataaaggcat tttggttaca gttaacccca 240

tcgcctcaac caatgatgat gaagtgctga ttgaggtgaa cccacctttt ggagacagct 300

acattatcgt tgggacagga gattcacgtc tcacttacca gtggcacaaa gagggaagct 360

caataggaaa gggaggtggc catcaccatc accatcactg atgaccggtt 410

<210> 9
<211> 410
<212> DNA
<213> Japanese encephalitis virus

<400> 9
taggcgcgcc agggtccctg gagggaggtg gcgggtctgc tctgaaaggc acaacctatg 60

gcatgtgtac agaaaaattc tcgttcgcga aaaatccggc ggacactggt cacggaacag 120

ttgtcattga actctcctac tctgggagtg atggcccctg caaaattccg attgtctccg 180

tcgcgagcct caatgacatg actcctgttg ggcggctggt gacagtgaac ccctttgtcg 240

cggcttccag tgccaactca aaggtgctgg tcgagatgga acccccccttc ggagactcct 300

atatcgtggt tggaagggga gacaagcaga tcaaccacca ttggcacaga gctggaagca 360

cgctgggcaa gggaggtggc catcaccatc accatcactg atgaccggtt 410

<210> 10
<211> 397
<212> DNA
<213> Zika virus

<400> 10
gcgcgccagg aggtggcggg tctcttagat tgaagggcgt gtcatactcc ttgtgtaccg 60

cagcgttcac attcaccaag atcccggctg aaacactgca cgggacagtc acagtggagg 120

tacagtacgc agggacagat ggaccctgca aggttccagc tcagatggcg gtggacatgc 180

aaactctgac cccagttggg aggctgataa ccgctaaccc tgtaatcact gaaagcactg 240

agaactctaa gatgatgctg gaacttgatc caccatttgg ggactcttac attgtcatag 300

gagtcgggga gaagaagatc acccatcact ggcacaggag tggcagcacc attggaaaag 360

gaggtggcca tcaccatcac catcactgat gaccggt 397

<210> 11
<211> 385
<212> DNA
<213> Wesselbron virus

<400> 11
gcgcgccagg aggtggcggg ctcatcctga aaggttcaac ctactcaatg tgcaaaagag 60

ggatgtcctt tgctaagcaa ccagttgaga cagaccatgg aacagcagtg atgcagataa 120

aagttacaac tggagctccg tgcagaattc cagtgattgc agcagattcc atggcgggaa 180

cagaaaaccg tggaagcgtc atcacaacca atcctattgc tgcgtcaaac aatgatgaag 240

tgttggtgga gatcagtcca ccatttggag agagttacat catcgttggt aatggagatg 300

ataaacttac ataccactgg caaagatcag gaagcaccat cgggaatgga ggtggccatc 360

accatcacca tcactgatga ccggt 385

<210> 12
<211> 397
<212> DNA
<213> Rocio virus

<400> 12
gcgcgccagg aggtggcggg tctctcaaaa tcaaagggtc aacatacctg atgtgcaagg 60

acaaatttgc ttttgccaag aacccagttg acacaggaca cggcacaatc gtgacggagg 120

tacagtacgc tggttctgat gggccatgca ggattccaat caccatgacc gagaacctac 180

atgatctcac tcccatcgga cgattggtga cggtcaatcc atttgttccc tcatccgaga 240

cggcacaaaa aattttgatt gaactcgagc ccccctttgg gacatccttc atactggtgg 300

gtacaggtcc caaccaggtg aaataccagt ggcataagtc tggtagtgtg atcggaaaag 360

gaggtggcca tcaccatcac catcactgat gaccggt 397

<210> 13
<211> 397
<212> DNA
<213> Murray encephalitis virus

<400> 13
gcgcgccagg aggtggcggg tctttgaaac tgaaaggaac cacttatggg atgtgcacag 60

aaaaatttac tttctcaaag aatccagccg acaccggaca tggcacggta gtactagaac 120

tgcagtacac cgggagtgat ggaccatgca aaattccaat atcctctgta gcaagtctca 180

atgacatgac gcctgtcgga agaatggtga cagctaatcc atatgtagct tcatcaactg 240

ccaatgctaa agttctggtg gagattgaac caccttcgg agactcatac attgtggtag 300

gcaggggaga caagcagatc aatcaccact ggcataagga gggtagttca attggcaaag 360

gaggtggcca tcaccatcac catcactgat gaccggt 397

<210> 14
<211> 397
<212> DNA
<213> Saint-Louis encephalitis virus

<400> 14
gcgcgccagg aggtggcggg tctgttaaaa tcaaggggac gacatatggt atgtgtgact 60

ctgctttcac cttcagcaag aaccctgctg acacagggca tgggacagtg atcgtggaac 120

tgcagtacac tggaagcaac ggaccatgcc gggttcccat ttctgtgact gcaaacctca 180

tggacttgac accagttgga agactggtca cggtcaatcc ctttattagc acagggggag 240

cgaacaacaa ggtcatgatc gaagttgaac cacccttgg cgactcttac atcgtcgtcg 300

gaagaggcac cacccagatc aactaccact ggcacaaaga gggaagcagc attgggaagg 360

gaggtggcca tcaccatcac catcactgat gaccggt 397

<210> 15
<211> 579
<212> DNA
<213> artificial

<220>
<223> DNA encoding original SNAP (normal G/C content)

<400> 15

agatctgaca aagactgcga aatgaagcgc accaccctgg atagccctct gggcaagctg 60

gaactgtctg ggtgcgaaca gggcctgcac gagatcaagc tgctgggcaa aggaacatct 120

gccgccgacg ccgtggaagt gcctgcccca gccgccgtgc tgggcggacc agagccactg 180

atgcaggcca ccgcctggct caacgcctac tttcaccagc ctgaggccat cgaggagttc 240

cctgtgccag ccctgcacca cccagtgttc cagcaggaga gctttacccg ccaggtgctg 300

tggaaactgc tgaaagtggt gaagttcgga gaggtcatca gctaccagca gctggccgcc 360

ctggccggca atcccgccgc caccgccgcc gtgaaaaccg ccctgagcgg aaatcccgtg 420

cccattctga tcccctgcca ccgggtggtg tctagctctg gcgccgtggg gggctacgag 480

ggcgggctcg ccgtgaaaga gtggctgctg gcccacgagg gccacagact gggcaagcct 540

gggctgggtc ctgcaggtat aggcgcgcca gggtcccta 579

<210> 16
<211> 235
<212> PRT
<213> artificial

<220>
<223> mutated N protein of schmallenberg virus

<400> 16

Asp Ile Ser Ser Gln Phe Ile Phe Glu Asp Val Pro Gln Arg Asn Ala
1 5 10 15

Ala Thr Phe Asn Pro Glu Val Gly Tyr Val Ala Phe Ile Gly Lys Tyr
20 25 30

Gly Gln Gln Leu Asn Phe Gly Val Ala Arg Val Phe Phe Leu Asn Gln
35 40 45

Lys Lys Ala Lys Met Val Leu His Lys Thr Ala Gln Pro Ser Val Asp
50 55 60

Leu Thr Phe Gly Gly Val Lys Phe Thr Val Val Asn Asn His Phe Pro
65 70 75 80

Gln Tyr Val Ser Asn Pro Val Pro Asp Asn Ala Ile Thr Leu His Arg
85 90 95

Met Ser Gly Tyr Leu Ala Arg Trp Ile Ala Asp Thr Cys Lys Ala Ser
100 105 110

Val Leu Lys Leu Ala Glu Ala Ser Ala Gln Ile Val Met Pro Leu Ala
115 120 125

Glu Val Lys Gly Cys Thr Trp Ala Asp Gly Tyr Thr Met Tyr Leu Gly
130 135 140

Phe Ala Pro Gly Ala Glu Met Phe Leu Asp Ala Phe Asp Phe Tyr Pro

145 150 155 160

Leu Val Ile Glu Met His Arg Val Leu Lys Asp Asn Met Asp Val Asn
165 170 175

Phe Met Lys Lys Val Leu Arg Gln Arg Tyr Gly Thr Met Thr Ala Glu
180 185 190

Glu Trp Met Thr Gln Lys Ile Thr Glu Ile Lys Ala Ala Phe Asn Ser
195 200 205

Val Gly Gln Leu Ala Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg
210 215 220

Thr Phe Leu Gln Gln Phe Gly Ile Asn Ile Pro
225 230 235

<210> 17
<211> 707
<212> DNA
<213> artificial

<220>
<223> Mutated DNA sequence encoding the N protein of schmallenberg virus

<400> 17
gatatctcaa gccaattcat ttttgaagat gtaccacaac ggaatgcagc tacatttaac 60

ccggaggtcg ggtatgtggc atttattggt aagtatgggc aacaactcaa cttcggtgtt 120

gctagagtct tcttcctcaa ccagaagaag gccaagatgg tcctacataa gacggcacaa 180

ccaagtgtcg atcttacttt tggtggggtc aaatttacag tggttaataa ccattttccc 240

caatatgtct caaatcctgt gccagacaat gccattacac ttcacaggat gtcaggttat 300

ctagcacgtt ggattgctga tacatgcaag gctagtgtcc tcaaactagc tgaagctagt 360

gctcagattg tcatgcccct tgctgaggtt aagggatgca cctgggccga tggttataca 420

atgtatcttg gatttgcacc tggggccgaa atgttccttg atgcttttga cttctatcca 480

ctagttattg aaatgcatag ggtcctcaag gacaatatgg atgtaaattt tatgaaaaaa 540

gtcctccgcc aacgctatgg aacaatgact gctgaagaat ggatgactca gaaaataaca 600

gaaataaaag ctgcttttaa ttctgttgga cagcttgcct gggccaaatc tggattctct 660

cctgctgcta gaaccttctt gcagcaattc ggtatcaaca tcccggg 707

<210> 18
<211> 211
<212> PRT
<213> mus musculus

<400> 18

Met Ala Glu Thr Cys Lys Met Lys Tyr Ser Val Leu Asp Ser Pro Leu
1 5 10 15

```
Gly Lys Met Glu Leu Ser Gly Cys Glu Arg Gly Leu His Gly Ile Arg
            20                  25                  30

Leu Leu Ser Gly Lys Thr Pro Asn Thr Asp Pro Thr Glu Ala Pro Ala
        35                  40                  45

Thr Pro Glu Val Leu Gly Gly Pro Glu Gly Val Pro Glu Pro Leu Val
    50                  55                  60

Gln Cys Thr Ala Trp Leu Glu Ala Tyr Phe Arg Glu Pro Ala Ala Thr
65                  70                  75                  80

Glu Gly Leu Pro Leu Pro Ala Leu His His Pro Val Phe Gln Gln Asp
                85                  90                  95

Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe
            100                 105                 110

Gly Glu Thr Val Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro
        115                 120                 125

Lys Ala Ala Arg Ala Val Gly Gly Ala Met Arg Ser Asn Pro Val Pro
    130                 135                 140

Ile Leu Ile Pro Cys His Arg Val Val Arg Ser Asp Gly Ala Ile Gly
145                 150                 155                 160

His Tyr Ser Gly Gly Gly Gln Ala Val Lys Glu Trp Leu Leu Ala His
                165                 170                 175

Glu Gly Ile Pro Thr Gly Gln Pro Ala Ser Lys Gly Leu Gly Leu Thr
            180                 185                 190

Gly Thr Trp Leu Lys Ser Ser Phe Glu Ser Thr Ser Ser Glu Pro Ser
        195                 200                 205

Gly Arg Asn
    210


<210>  19
<211>  209
<212>  PRT
<213>  Rattus norvegicus

<400>  19

Met Ala Glu Ile Cys Lys Met Lys Tyr Thr Val Leu Asp Ser Pro Leu
1               5                   10                  15

Gly Lys Ile Glu Leu Ser Gly Cys Glu Arg Gly Leu His Gly Ile Arg
            20                  25                  30
```

Phe Leu Ser Gly Lys Thr Pro Asn Thr Asp Pro Thr Glu Ala Pro Ala
35 40 45

Cys Pro Glu Val Leu Gly Gly Pro Glu Gly Val Pro Glu Pro Leu Val
50 55 60

Gln Cys Thr Ala Trp Leu Glu Ala Tyr Phe His Glu Pro Ala Ala Thr
65 70 75 80

Glu Gly Leu Pro Leu Pro Ala Leu His His Pro Val Phe Gln Gln Asp
85 90 95

Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe
100 105 110

Gly Glu Met Val Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro
115 120 125

Lys Ala Ala Arg Ala Val Gly Gly Ala Met Arg Ser Asn Pro Val Pro
130 135 140

Ile Leu Ile Pro Cys His Arg Val Ile Arg Ser Asp Gly Ala Ile Gly
145 150 155 160

Asn Tyr Ser Gly Gly Gly Gln Thr Val Lys Glu Trp Leu Leu Ala His
165 170 175

Glu Gly Ile Pro Thr Gly Gln Pro Ala Ser Lys Gly Leu Gly Leu Ile
180 185 190

Gly Ser Trp Leu Lys Pro Ser Phe Glu Ser Ser Ser Pro Lys Pro Ser
195 200 205

Gly

<210> 20
<211> 963
<212> DNA
<213> artificial

<220>
<223> DNA sequence coding for SNAP - linker - DEN1 EDIII - linker-Histag

<400> 20
agatctgaca aagactgcga aatgaagcgc accaccctgg atagccctct gggcaagctg 60

gaactgtctg ggtgcgaaca gggcctgcac gagatcaagc tgctgggcaa aggaacatct 120

gccgccgacg ccgtggaagt gcctgcccca gccgccgtgc tgggcggacc agagccactg 180

atgcaggcca ccgcctggct caacgcctac tttcaccagc ctgaggccat cgaggagttc 240

cctgtgccag ccctgcacca cccagtgttc cagcaggaga gctttacccg ccaggtgctg 300

tggaaactgc tgaaagtggt gaagttcgga gaggtcatca gctaccagca gctggccgcc 360

ctggccggca atcccgccgc caccgccgcc gtgaaaaccg ccctgagcgg aaatcccgtg 420

cccattctga tcccctgcca ccgggtggtg tctagctctg gcgccgtggg gggctacgag 480

ggcgggctcg ccgtgaaaga gtggctgctg gcccacgagg gccacagact gggcaagcct 540

gggctgggtc ctgcaggtat aggcgcgcca gggtccctgg agggaggtgg cgggtctctg 600

actttaaaag ggatgtcata tgtgatgtgc acaggctcat ttaagctaga gaaggaagtg 660

gctgagaccc agcatggaac tgtcctagtg caggttaaat acgaaggaac agatgcgcca 720

tgcaagatcc ccttttcgac ccaagatgag aaaggagtga cccagaatgg gagattgata 780

acagccaatc ccatagttac tgacaaagaa aaaccaatca acattgagac agaaccacct 840

tttggtgaga gctacatcat agtaggggca ggtgaaaaag ctttgaaact aagctggttc 900

aagaaaggaa gcagcatagg gaaaggaggt ggccatcacc atcaccatca ctgatgaccg 960

gtt 963

<210> 21
<211> 316
<212> PRT
<213> artificial

<220>
<223> SNAP - linker - DEN1 EDIII - linker- Histag

<400> 21

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala
35 40 45

Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala
50 55 60

Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro
65 70 75 80

Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg
85 90 95

Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile
100 105 110

Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala
115 120 125

Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro

130 135 140

Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly
145 150 155 160

Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu
165 170 175

Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu
180 185 190

Glu Gly Gly Gly Gly Ser Leu Thr Leu Lys Gly Met Ser Tyr Val Met
195 200 205

Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val Ala Glu Thr Gln His
210 215 220

Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly Thr Asp Ala Pro Cys
225 230 235 240

Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly Val Thr Gln Asn Gly
245 250 255

Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp Lys Glu Lys Pro Ile
260 265 270

Asn Ile Glu Thr Glu Pro Pro Phe Gly Glu Ser Tyr Ile Ile Val Gly
275 280 285

Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe Lys Lys Gly Ser Ser
290 295 300

Ile Gly Lys Gly Gly Gly His His His His His His
305 310 315

<210> 22
<211> 54
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding the ssBiP sequence

<400> 22
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cggg 54

<210> 23
<211> 66
<212> DNA
<213> artificial

<220>
<223> DNA artificial encoding BiP-like signal = insect ssBiP signal peptide + cleavage site DEN1prM signal sequence

<400> 23
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt acctacagct 60

ctggca 66

<210> 24
<211> 22
<212> PRT
<213> artificial

<220>
<223> BiP-like signal = insect ssBiP signal peptide + cleavage site DEN1prM signal sequence

<400> 24

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1 5 10 15

Leu Pro Thr Ala Leu Ala
20

<210> 25
<211> 4
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the spacer

<400> 25

Gly Gly Gly Ser
1

<210> 26
<211> 12
<212> DNA
<213> artificial

<220>
<223> DNA sequence of the spacer

<400> 26
ggtggcggat ct 12

<210> 27
<211> 6
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of HIS TAG

<400> 27

His His His His His His
1 5

<210> 28
<211> 18
<212> DNA

<213> artificial

<220>
<223> DNA sequence encoding His Tag

<400> 28
catcatcatc atcatcat 18

<210> 29
<211> 21
<212> DNA
<213> artificial

<220>
<223> DNA encoding pro-TEV cleavage site 1

<400> 29
gaaaacctgt acttccagag c 21

<210> 30
<211> 21
<212> DNA
<213> artificial

<220>
<223> DNA encoding pro-TEV cleavage site 2

<400> 30
gagaatctat attttcaagg g 21

<210> 31
<211> 530
<212> DNA
<213> artificial

<220>
<223> SNAP-like sequence G/C low content

<400> 31
gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 60

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 120

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 180

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 240

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 300

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 360

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 420

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 480

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa 530

<210> 32
<211> 7
<212> PRT
<213> artificial

<220>
<223> Pro-TEV cleavage site 1

<400> 32

Glu Asn Leu Tyr Phe Gln Ser
1 5

<210> 33
<211> 7
<212> PRT
<213> artificial

<220>
<223> Pro-TEV cleavage site 2

<400> 33

Glu Asn Leu Tyr Phe Gln Gly
1 5

<210> 34
<211> 798
<212> DNA
<213> artificial

<220>
<223> Cassette DNA pDeSNAP Univ (BIPlike - SNAPlike- proTEV/Histag)

<400> 34
gatcgcgagc tagcaccatg aaactatgta ttctacttgc agttgttgcg ttcgtaggat 60

tgtccttacc tacagctctg gcaagatctg acaaagactg cgaaatgaaa agaactacat 120

tggattcacc acttgggaag ttggaactga gtggatgcga gcaaggattg catgaaatta 180

agctactggg aaaaggaact tctgctgctg atgcagttga agttccagca ccagcagctg 240

ttcttggagg tcctgagccc ctcatgcaag ccacagcctg gcttaacgca tatttccacc 300

agcctgaggc cattgaggaa tttccagtcc ccgcccttca ccatcctgtg tttcagcagg 360

agagcttcac ccgccaggtc ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga 420

tttcatatca gcaacttgct gcattggccg gtaaccccgc agctacagct gccgtgaaaa 480

ctgctctcag cggaaatcct gtgcccatcc tgatcccttg tcacagagtc gtttcatctt 540

ccggagctgt aggtggctat gaaggaggac tggcagttaa ggagtggctg ctggctcatg 600

aaggtcatag acttggaaag cctgggctgg gtcctgctgg tataggcgcg ccagggtccc 660

taggtggcgg atccgaaaac ctgtacttcc agagcgatat cggaggtgga ggcccgggag 720

agaatctata ttttcaaggg cccggcggag gtagtcacca tcatcaccat cactaatgac 780

cggtgcggcc gcaagctt 798

<210> 35
<211> 1439
<212> DNA
<213> artificial

<220>
<223> Cassette DNA pDeSNAP Univ +SBV.N (ssBIP - SNAPlike- SBV.N-
proTEV/Histag)

<400> 35
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tctcaagcca attcattttt gaagatgtac cacaacggaa tgcagctaca 720

tttaacccgg aggtcgggta tgtggcattt attggtaagt atgggcaaca actcaacttc 780

ggtgttgcta gagtcttctt cctcaaccag aagaaggcca agatggtcct acataagacg 840

gcacaaccaa gtgtcgatct tacttttggt ggggtcaaat ttacagtggt taataaccat 900

tttccccaat atgtctcaaa tcctgtgcca gacaatgcca ttacacttca caggatgtca 960

ggttatctag cacgttggat tgctgataca tgcaaggcta gtgtcctcaa actagctgaa 1020

gctagtgctc agattgtcat gccccttgct gaggttaagg gatgcacctg ggccgatggt 1080

tatacaatgt atcttggatt tgcacctggg gccgaaatgt tccttgatgc ttttgacttc 1140

tatccactag ttattgaaat gcatagggtc ctcaaggaca atatggatgt aaattttatg 1200

aaaaaagtcc tccgccaacg ctatggaaca atgactgctg aagaatggat gactcagaaa 1260

ataacagaaa taaaagctgc ttttaattct gttggacagc ttgcctgggc caaatctgga 1320

ttctctcctg ctgctagaac cttcttgcag caattcggta tcaacatccc gggagagaat 1380

ctatattttc aagggcccgg cggaggtagt caccatcatc accatcacta atgaccggt 1439

<210> 36
<211> 1480
<212> DNA
<213> artificial

<220>
<223> Cassette DNA pDeSNAP Univ +SBV.N (BIPlike - SNAPlike-SBV.N -proTEV/Histag)

<400> 36
tcgcgagcta gcaccatgaa actatgtatt ctacttgcag ttgttgcgtt cgtaggattg 60

tccttaccta cagctctggc aagatctgac aaagactgcg aaatgaaaag aactacattg 120

gattcaccac ttgggaagtt ggaactgagt ggatgcgagc aaggattgca tgaaattaag 180

ctactgggaa aaggaacttc tgctgctgat gcagttgaag ttccagcacc agcagctgtt 240

cttggaggtc ctgagcccct catgcaagcc acagcctggc ttaacgcata tttccaccag 300

cctgaggcca ttgaggaatt tccagtcccc gcccttcacc atcctgtgtt tcagcaggag 360

agcttcaccc gccaggtcct gtggaaattg ctgaaggtgg tcaagtttgg tgaagtgatt 420

tcatatcagc aacttgctgc attggccggt aaccccgcag ctacagctgc cgtgaaaact 480

gctctcagcg gaaatcctgt gcccatcctg atcccttgtc acagagtcgt ttcatcttcc 540

ggagctgtag gtggctatga aggaggactg gcagttaagg agtggctgct ggctcatgaa 600

ggtcatagac ttggaaagcc tgggctgggt cctgctggta taggcgcgcc agggtcccta 660

ggtggcggat ccgaaaacct gtacttccag agcgatatct caagccaatt cattttgaa 720

gatgtaccac aacggaatgc agctacattt aacccggagg tcgggtatgt ggcatttatt 780

ggtaagtatg ggcaacaact caacttcggt gttgctagag tcttcttcct caaccagaag 840

aaggccaaga tggtcctaca taagacggca caaccaagtg tcgatcttac ttttggtggg 900

gtcaaattta cagtggttaa taaccatttt ccccaatatg tctcaaatcc tgtgccagac 960

aatgccatta cacttcacag gatgtcaggt tatctagcac gttggattgc tgatacatgc 1020

aaggctagtg tcctcaaact agctgaagct agtgctcaga ttgtcatgcc ccttgctgag 1080

gttaagggat gcacctgggc cgatggttat acaatgtatc ttggatttgc acctggggcc 1140

gaaatgttcc ttgatgcttt tgacttctat ccactagtta ttgaaatgca tagggtcctc 1200

aaggacaata tggatgtaaa ttttatgaaa aaagtcctcc gccaacgcta tggaacaatg 1260

actgctgaag aatggatgac tcagaaaata acagaaataa aagctgcttt taattctgtt 1320

ggacagcttg cctgggccaa atctggattc tctcctgctg ctagaacctt cttgcagcaa 1380

ttcggtatca acatcccggg agagaatcta tattttcaag ggcccggcgg aggtagtcac 1440

catcatcacc atcactaatg accggtgcgg ccgcaagctt 1480

<210> 37
<211> 18
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the ssBiP sequence

<400> 37

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1 5 10 15

Leu Gly

<210> 38
<211> 17
<212> PRT
<213> West Nile virus strain IS-98-ST1

<400> 38

Met Val Val Phe Val Val Leu Leu Leu Val Ala Pro Ala Tyr Ser
1 5 10 15

Leu

<210> 39
<211> 5
<212> PRT
<213> Dengue virus

<400> 39

Pro Thr Ala Leu Ala
1 5

<210> 40
<211> 6
<212> PRT
<213> artificial

<220>
<223> Cleavage site of enterokinase

<400> 40

Asp Asp Asp Asp Lys Asp
1 5

<210> 41
<211> 480
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of
BiPlike+SNAPlike+proTEV+SBV.N+proTEV+Histag

<400> 41

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1 5 10 15

Leu Pro Thr Ala Leu Ala Arg Ser Asp Lys Asp Cys Glu Met Lys Arg
20 25 30

Thr Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu
35 40 45

Gln Gly Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala
50 55 60

Asp Ala Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu
65 70 75 80

Pro Leu Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro
85 90 95

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Glu Ala Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe
            100                 105                 110

Gln Gln Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val
        115                 120                 125

Val Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala
    130                 135                 140

Gly Asn Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn
145                 150                 155                 160

Pro Val Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly
                165                 170                 175

Ala Val Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu
            180                 185                 190

Ala His Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly
        195                 200                 205

Ile Gly Ala Pro Gly Ser Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe
    210                 215                 220

Gln Ser Asp Ile Ser Ser Gln Phe Ile Phe Glu Asp Val Pro Gln Arg
225                 230                 235                 240

Asn Ala Ala Thr Phe Asn Pro Glu Val Gly Tyr Val Ala Phe Ile Gly
                245                 250                 255

Lys Tyr Gly Gln Gln Leu Asn Phe Gly Val Ala Arg Val Phe Phe Leu
            260                 265                 270

Asn Gln Lys Lys Ala Lys Met Val Leu His Lys Thr Ala Gln Pro Ser
        275                 280                 285

Val Asp Leu Thr Phe Gly Gly Val Lys Phe Thr Val Val Asn Asn His
    290                 295                 300

Phe Pro Gln Tyr Val Ser Asn Pro Val Pro Asp Asn Ala Ile Thr Leu
305                 310                 315                 320

His Arg Met Ser Gly Tyr Leu Ala Arg Trp Ile Ala Asp Thr Cys Lys
                325                 330                 335

Ala Ser Val Leu Lys Leu Ala Glu Ala Ser Ala Gln Ile Val Met Pro
            340                 345                 350

Leu Ala Glu Val Lys Gly Cys Thr Trp Ala Asp Gly Tyr Thr Met Tyr
        355                 360                 365
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Leu Gly Phe Ala Pro Gly Ala Glu Met Phe Leu Asp Ala Phe Asp Phe
370 375 380

Tyr Pro Leu Val Ile Glu Met His Arg Val Leu Lys Asp Asn Met Asp
385 390 395 400

Val Asn Phe Met Lys Lys Val Leu Arg Gln Arg Tyr Gly Thr Met Thr
405 410 415

Ala Glu Glu Trp Met Thr Gln Lys Ile Thr Glu Ile Lys Ala Ala Phe
420 425 430

Asn Ser Val Gly Gln Leu Ala Trp Ala Lys Ser Gly Phe Ser Pro Ala
435 440 445

Ala Arg Thr Phe Leu Gln Gln Phe Gly Ile Asn Ile Pro Gly Glu Asn
450 455 460

Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His His His
465 470 475 480

<210> 42
<211> 438
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein SNAP+SCHM.N (without BiPlike + HisTag

<400> 42

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr

115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ser Ser
195 200 205

Gln Phe Ile Phe Glu Asp Val Pro Gln Arg Asn Ala Ala Thr Phe Asn
210 215 220

Pro Glu Val Gly Tyr Val Ala Phe Ile Gly Lys Tyr Gly Gln Gln Leu
225 230 235 240

Asn Phe Gly Val Ala Arg Val Phe Phe Leu Asn Gln Lys Lys Ala Lys
245 250 255

Met Val Leu His Lys Thr Ala Gln Pro Ser Val Asp Leu Thr Phe Gly
260 265 270

Gly Val Lys Phe Thr Val Val Asn Asn His Phe Pro Gln Tyr Val Ser
275 280 285

Asn Pro Val Pro Asp Asn Ala Ile Thr Leu His Arg Met Ser Gly Tyr
290 295 300

Leu Ala Arg Trp Ile Ala Asp Thr Cys Lys Ala Ser Val Leu Lys Leu
305 310 315 320

Ala Glu Ala Ser Ala Gln Ile Val Met Pro Leu Ala Glu Val Lys Gly
325 330 335

Cys Thr Trp Ala Asp Gly Tyr Thr Met Tyr Leu Gly Phe Ala Pro Gly
340 345 350

Ala Glu Met Phe Leu Asp Ala Phe Asp Phe Tyr Pro Leu Val Ile Glu
355 360 365

Met His Arg Val Leu Lys Asp Asn Met Asp Val Asn Phe Met Lys Lys
370 375 380

Val Leu Arg Gln Arg Tyr Gly Thr Met Thr Ala Glu Glu Trp Met Thr

385 390 395 400

Gln Lys Ile Thr Glu Ile Lys Ala Ala Phe Asn Ser Val Gly Gln Leu
405 410 415

Ala Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg Thr Phe Leu Gln
420 425 430

Gln Phe Gly Ile Asn Ile
435

<210> 43
<211> 4194
<212> DNA
<213> artificial

<220>
<223> pMT/BiP/SNAP-Histag avec cassette DeSNAP Univ

<400> 43
tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgccag tgaattttaa cgttgcagga 420

caggatgtgg tgcccgatgt gactagctct ttgctgcagg ccgtcctatc ctctggttcc 480

gataagagac ccagaactcc ggccccccac cgcccaccgc cacccccata catatgtggt 540

acgcaagtaa gagtgcctgc gcatgcccca tgtgccccac caagagtttt gcatcccata 600

caagtcccca aagtggagaa ccgaaccaat tcttcgcggg cagaacaaaa gcttctgcac 660

acgtctccac tcgaatttgg agccggccgg cgtgtgcaaa agaggtgaat cgaacgaaag 720

acccgtgtgt aaagccgcgt ttccaaaatg tataaaaccg agagcatctg gccaatgtgc 780

atcagttgtg gtcagcagca aaatcaagtg aatcatctca gtgcaactaa agggggggatc 840

cgatctcaat atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct 900

cgggagatct gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa 960

gttggaactg agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac 1020

ttctgctgct gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc 1080

cctcatgcaa gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga 1140

atttccagtc cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt 1200

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gcccggcgga ggtagtcacc atcatcacca tcactaatga ccggtcatca tcaccatcac 1620

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tcagcctcga tcgaggatcc agacatgata agatacattg atgagtttgg acaaaccaca 1740

actagaatgc agtgaaaaaa atgctttatt tgtgaaattt gtgatgctat tgctttattt 1800

gtaaccatta taagctgcaa taaacaagtt aacaacaaca attgcattca ttttatgttt 1860

caggttcagg gggaggtgtg ggaggttttt taaagcaagt aaaacctcta caaatgtggt 1920

atggctgatt atgatcagtc gacctgcagg catgcaagct tggcgtaatc atggtcatag 1980

ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc 2040

ataaagtgta aagcctgggg tgcctaatga gtgagctaac tcacattaat tgcgttgcgc 2100

tcactgcccg ctttccagtc gggaaacctg tcgtgccagc tgcattaatg aatcggccaa 2160

cgcgcgggga gaggcggttt gcgtattggg cgctcttccg cttcctcgct cactgactcg 2220

ctgcgctcgg tcgttcggct gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg 2280

ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg ccagcaaaag 2340

gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg ccccccctgac 2400

gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg actataaaga 2460

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tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtgt gcacgaaccc 2640

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cagtggaacg aaaactcacg ttaagggatt ttggtcatga gattatcaaa aaggatcttc 3060

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gcagtgttat cactcatggt tatggcagca ctgcataatt ctcttactgt catgccatcc 3660

gtaagatgct tttctgtgac tggtgagtac tcaaccaagt cattctgaga atagtgtatg 3720

cggcgaccga gttgctcttg cccggcgtca atacgggata ataccgcgcc acatagcaga 3780

actttaaaag tgctcatcat tggaaaacgt tcttcggggc gaaaactctc aaggatctta 3840

ccgctgttga gatccagttc gatgtaaccc actcgtgcac ccaactgatc ttcagcatct 3900

tttactttca ccagcgtttc tgggtgagca aaaacaggaa ggcaaaatgc cgcaaaaaag 3960

ggaataaggg cgacacggaa atgttgaata ctcatactct tccttttca atattattga 4020

agcatttatc agggttattg tctcatgagc ggatacatat ttgaatgtat ttagaaaaat 4080

aaacaaatag gggttccgcg cacatttccc cgaaaagtgc cacctgacgt ctaagaaacc 4140

attattatca tgacattaac ctataaaaat aggcgtatca cgaggccctt tcgt 4194

<210> 44
<211> 3458
<212> DNA
<213> artificial

<220>
<223> pUC57 with DeSNAP Univ cassette

<400> 44
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cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cgagctcggt acctcgcgaa 420

tgcatctaga tcgagctagc accatgaaac tatgtattct acttgcagtt gttgcgttcg 480

taggattgtc cttacctaca gctctggcaa gatctgacaa agactgcgaa atgaaaagaa 540

ctacattgga ttcaccactt gggaagttgg aactgagtgg atgcgagcaa ggattgcatg 600

aaattaagct actgggaaaa ggaacttctg ctgctgatgc agttgaagtt ccagcaccag 660

cagctgttct tggaggtcct gagcccctca tgcaagccac agcctggctt aacgcatatt 720

tccaccagcc tgaggccatt gaggaatttc cagtccccgc ccttcaccat cctgtgtttc 780

agcaggagag cttcacccgc caggtcctgt ggaaattgct gaaggtggtc aagtttggtg 840

aagtgatttc atatcagcaa cttgctgcat tggccggtaa ccccgcagct acagctgccg 900

tgaaaactgc tctcagcgga aatcctgtgc ccatcctgat cccttgtcac agagtcgttt 960

catcttccgg agctgtaggt ggctatgaag gaggactggc agttaaggag tggctgctgg 1020

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<212> DNA
<213> artificial

<220>
<223> pcDNA3 with DeSNAP Univ cassette

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<211> 476
<212> PRT
<213> artificial

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<223> Amino acid sequence of ssBiP+SNAPlike+proTEV+SBV.N+proTEV+Histag (encoded by SEQ ID NO:35)

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Leu Gly Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp
20 25 30

Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His
35 40 45

Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu
50 55 60

Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln
65 70 75 80

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Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu
                85                  90                  95

Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser
            100                 105                 110

Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly
        115                 120                 125

Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala
    130                 135                 140

Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile
145                 150                 155                 160

Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly
                165                 170                 175

Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly
            180                 185                 190

His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro
        195                 200                 205

Gly Ser Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile
    210                 215                 220

Ser Ser Gln Phe Ile Phe Glu Asp Val Pro Gln Arg Asn Ala Ala Thr
225                 230                 235                 240

Phe Asn Pro Glu Val Gly Tyr Val Ala Phe Ile Gly Lys Tyr Gly Gln
                245                 250                 255

Gln Leu Asn Phe Gly Val Ala Arg Val Phe Phe Leu Asn Gln Lys Lys
            260                 265                 270

Ala Lys Met Val Leu His Lys Thr Ala Gln Pro Ser Val Asp Leu Thr
        275                 280                 285

Phe Gly Gly Val Lys Phe Thr Val Val Asn Asn His Phe Pro Gln Tyr
    290                 295                 300

Val Ser Asn Pro Val Pro Asp Asn Ala Ile Thr Leu His Arg Met Ser
305                 310                 315                 320

Gly Tyr Leu Ala Arg Trp Ile Ala Asp Thr Cys Lys Ala Ser Val Leu
                325                 330                 335

Lys Leu Ala Glu Ala Ser Ala Gln Ile Val Met Pro Leu Ala Glu Val
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Lys Gly Cys Thr Trp Ala Asp Gly Tyr Thr Met Tyr Leu Gly Phe Ala
355 360 365

Pro Gly Ala Glu Met Phe Leu Asp Ala Phe Asp Phe Tyr Pro Leu Val
370 375 380

Ile Glu Met His Arg Val Leu Lys Asp Asn Met Asp Val Asn Phe Met
385 390 395 400

Lys Lys Val Leu Arg Gln Arg Tyr Gly Thr Met Thr Ala Glu Glu Trp
405 410 415

Met Thr Gln Lys Ile Thr Glu Ile Lys Ala Ala Phe Asn Ser Val Gly
420 425 430

Gln Leu Ala Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg Thr Phe
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Leu Gln Gln Phe Gly Ile Asn Ile Pro Gly Glu Asn Leu Tyr Phe Gln
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Gly Pro Gly Gly Gly Ser His His His His His His
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<210> 47
<211> 891
<212> DNA
<213> artificial

<220>
<223> Modified DNA sequence encoding enterovirus 71 VP1 (strain JL-AFP-EV71-07-03)

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acaggcaagg ttccagcact ccaagctgct gaaattggag catcatcaaa tgctagtgat 180

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ggcaactcca ttaagccaac tggtaccagt cgcacagcga tcactactct c 891

<210> 48
<211> 1643
<212> DNA
<213> artificial

<220>
<223> DNA Sequence of chimeric DeSNAPuniv-EV71.VP1 (ssBiP-SNAPlike-proTEVcleavage site - modified EV71-VP1-proTEVcleavage site -Histag) for expression in S2 cells

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<210> 49
<211> 523
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike - proTEV - VP1 EV71 - proTEV- Histag]

<400> 49

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Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Gly Asp
195 200 205

```
Arg Val Ala Asp Val Ile Glu Ser Ser Lys Gly Asn Ser Val Ser Arg
    210                 215                 220

Ala Leu Thr Gln Ala Leu Pro Ala Pro Thr Gly Gln Asn Thr Gln Val
225                 230                 235                 240

Ser Ser His Arg Leu Asp Thr Gly Lys Val Pro Ala Leu Gln Ala Ala
                245                 250                 255

Glu Ile Gly Ala Ser Ser Asn Ala Ser Asp Glu Ser Met Ile Glu Thr
            260                 265                 270

Arg Cys Val Leu Asn Ser His Ser Thr Ala Glu Thr Thr Leu Asp Ser
        275                 280                 285

Phe Phe Ser Arg Ala Gly Leu Val Gly Glu Ile Asp Leu Pro Leu Glu
    290                 295                 300

Gly Thr Thr Asn Pro Asn Gly Tyr Ala Asn Trp Asp Ile Asp Ile Thr
305                 310                 315                 320

Gly Tyr Ala Gln Met Arg Arg Lys Val Glu Leu Phe Thr Tyr Met Arg
                325                 330                 335

Phe Asp Ala Glu Phe Thr Phe Val Ala Cys Thr Pro Thr Gly Glu Val
            340                 345                 350

Val Pro Gln Leu Leu Gln Tyr Met Phe Val Pro Pro Gly Ala Pro Lys
        355                 360                 365

Pro Asp Ser Arg Glu Ser Leu Ala Trp Gln Thr Ala Thr Asn Pro Ser
    370                 375                 380

Val Phe Val Lys Leu Ser Asp Pro Pro Ala Gln Val Ser Val Pro Phe
385                 390                 395                 400

Met Ser Pro Ala Ser Ala Tyr Gln Trp Phe Tyr Asp Gly Tyr Pro Thr
                405                 410                 415

Phe Gly Glu His Lys Gln Glu Lys Asp Leu Glu Tyr Gly Ala Cys Pro
            420                 425                 430

Asn Asn Met Met Gly Thr Phe Ser Val Arg Thr Val Gly Thr Ser Lys
        435                 440                 445

Ser Lys Tyr Pro Leu Val Val Arg Ile Tyr Met Arg Met Lys His Val
    450                 455                 460

Arg Ala Trp Ile Pro Arg Pro Met Arg Asn Gln Asn Tyr Leu Phe Lys
465                 470                 475                 480
```

Ala Asn Pro Asn Tyr Ala Gly Asn Ser Ile Lys Pro Thr Gly Thr Ser
485 490 495

Arg Thr Ala Ile Thr Thr Leu Pro Gly Glu Asn Leu Tyr Phe Gln Gly
500 505 510

Pro Gly Gly Gly Ser His His His His His His
515 520

<210> 50
<211> 2386
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding chimeric DeSNAPuniv-JE. -sE (ssBiP- sE from JEV strain SA-14 -SNAPlike) for expression in S2 cells

<400> 50
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

atgaagttgt cgaatttcca ggggaagctt ttgatgacca tcaacaacac ggacattgca 120

gacgttatcg tgattcccac ctcaaaagga gagaacagat gctgggtccg ggcaatcgac 180

gtcggctaca tgtgtgagga cactatcacg tacgaatgtc ctaagcttac catgggcaat 240

gatccagagg atgtggattg ctggtgtgac aaccaagaag tctacgtcca atatggacgg 300

tgcacgcgga ccaggcattc caagcgaagc aggagatccg tgtcggtcca aacacatggg 360

gagagttcac tagtgaataa aaaagaggct tggctggatt caacgaaagc cacacgatat 420

ctcatgaaaa ctgagaactg gatcataagg aatcctggct atgctttcct ggcggcggta 480

cttggctgga tgcttggcag taacaacggt caacgcgtgg tatttaccat cctcctgctg 540

ttggtcgctc cggcttacag ttttaattgt ctgggaatgg gcaatcgtga cttcatagaa 600

ggagccagtg gagccacttg ggtggacttg gtgctagaag gagatagctg cttgacaatc 660

atggcaaacg acaaaccaac attggacgtc cgcatgatta acatcgaagc tagccaactt 720

gctgaggtca gaagttactg ctatcatgct tcagtcactg acatctcgac ggtggctcgg 780

tgccccacga ctggagaagc tcacaacgag aagcgagctg atagtagcta tgtgtgcaaa 840

caaggcttca ctgaccgtgg gtggggcaac ggatgtggac ttttcgggaa gggaagcatt 900

gacacatgtg caaaattctc ctgcaccagt aaagcgattg ggagaacaat ccagccagaa 960

aacatcaaat acgaagttgg cattttgtg catggaacca ccacttcgga aaaccatggg 1020

aattattcag cgcaagttgg ggcgtcccag gcggcaaagt ttacagtaac acccaatgct 1080

ccttcgataa ccctcaaact tggtgactac ggagaagtca cactggactg tgagccaagg 1140

agtggactga acactgaagc gttttacgtc atgaccgtgg ggtcaaagtc atttctggtc 1200

catagggagt ggtttcatga cctcgctctc ccctggacgt ccccttcgag cacagcgtgg 1260

agaaacagag aactcctcat ggaatttgaa ggggcgcacg ccacaaaaca gtccgttgtt 1320

gctcttgggt cacaggaagg aggcctccat caggcgttgg caggagccat cgtggtggag 1380

tactcaagct cagtgaagtt aacatcaggc cacctgaaat gtaggctgaa aatggacaaa 1440

ctggctctga aaggcacaac ctatggcatg tgtacagaaa aattctcgtt cgcgaaaaat 1500

ccggcggaca ctggtcacgg aacagttgtc attgaactct cctactctgg gagtgatggc 1560

tcctgcaaaa ttccgattgt ttccgttgcg agcctcaatg acatgacccc cgttgggcgg 1620

ctggtgacag tgaacccctt cgtcgcgact tccagtgcca actcaaaggt gctggtcgag 1680

atggaacccc ccttcggaga ctcctacatc gtagttggaa ggggagacaa gcagatcaac 1740

caccattggc acaaagctgg gcggccgcac ggcggaggta gcaaagactg cgaaatgaag 1800

cgcaccaccc tggatagccc tctgggcaag ctggaactgt ctgggtgcga acagggcctg 1860

cacgagatca agctgctggg caaaggaaca tctgccgccg acgccgtgga agtgcctgcc 1920

ccagccgccg tgctgggcgg accagagcca ctgatgcagg ccaccgcctg gctcaacgcc 1980

tactttcacc agcctgaggc catcgaggag ttccctgtgc cagccctgca ccacccagtg 2040

ttccagcagg agagctttac ccgccaggtg ctgtggaaac tgctgaaagt ggtgaagttc 2100

ggagaggtca tcagctacca gcagctggcc gccctggccg gcaatcccgc cgccaccgcc 2160

gccgtgaaaa ccgccctgag cggaaatccc gtgcccattc tgatcccctg ccaccgggtg 2220

gtgtctagct ctggcgccgt gggggggctac gagggcgggc tcgccgtgaa agagtggctg 2280

ctggcccacg agggccacag actgggcaag cctgggctgg gtcctgcagg tataggcgcg 2340

ccagggtccc tggagcatca tcatcatcat cattgatgac gggccc 2386

<210> 51
<211> 773
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of fusion protein [sE from JEV strain SA-14 - SNAPlike - Histag]

<400> 51

Arg Ser Met Lys Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile
1 5 10 15

Asn Asn Thr Asp Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly
20 25 30

Glu Asn Arg Cys Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu
35 40 45

Asp Thr Ile Thr Tyr Glu Cys Pro Lys Leu Thr Met Gly Asn Asp Pro
50 55 60

Glu Asp Val Asp Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr
65 70 75 80

```
Gly Arg Cys Thr Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val
                85                  90                  95

Ser Val Gln Thr His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala
            100                 105                 110

Trp Leu Asp Ser Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn
        115                 120                 125

Trp Ile Ile Arg Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly
    130                 135                 140

Trp Met Leu Gly Ser Asn Asn Gly Gln Arg Val Val Phe Thr Ile Leu
145                 150                 155                 160

Leu Leu Leu Val Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly
                165                 170                 175

Asn Arg Asp Phe Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu
            180                 185                 190

Val Leu Glu Gly Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro
        195                 200                 205

Thr Leu Asp Val Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu
    210                 215                 220

Val Arg Ser Tyr Cys Tyr His Ala Ser Val Thr Asp Ile Ser Thr Val
225                 230                 235                 240

Ala Arg Cys Pro Thr Thr Gly Glu Ala His Asn Glu Lys Arg Ala Asp
                245                 250                 255

Ser Ser Tyr Val Cys Lys Gln Gly Phe Thr Asp Arg Gly Trp Gly Asn
            260                 265                 270

Gly Cys Gly Leu Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys Phe
        275                 280                 285

Ser Cys Thr Ser Lys Ala Ile Gly Arg Thr Ile Gln Pro Glu Asn Ile
    290                 295                 300

Lys Tyr Glu Val Gly Ile Phe Val His Gly Thr Thr Thr Ser Glu Asn
305                 310                 315                 320

His Gly Asn Tyr Ser Ala Gln Val Gly Ala Ser Gln Ala Ala Lys Phe
                325                 330                 335

Thr Val Thr Pro Asn Ala Pro Ser Ile Thr Leu Lys Leu Gly Asp Tyr
            340                 345                 350
```

```
Gly Glu Val Thr Leu Asp Cys Glu Pro Arg Ser Gly Leu Asn Thr Glu
        355                 360                 365

Ala Phe Tyr Val Met Thr Val Gly Ser Lys Ser Phe Leu Val His Arg
    370                 375                 380

Glu Trp Phe His Asp Leu Ala Leu Pro Trp Thr Ser Pro Ser Ser Thr
385                 390                 395                 400

Ala Trp Arg Asn Arg Glu Leu Leu Met Glu Phe Glu Gly Ala His Ala
                405                 410                 415

Thr Lys Gln Ser Val Val Ala Leu Gly Ser Gln Glu Gly Gly Leu His
            420                 425                 430

Gln Ala Leu Ala Gly Ala Ile Val Val Glu Tyr Ser Ser Ser Val Lys
        435                 440                 445

Leu Thr Ser Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Ala
    450                 455                 460

Leu Lys Gly Thr Thr Tyr Gly Met Cys Thr Glu Lys Phe Ser Phe Ala
465                 470                 475                 480

Lys Asn Pro Ala Asp Thr Gly His Gly Thr Val Val Ile Glu Leu Ser
                485                 490                 495

Tyr Ser Gly Ser Asp Gly Ser Cys Lys Ile Pro Ile Val Ser Val Ala
            500                 505                 510

Ser Leu Asn Asp Met Thr Pro Val Gly Arg Leu Val Thr Val Asn Pro
        515                 520                 525

Phe Val Ala Thr Ser Ser Ala Asn Ser Lys Val Leu Val Glu Met Glu
    530                 535                 540

Pro Pro Phe Gly Asp Ser Tyr Ile Val Val Gly Arg Gly Asp Lys Gln
545                 550                 555                 560

Ile Asn His His Trp His Lys Ala Gly Arg Pro His Gly Gly Gly Ser
                565                 570                 575

Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly Lys
            580                 585                 590

Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu Leu
        595                 600                 605

Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro Ala
    610                 615                 620
```

```
Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp Leu
625                 630                 635                 640

Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val Pro
                645                 650                 655

Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln Val
            660                 665                 670

Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser Tyr
        675                 680                 685

Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala Val
    690                 695                 700

Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys His
705                 710                 715                 720

Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly Leu
                725                 730                 735

Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly Lys
            740                 745                 750

Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu His
        755                 760                 765

His His His His His
    770
```

<210> 52
<211> 1020
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein BiPlike-SNAPlike-EDIII from Japanese encephalitis virus genotype I cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 52

```
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc      300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg      360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt      420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg      480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg      540
```

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctgc tctgaagggc 660

acgacttacg gcatgtgtac agaaaaattc tcgttcgcga aaaatccagc ggacacaggc 720

catggaacag ttgtcattga gctcacatac tctggaagcg atggtccctg taaaattccg 780

attgtctcag tcgcgagttt aaacgacatg acccctgtgg ggaggctggt aacagtaaac 840

cccttcgtcg cgacatctag ctccaactca aaggtgctgg ttgagatgga acctcccttc 900

ggagactctt acatcgtggt tggaagaggg gataagcaga ttaaccatca ctggcacaaa 960

gctggaagca cgctgggtaa aggaggtggc catcaccatc accatcactg atgaccggtt 1020

<210> 53
<211> 319
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from Japanese encephalitis virus genotype I - Histag

<400> 53

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

```
Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Glu Gly Gly Gly Gly Ser Ala Leu Lys Gly Thr Thr Tyr Gly Met
        195                 200                 205

Cys Thr Glu Lys Phe Ser Phe Ala Lys Asn Pro Ala Asp Thr Gly His
    210                 215                 220

Gly Thr Val Val Ile Glu Leu Thr Tyr Ser Gly Ser Asp Gly Pro Cys
225                 230                 235                 240

Lys Ile Pro Ile Val Ser Val Ala Ser Leu Asn Asp Met Thr Pro Val
                245                 250                 255

Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala Thr Ser Ser Ser Asn
            260                 265                 270

Ser Lys Val Leu Val Glu Met Glu Pro Pro Phe Gly Asp Ser Tyr Ile
        275                 280                 285

Val Val Gly Arg Gly Asp Lys Gln Ile Asn His His Trp His Lys Ala
    290                 295                 300

Gly Ser Thr Leu Gly Lys Gly Gly Gly His His His His His His
305                 310                 315
```

<210> 54
<211> 406
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding Domain III of envelope E protein from Japanese encephalitis virus of genotype 1 (JE-1.EDIII)

<400> 54

```
gcgcgccagg gtccctggag ggaggtggcg ggtctgctct gaagggcacg acttacggca       60

tgtgtacaga aaaattctcg ttcgcgaaaa atccagcgga cacaggccat ggaacagttg      120

tcattgagct cacatactct ggaagcgatg gtccctgtaa aattccgatt gtctcagtcg      180

cgagtttaaa cgacatgacc cctgtgggga ggctggtaac agtaaacccc ttcgtcgcga      240

catctagctc caactcaaag gtgctggttg agatggaacc tcccttcgga gactcttaca      300

tcgtggttgg aagaggggat aagcagatta accatcactg gcacaaagct ggaagcacgc      360

tgggtaaagg aggtggccat caccatcacc atcactgatg accggt                     406
```

<210> 55
<211> 406

<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding Domain III of envelope E protein from Japanese encephalitis virus of genotype 2 (JE-2.EDIII)

<400> 55
gcgcgccagg gtccctggag ggaggtggcg ggtctgctct gaaaggcaca acctatggta 60

tgtgcacaga aaactcctcg ttccgaaaaa atccagcgga cacaggccat ggaacagttg 120

tcattgagct cacatactct gggagtgatg gtccctgtaa gattccaaat gtctccgttg 180

cgagcctgaa tgacatgacc cctgtaggga ggctggtaac agtaaacccc tttgtcgcga 240

catccagcgc caactcaaaa gtgctggttg aaatggaacc ccctttgga gattcttaca 300

tcgtggtcgg aagaggtgac aagcagatca atcatcactg gcacaaagct ggaagcacgc 360

tgggcaaagg aggtggccat caccatcacc atcactgatg accggt 406

<210> 56
<211> 406
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding Domain III of envelope E protein from Japanese encephalitis virus of genotype 4 (JE-4.EDIII)

<400> 56
gcgcgccagg gtccctggag ggaggtggcg ggtctgctct gaaaggcaca acctatggaa 60

tgtgcacaga aaagttctcg tttgcaaaga atccagcaga cactggtcat ggaacagttg 120

tcattgaact cctgtattct ggaagtgacg gcccctgtaa catcccaatt gtctcagtgg 180

tcagtctaaa cgacatgact ccagttggaa ggttggtgac agtgaacccc ttcgttgcca 240

catccagttc caattcaaag gtcttagttg agatggaacc tcctttgga gactcctaca 300

ttgtggtcgg gagaggagaa aaacaaatca accaccactg gcacaaacct ggaagcacat 360

tgggcaaagg aggtggccat caccatcacc atcactgatg accggt 406

<210> 57
<211> 406
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding Domain III of envelope E protein from Japanese encephalitis virus of genotype 5 (JE-5.EDIII)

<400> 57
gcgcgccagg gtccctggag ggaggtggcg ggtctgcgtt gaaagggacc acctatggta 60

tgtgcacaga gaagttctct ttttccaaga atccagctga cactggtcat ggtacggttg 120

tcatagaatt gcagtacacc ggcactgacg gaccttgcaa gatacccatc tcttcggtgg 180

ccagtctgaa tgatttaact ccagttggta gattggtgac agtcaatcct tttgttgcca 240

catccaccgc caattcgaag gttttggtag aattggaacc accatttgga gattcattca 300

ttgttgtcgg aagaggagat aagcagatca atcaccattg gcacaaggct ggcagttcac 360

tgggaaaggg aggtggccat caccatcacc atcactgatg accggt 406

<210> 58
<211> 394
<212> DNA
<213> artificial

<220>
<223> DNA sequence of Domain III encoding envelope E protein from Rabensburg virus (RabV.EDIII)

<400> 58
gcgcgccagg aggtggcggg tctcagctca aaggaacgac ctatggagta tgcgcaaaag 60

ccttcaagtt ttctgggaat ccagctgaca cagggcatgg caccgtggtc ttagagttgc 120

aatacaccgg aaccgatggt ccttgtaagg tgcctgtctc ttccgtggct tcactcaacg 180

acctaactcc cgttgggaga ctggtgacag tgaatccctt tgttgctgca gctactgcta 240

attcaaaggt tctgatagaa ctggaacctc cattcggtga ctcatacatt gtggtaggta 300

gaggagaaca ccagataaac caccattggc acaagtctgg aagcagtatt ggaaagggag 360

gtggccatca ccatcaccat cactgatgac cggt 394

<210> 59
<211> 1020
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein BiPlike -SNAPlike-EDIII from Japanese encephalitis virus genotype 2 cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 59
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac 60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt 120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat 180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc 240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctgc tctgaaaggc 660

acaacctatg gtatgtgcac agaaaactcc tcgttccgaa aaaatccagc ggacacaggc 720

catggaacag ttgtcattga gctcacatac tctgggagtg atggtccctg taagattcca 780

aatgtctccg ttgcgagcct gaatgacatg acccctgtag ggaggctggt aacagtaaac 840

ccctttgtcg cgacatccag cgccaactca aaagtgctgg ttgaaatgga acccccttt 900

ggagattctt acatcgtggt cggaagaggt gacaagcaga tcaatcatca ctggcacaaa 960

gctggaagca cgctgggcaa aggaggtggc catcaccatc accatcactg atgaccggtt 1020

<210> 60
<211> 319
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from Japanese encephalitis virus genotype 2 - Histag

<400> 60

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Glu Gly Gly Gly Gly Ser Ala Leu Lys Gly Thr Thr Tyr Gly Met

195 200 205

Cys Thr Glu Asn Ser Ser Phe Arg Lys Asn Pro Ala Asp Thr Gly His
210 215 220

Gly Thr Val Val Ile Glu Leu Thr Tyr Ser Gly Ser Asp Gly Pro Cys
225 230 235 240

Lys Ile Pro Asn Val Ser Val Ala Ser Leu Asn Asp Met Thr Pro Val
245 250 255

Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala Thr Ser Ser Ala Asn
260 265 270

Ser Lys Val Leu Val Glu Met Glu Pro Pro Phe Gly Asp Ser Tyr Ile
275 280 285

Val Val Gly Arg Gly Asp Lys Gln Ile Asn His His Trp His Lys Ala
290 295 300

Gly Ser Thr Leu Gly Lys Gly Gly Gly His His His His His His
305 310 315

<210> 61
<211> 1020
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein BiPlike -SNAPlike-EDIII from Japanese encephalitis virus genotype 4 cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 61
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac 60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt 120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat 180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc 240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctgc tctgaaaggc 660

acaacctatg gaatgtgcac agaaaagttc tcgtttgcaa agaatccagc agacactggt 720

catggaacag ttgtcattga actcctgtat tctggaagtg acggcccctg taacatccca 780

attgtctcag tggtcagtct aaacgacatg actccagttg gaaggttggt gacagtgaac 840

cccttcgttg ccacatccag ttccaattca aaggtcttag ttgagatgga acctcctttt 900

ggagactcct acattgtggt cgggagagga gaaaaacaaa tcaaccacca ctggcacaaa 960

cctggaagca cattgggcaa aggaggtggc catcaccatc accatcactg atgaccggtt 1020

<210> 62
<211> 319
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from Japanese encephalitis virus genotype 4 - Histag

<400> 62

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Glu Gly Gly Gly Gly Ser Ala Leu Lys Gly Thr Thr Tyr Gly Met
195 200 205

Cys Thr Glu Lys Phe Ser Phe Ala Lys Asn Pro Ala Asp Thr Gly His
210 215 220

Gly Thr Val Val Ile Glu Leu Leu Tyr Ser Gly Ser Asp Gly Pro Cys
225 230 235 240

Asn Ile Pro Ile Val Ser Val Val Ser Leu Asn Asp Met Thr Pro Val
245 250 255

Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala Thr Ser Ser Ser Asn
260 265 270

Ser Lys Val Leu Val Glu Met Glu Pro Pro Phe Gly Asp Ser Tyr Ile
275 280 285

Val Val Gly Arg Gly Glu Lys Gln Ile Asn His His Trp His Lys Pro
290 295 300

Gly Ser Thr Leu Gly Lys Gly Gly Gly His His His His His His
305 310 315

<210> 63
<211> 1020
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein BiPlike -SNAPlike-EDIII from Japanese encephalitis virus genotype 5 cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 63
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac 60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt 120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat 180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc 240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctgc gttgaaaggg 660

accacctatg gtatgtgcac agagaagttc tcttttcca agaatccagc tgacactggt 720

catggtacgg ttgtcataga attgcagtac accggcactg acggaccttg caagataccc 780

atctcttcgg tggccagtct gaatgattta actccagttg gtagattggt gacagtcaat 840

ccttttgttg ccacatccac cgccaattcg aaggttttgg tagaattgga accaccattt 900

ggagattcat tcattgttgt cggaagagga gataagcaga tcaatcacca ttggcacaag 960

gctggcagtt cactgggaaa gggaggtggc catcaccatc accatcactg atgaccggtt 1020

<210> 64
<211> 319
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from Japanese encephalitis virus genotype 5 - Histag

<400> 64

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

```
Leu Glu Gly Gly Gly Gly Ser Ala Leu Lys Gly Thr Thr Tyr Gly Met
        195                 200                 205

Cys Thr Glu Lys Phe Ser Phe Ser Lys Asn Pro Ala Asp Thr Gly His
    210                 215                 220

Gly Thr Val Val Ile Glu Leu Gln Tyr Thr Gly Thr Asp Gly Pro Cys
225                 230                 235                 240

Lys Ile Pro Ile Ser Ser Val Ala Ser Leu Asn Asp Leu Thr Pro Val
                245                 250                 255

Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala Thr Ser Thr Ala Asn
            260                 265                 270

Ser Lys Val Leu Val Glu Leu Glu Pro Pro Phe Gly Asp Ser Phe Ile
        275                 280                 285

Val Val Gly Arg Gly Asp Lys Gln Ile Asn His His Trp His Lys Ala
    290                 295                 300

Gly Ser Ser Leu Gly Lys Gly Gly Gly His His His His His His
305                 310                 315
```

<210> 65
<211> 1008
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein BiPlike -SNAPlike-EDIII from Rabensburg virus cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 65

```
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc      300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg      360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt      420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg      480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg      540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt      600

cctgctggta taggcgcgcc aggaggtggc gggtctcagc tcaaaggaac gacctatgga      660

gtatgcgcaa aagccttcaa gttttctggg aatccagctg acacagggca tggcaccgtg      720
```

gtcttagagt tgcaatacac cggaaccgat ggtccttgta aggtgcctgt ctcttccgtg 780

gcttcactca acgacctaac tcccgttggg agactggtga cagtgaatcc ctttgttgct 840

gcagctactg ctaattcaaa ggttctgata gaactggaac ctccattcgg tgactcatac 900

attgtggtag gtagaggaga acaccagata aaccaccatt ggcacaagtc tggaagcagt 960

attggaaagg gaggtggcca tcaccatcac catcactgat gaccggtt 1008

<210> 66
<211> 314
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from Rabensburg virus - Histag

<400> 66

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Gly
180 185 190

```
Gly Gly Ser Gln Leu Lys Gly Thr Thr Tyr Gly Val Cys Ala Lys Ala
        195                 200                 205

Phe Lys Phe Ser Gly Asn Pro Ala Asp Thr Gly His Gly Thr Val Val
    210                 215                 220

Leu Glu Leu Gln Tyr Thr Gly Thr Asp Gly Pro Cys Lys Val Pro Val
225                 230                 235                 240

Ser Ser Val Ala Ser Leu Asn Asp Leu Thr Pro Val Gly Arg Leu Val
                245                 250                 255

Thr Val Asn Pro Phe Val Ala Ala Ala Thr Ala Asn Ser Lys Val Leu
            260                 265                 270

Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser Tyr Ile Val Val Gly Arg
        275                 280                 285

Gly Glu His Gln Ile Asn His His Trp His Lys Ser Gly Ser Ser Ile
    290                 295                 300

Gly Lys Gly Gly Gly His His His His His
305                 310
```

<210> 67
<211> 977
<212> DNA
<213> artificial

<220>
<223> DNA Sequence encoding the fusion protein ssBiP-SNAPlike-EDIII from an insect flavivirus virus cloned into pMT/BiP/SNAP for expression in S2 cells

<400> 67

```
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc      300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg      360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt      420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg      480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg      540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt      600

cctgctggta taggcgcgcc aggaggtggc ggggaagcgc agacagtgtt ctctcaatcc      660

ccttgggggt tcgaaggaat agcggagata acactaaaag aggcccaaaa gagcatttgt      720
```

tcactacctt tgtcttgtgt gggctgtagc ttgttgtctt ccaaggtcgt tttccttgag 780

acaacaacga aagctgccgt ccacgttgga tgtgggaatg gaacttctgt tctaacagtt 840

ggaactactc ctgtgagtat cgactgtgta gtaacgcccc tgtcgcaggt gtggaggctc 900

gtgtcgcacg tcaccggaag atacaccaaa cttgggtttg gaggtggcca tcaccatcac 960

catcactgat gaccggt 977

<210> 68
<211> 305
<212> PRT
<213> artificial

<220>
<223> Amino acid Sequence of the fusion protein SNAPlike-EDIII from a insect flavivirus - Histag

<400> 68

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Gly

180 185 190

Gly Gly Glu Ala Gln Thr Val Phe Ser Gln Ser Pro Trp Gly Phe Glu
195 200 205

Gly Ile Ala Glu Ile Thr Leu Lys Glu Ala Gln Lys Ser Ile Cys Ser
210 215 220

Leu Pro Leu Ser Cys Val Gly Cys Ser Leu Leu Ser Ser Lys Val Val
225 230 235 240

Phe Leu Glu Thr Thr Thr Lys Ala Ala Val His Val Gly Cys Gly Asn
245 250 255

Gly Thr Ser Val Leu Thr Val Gly Thr Thr Pro Val Ser Ile Asp Cys
260 265 270

Val Val Thr Pro Leu Ser Gln Val Trp Arg Leu Val Ser His Val Thr
275 280 285

Gly Arg Tyr Thr Lys Leu Gly Phe Gly Gly Gly His His His His His
290 295 300

His
305

<210> 69
<211> 1783
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-sE2 from Ross River virus strain QML-1 -SNAPlike-Histag for expression in S2 cells

<400> 69
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

agtgtaacag agcacttcaa tgtgtataag gctactagac catacctagc acattgcgct 120

gattgcgggg acgggtactt ctgctatagc ccagttgcca tcgagaagat ccgagatgag 180

gcgtctgatg gcatgctcaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc 240

acccacgccc acacgaagct ccgatatatg gctggtcacg atgttcagga atctaagaga 300

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc 360

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg 420

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag 480

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg 540

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg 600

ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaat 660

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc 720

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca 780

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg 840

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgtcaccta tggtaagaag 900

gaggtgaccc tgagattaca cccagatcat ccgacactct tctcctatag gagtttagga 960

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg 1020

acggaagaag ggattgaata ccagtggggc aacaacccgc cggtccgcct gtgggcgcaa 1080

ctgacgaccg agggcaaacc ccatggatgg ccacatgaaa tcattcagta ctattatgga 1140

ctataccccg ccgccacgcg gccgcacggc ggaggtagca aagactgcga aatgaagcgc 1200

accaccctgg atagccctct gggcaagctg gaactgtctg ggtgcgaaca gggcctgcac 1260

gagatcaagc tgctgggcaa aggaacatct gccgccgacg ccgtggaagt gcctgcccca 1320

gccgccgtgc tgggcggacc agagccactg atgcaggcca ccgcctggct caacgcctac 1380

tttcaccagc ctgaggccat cgaggagttc cctgtgccag ccctgcacca cccagtgttc 1440

cagcaggaga gctttacccg ccaggtgctg tggaaactgc tgaaagtggt gaagttcgga 1500

gaggtcatca gctaccagca gctggccgcc ctggccggca atcccgccgc caccgccgcc 1560

gtgaaaaccg ccctgagcgg aaatcccgtg cccattctga tcccctgcca ccgggtggtg 1620

tctagctctg gcgccgtggg gggctacgag ggcgggctcg ccgtgaaaga gtggctgctg 1680

gcccacgagg gccacagact gggcaagcct gggctgggtc ctgcaggtat aggcgcgcca 1740

gggtccctgg agcatcatca tcatcatcat tgatgacggg ccc 1783

<210> 70
<211> 572
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein [sE2 from Ross River virus strain QML-1 -SNAPlike-Histag]

<400> 70

Arg Ser Ser Val Thr Glu His Phe Asn Val Tyr Lys Ala Thr Arg Pro
1 5 10 15

Tyr Leu Ala His Cys Ala Asp Cys Gly Asp Gly Tyr Phe Cys Tyr Ser
20 25 30

Pro Val Ala Ile Glu Lys Ile Arg Asp Glu Ala Ser Asp Gly Met Leu
35 40 45

Lys Ile Gln Val Ser Ala Gln Ile Gly Leu Asp Lys Ala Gly Thr His
50 55 60

Ala His Thr Lys Leu Arg Tyr Met Ala Gly His Asp Val Gln Glu Ser
65 70 75 80

```
Lys Arg Asp Ser Leu Arg Val Tyr Thr Ser Ala Ala Cys Ser Ile His
                85                  90                  95

Gly Thr Met Gly His Phe Ile Val Ala His Cys Pro Pro Gly Asp Tyr
            100                 105                 110

Leu Lys Val Ser Phe Glu Asp Ala Asp Ser His Val Lys Ala Cys Lys
        115                 120                 125

Val Gln Tyr Lys His Asn Pro Leu Pro Val Gly Arg Glu Lys Phe Val
    130                 135                 140

Val Arg Pro His Phe Gly Val Glu Leu Pro Cys Thr Ser Tyr Gln Leu
145                 150                 155                 160

Thr Thr Ala Pro Thr Asp Glu Glu Ile Asp Met His Thr Pro Pro Asp
                165                 170                 175

Ile Pro Asp Arg Thr Leu Leu Ser Gln Thr Ala Gly Asn Val Lys Ile
            180                 185                 190

Thr Ala Gly Gly Arg Thr Ile Arg Tyr Asn Cys Thr Cys Gly Arg Asp
        195                 200                 205

Asn Val Gly Thr Thr Ser Thr Asp Lys Thr Ile Asn Thr Cys Lys Ile
    210                 215                 220

Asp Gln Cys His Ala Ala Val Thr Ser His Asp Lys Trp Gln Phe Thr
225                 230                 235                 240

Ser Pro Phe Val Pro Arg Ala Asp Gln Thr Ala Arg Lys Gly Lys Val
                245                 250                 255

His Val Pro Phe Pro Leu Thr Asn Val Thr Cys Arg Val Pro Leu Ala
            260                 265                 270

Arg Ala Pro Asp Val Thr Tyr Gly Lys Lys Glu Val Thr Leu Arg Leu
        275                 280                 285

His Pro Asp His Pro Thr Leu Phe Ser Tyr Arg Ser Leu Gly Ala Glu
    290                 295                 300

Pro His Pro Tyr Glu Glu Trp Val Asp Lys Phe Ser Glu Arg Ile Ile
305                 310                 315                 320

Pro Val Thr Glu Glu Gly Ile Glu Tyr Gln Trp Gly Asn Asn Pro Pro
                325                 330                 335

Val Arg Leu Trp Ala Gln Leu Thr Thr Glu Gly Lys Pro His Gly Trp
            340                 345                 350
```

```
Pro His Glu Ile Ile Gln Tyr Tyr Tyr Gly Leu Tyr Pro Ala Ala Thr
        355                 360                 365

Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr
    370                 375                 380

Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly
385                 390                 395                 400

Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala
                405                 410                 415

Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu
            420                 425                 430

Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala
        435                 440                 445

Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln
    450                 455                 460

Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys
465                 470                 475                 480

Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn
                485                 490                 495

Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val
            500                 505                 510

Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val
        515                 520                 525

Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His
    530                 535                 540

Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly
545                 550                 555                 560

Ala Pro Gly Ser Leu Glu His His His His His His
                565                 570
```

<210> 71
<211> 1783
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-sE2 from Mayaro virus strain IQD2668 -SNAPlike-Histag for expression in S2 cells

<400> 71
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

agtgtaacag agcacttcaa tgtgtataag gctactagac catacctagc acattgcgct 120

gattgcgggg acgggtactt ctgctatagc ccagttgcca tcgagaagat ccgagatgag 180

gcgtctgatg gcatgctcaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc 240

acccacgccc acacgaagct ccgatatatg gctggtcacg atgttcagga atctaagaga 300

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc 360

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg 420

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag 480

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg 540

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg 600

ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaat 660

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc 720

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca 780

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg 840

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgtcaccta tggtaagaag 900

gaggtgaccc tgagattaca cccagatcat ccgacactct tctcctatag gagtttagga 960

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg 1020

acggaagaag ggattgaata ccagtggggc aacaacccgc cggtccgcct gtgggcgcaa 1080

ctgacgaccg agggcaaacc ccatggatgg ccacatgaaa tcattcagta ctattatgga 1140

ctataccccg ccgccacgcg gccgcacggc ggaggtagca aagactgcga aatgaagcgc 1200

accaccctgg atagccctct gggcaagctg gaactgtctg ggtgcgaaca gggcctgcac 1260

gagatcaagc tgctgggcaa aggaacatct gccgccgacg ccgtggaagt gcctgcccca 1320

gccgccgtgc tgggcggacc agagccactg atgcaggcca ccgcctggct caacgcctac 1380

tttcaccagc ctgaggccat cgaggagttc cctgtgccag ccctgcacca cccagtgttc 1440

cagcaggaga gctttacccg ccaggtgctg tggaaactgc tgaaagtggt gaagttcgga 1500

gaggtcatca gctaccagca gctggccgcc ctggccggca atcccgccgc caccgccgcc 1560

gtgaaaaccg ccctgagcgg aaatcccgtg cccattctga tcccctgcca ccgggtggtg 1620

tctagctctg gcgccgtggg gggctacgag ggcgggctcg ccgtgaaaga gtggctgctg 1680

gcccacgagg gccacagact gggcaagcct gggctgggtc ctgcaggtat aggcgcgcca 1740

gggtccctgg agcatcatca tcatcatcat tgatgacggg ccc 1783

<210> 72
<211> 572
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein [sE2 from Mayaro virus strain IQD2668 -SNAPlike-Histag]

<400> 72

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Arg Ser Ser Val Thr Glu His Phe Asn Val Tyr Lys Ala Thr Arg Pro
1               5                  10                  15

Tyr Leu Ala His Cys Ala Asp Cys Gly Asp Gly Tyr Phe Cys Tyr Ser
            20                  25                  30

Pro Val Ala Ile Glu Lys Ile Arg Asp Glu Ala Ser Asp Gly Met Leu
        35                  40                  45

Lys Ile Gln Val Ser Ala Gln Ile Gly Leu Asp Lys Ala Gly Thr His
    50                  55                  60

Ala His Thr Lys Leu Arg Tyr Met Ala Gly His Asp Val Gln Glu Ser
65                  70                  75                  80

Lys Arg Asp Ser Leu Arg Val Tyr Thr Ser Ala Ala Cys Ser Ile His
                85                  90                  95

Gly Thr Met Gly His Phe Ile Val Ala His Cys Pro Pro Gly Asp Tyr
            100                 105                 110

Leu Lys Val Ser Phe Glu Asp Ala Asp Ser His Val Lys Ala Cys Lys
        115                 120                 125

Val Gln Tyr Lys His Asn Pro Leu Pro Val Gly Arg Glu Lys Phe Val
    130                 135                 140

Val Arg Pro His Phe Gly Val Glu Leu Pro Cys Thr Ser Tyr Gln Leu
145                 150                 155                 160

Thr Thr Ala Pro Thr Asp Glu Glu Ile Asp Met His Thr Pro Pro Asp
                165                 170                 175

Ile Pro Asp Arg Thr Leu Leu Ser Gln Thr Ala Gly Asn Val Lys Ile
            180                 185                 190

Thr Ala Gly Gly Arg Thr Ile Arg Tyr Asn Cys Thr Cys Gly Arg Asp
        195                 200                 205

Asn Val Gly Thr Thr Ser Thr Asp Lys Thr Ile Asn Thr Cys Lys Ile
    210                 215                 220

Asp Gln Cys His Ala Ala Val Thr Ser His Asp Lys Trp Gln Phe Thr
225                 230                 235                 240

Ser Pro Phe Val Pro Arg Ala Asp Gln Thr Ala Arg Lys Gly Lys Val
                245                 250                 255

His Val Pro Phe Pro Leu Thr Asn Val Thr Cys Arg Val Pro Leu Ala
```

260 265 270

Arg Ala Pro Asp Val Thr Tyr Gly Lys Lys Glu Val Thr Leu Arg Leu
275 280 285

His Pro Asp His Pro Thr Leu Phe Ser Tyr Arg Ser Leu Gly Ala Glu
290 295 300

Pro His Pro Tyr Glu Glu Trp Val Asp Lys Phe Ser Glu Arg Ile Ile
305 310 315 320

Pro Val Thr Glu Glu Gly Ile Glu Tyr Gln Trp Gly Asn Asn Pro Pro
325 330 335

Val Arg Leu Trp Ala Gln Leu Thr Thr Glu Gly Lys Pro His Gly Trp
340 345 350

Pro His Glu Ile Ile Gln Tyr Tyr Tyr Gly Leu Tyr Pro Ala Ala Thr
355 360 365

Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr
370 375 380

Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly
385 390 395 400

Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala
405 410 415

Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu
420 425 430

Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala
435 440 445

Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln
450 455 460

Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys
465 470 475 480

Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn
485 490 495

Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val
500 505 510

Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val
515 520 525

Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His

530 535 540

Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly
545 550 555 560

Ala Pro Gly Ser Leu Glu His His His His His His
565 570

<210> 73
<211> 1786
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-sE2 from Western Equine Encephalitis virus -SNAPlike-Histag for expression in S2 cells

<400> 73
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

agcattaccg atgacttcac actgaccagt ccctacctgg ggttctgccc gtattgcaga 120

cactcaacgc cgtgtttcag cccaataaaa attgagaacg tgtgggacga atctgatgat 180

ggatcgatta gaatccaggt ctcggcacaa ttcggctaca atcaggcagg cactgcggat 240

gtcaccaaat tccgttacat gtctttcgac cacgaccatg acatcaagga agacagtatg 300

gagaaaatag ctatcagcac atctggaccc tgccgtcgtc ttggccacaa agggtacttc 360

ctgttagctc aatgtcctcc aggtgacagt gtaaccgtca gtatcacgag cggagcatct 420

gagaattcat gcaccgtgga gaaaaagatc aggaggaagt ttgtcggtag agaggagtac 480

ttgttcccac ccgtccatgg aaagctggta aagtgccacg tttacgatca cttgaaggag 540

acgtctgccg ggtacataac catgcacagg ccaggcccac acgcgtataa gtcctatctg 600

gaggaagcgt caggcgaagt gtacattaaa ccaccttctg gcaagaacgt cacctacgaa 660

tgtaagtgtg gcgactacag cacaggtatc gtgagcacgc gaacgaagat gaacggctgc 720

actaaagcaa aacagtgcat tgcctacaag agcgaccaaa cgaaatgggt cttcaactcg 780

ccggatctta ttaggcacac agaccactca gtgcaaggta aattgcacat tccattccgc 840

ttgacaccga cagtctgccc ggttccgtta gctcacacgc ctacagtcac gaagtggttc 900

aaaggcatca ccctccacct gactgcaatg cgaccaacat tgctgacaac gagaaaattg 960

gggctgcgag cagacgcaac agcagaatgg attacagggt ctacatccag gaatttttct 1020

gtggggcgag aagggctgga gtacgtatgg ggtaaccatg aaccagtcag agtctgggcc 1080

caggagtcgg caccaggcga cccacatgga tggccgcatg agatcatcat ccactattat 1140

catcggcatc cagtctacac gcggccgcac ggcggaggta gcaaagactg cgaaatgaag 1200

cgcaccaccc tggatagccc tctgggcaag ctggaactgt ctgggtgcga acagggcctg 1260

cacgagatca agctgctggg caaaggaaca tctgccgccg acgccgtgga agtgcctgcc 1320

ccagccgccg tgctgggcgg accagagcca ctgatgcagg ccaccgcctg gctcaacgcc 1380

tactttcacc agcctgaggc catcgaggag ttccctgtgc cagccctgca ccacccagtg 1440

ttccagcagg agagctttac ccgccaggtg ctgtggaaac tgctgaaagt ggtgaagttc 1500

ggagaggtca tcagctacca gcagctggcc gccctggccg gcaatcccgc cgccaccgcc 1560

gccgtgaaaa ccgccctgag cggaaatccc gtgcccattc tgatcccctg ccaccgggtg 1620

gtgtctagct ctggcgccgt gggggggctac gagggcgggc tcgccgtgaa agagtggctg 1680

ctggcccacg agggccacag actgggcaag cctgggctgg gtcctgcagg tataggcgcg 1740

ccagggtccc tggagcatca tcatcatcat cattgatgac gggccc 1786

<210> 74
<211> 573
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein [sE2 from Western Equine Encephalitis -SNAPlike-Histag]

<400> 74

Arg Ser Ser Ile Thr Asp Asp Phe Thr Leu Thr Ser Pro Tyr Leu Gly
1 5 10 15

Phe Cys Pro Tyr Cys Arg His Ser Thr Pro Cys Phe Ser Pro Ile Lys
20 25 30

Ile Glu Asn Val Trp Asp Glu Ser Asp Asp Gly Ser Ile Arg Ile Gln
35 40 45

Val Ser Ala Gln Phe Gly Tyr Asn Gln Ala Gly Thr Ala Asp Val Thr
50 55 60

Lys Phe Arg Tyr Met Ser Phe Asp His Asp His Asp Ile Lys Glu Asp
65 70 75 80

Ser Met Glu Lys Ile Ala Ile Ser Thr Ser Gly Pro Cys Arg Arg Leu
85 90 95

Gly His Lys Gly Tyr Phe Leu Leu Ala Gln Cys Pro Pro Gly Asp Ser
100 105 110

Val Thr Val Ser Ile Thr Ser Gly Ala Ser Glu Asn Ser Cys Thr Val
115 120 125

Glu Lys Lys Ile Arg Arg Lys Phe Val Gly Arg Glu Glu Tyr Leu Phe
130 135 140

Pro Pro Val His Gly Lys Leu Val Lys Cys His Val Tyr Asp His Leu
145 150 155 160

Lys Glu Thr Ser Ala Gly Tyr Ile Thr Met His Arg Pro Gly Pro His
165 170 175

```
Ala Tyr Lys Ser Tyr Leu Glu Glu Ala Ser Gly Glu Val Tyr Ile Lys
            180                 185                 190

Pro Pro Ser Gly Lys Asn Val Thr Tyr Glu Cys Lys Cys Gly Asp Tyr
        195                 200                 205

Ser Thr Gly Ile Val Ser Thr Arg Thr Lys Met Asn Gly Cys Thr Lys
    210                 215                 220

Ala Lys Gln Cys Ile Ala Tyr Lys Ser Asp Gln Thr Lys Trp Val Phe
225                 230                 235                 240

Asn Ser Pro Asp Leu Ile Arg His Thr Asp His Ser Val Gln Gly Lys
                245                 250                 255

Leu His Ile Pro Phe Arg Leu Thr Pro Thr Val Cys Pro Val Pro Leu
            260                 265                 270

Ala His Thr Pro Thr Val Thr Lys Trp Phe Lys Gly Ile Thr Leu His
        275                 280                 285

Leu Thr Ala Met Arg Pro Thr Leu Leu Thr Thr Arg Lys Leu Gly Leu
    290                 295                 300

Arg Ala Asp Ala Thr Ala Glu Trp Ile Thr Gly Ser Thr Ser Arg Asn
305                 310                 315                 320

Phe Ser Val Gly Arg Glu Gly Leu Glu Tyr Val Trp Gly Asn His Glu
                325                 330                 335

Pro Val Arg Val Trp Ala Gln Glu Ser Ala Pro Gly Asp Pro His Gly
            340                 345                 350

Trp Pro His Glu Ile Ile Ile His Tyr Tyr His Arg His Pro Val Tyr
        355                 360                 365

Thr Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr
    370                 375                 380

Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln
385                 390                 395                 400

Gly Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp
                405                 410                 415

Ala Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro
            420                 425                 430

Leu Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu
        435                 440                 445
```

```
Ala Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln
    450                 455                 460

Gln Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val
465                 470                 475                 480

Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly
                485                 490                 495

Asn Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro
            500                 505                 510

Val Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala
        515                 520                 525

Val Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala
    530                 535                 540

His Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile
545                 550                 555                 560

Gly Ala Pro Gly Ser Leu Glu His His His His His His
                565                 570
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<210> 75
<211> 1786
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-sE2 from Eastern Equine Encephalitis virus -SNAPlike-Histag for expression in S2 cells

<400> 75

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gatttggaca ctcatttcac ccagtataag ttggcacgcc cgtatattgc tgattgccct      120

aactgtgggc atagtcggtg cgacagccct atagctatag aagaagtcag aggggatgcg      180

cacgcaggag tcatccgcat ccagacatca gctatgttcg gtctgaagac ggatggagtc      240

gatttggcct acatgagttt catgaacggc aaaacgcaga aatcaataaa gatcgacaac      300

ctgcatgtgc gcacctcagc cccttgttcc ctcgtgtcgc accacggcta ttacatcctg      360

gctcaatgcc caccagggga cacggttaca gttgggtttc acgacgggcc taaccgccat      420

acgtgcacag ttgcccataa ggtagaattc aggccagtgg gtagagagaa ataccgtcac      480

ccacctgaac atggagttga attaccgtgt aaccgttaca ctcacaagcg tgcagaccaa      540

ggacactatg ttgagatgca tcaacccggg ctagttgccg accactctct ccttagcatc      600

cacagtgcca aggtgaaaat tacggtaccg agcggcgccc aagtgaaata ctactgcaag      660

tgcccagatg tacgagaggg aattaccagc agcgaccata caaccacctg cacggatgtc      720

aaacaatgca gggcttacct gattgacaac aaaaaatggg tgtacaactc tggaagactg      780
```

cctcgaggag agggcgacac ttttaaagga aaacttcatg tgccctttgt gcctgttaag 840

gccaagtgca tcgccacgct ggcaccggag cctctagttg agcacaaaca ccgcaccctg 900

attttacacc tgcacccgga ccatccgacc ttgctgacga ccaggtcact tggaagtgat 960

gcaaatccaa ctcgacaatg gattgagcga ccaacaactg tcaatttcac agtcaccgga 1020

gaagggttgg agtatacctg gggaaaccat ccaccaaaaa gagtatgggc tcaagagtca 1080

ggagaaggga atccacatgg atggccgcac gaagtggtag tctattacta caacaggtac 1140

ccgttaacca caattatcgg gcggccgcac ggcggaggta gcaaagactg cgaaatgaag 1200

cgcaccaccc tggatagccc tctgggcaag ctggaactgt ctgggtgcga acagggcctg 1260

cacgagatca agctgctggg caaaggaaca tctgccgccg acgccgtgga agtgcctgcc 1320

ccagccgccg tgctgggcgg accagagcca ctgatgcagg ccaccgcctg gctcaacgcc 1380

tactttcacc agcctgaggc catcgaggag ttccctgtgc cagccctgca ccacccagtg 1440

ttccagcagg agagctttac ccgccaggtg ctgtggaaac tgctgaaagt ggtgaagttc 1500

ggagaggtca tcagctacca gcagctggcc gccctggccg gcaatcccgc cgccaccgcc 1560

gccgtgaaaa ccgccctgag cggaaatccc gtgcccattc tgatcccctg ccaccgggtg 1620

gtgtctagct ctggcgccgt gggggggctac gagggcgggc tcgccgtgaa agagtggctg 1680

ctggcccacg agggccacag actgggcaag cctgggctgg gtcctgcagg tataggcgcg 1740

ccagggtccc tggagcatca tcatcatcat cattgatgac gggccc 1786

<210> 76
<211> 573
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein [sE2 from Eastern Equine Encephalitis -SNAPlike-Histag]

<400> 76

Arg Ser Asp Leu Asp Thr His Phe Thr Gln Tyr Lys Leu Ala Arg Pro
1 5 10 15

Tyr Ile Ala Asp Cys Pro Asn Cys Gly His Ser Arg Cys Asp Ser Pro
20 25 30

Ile Ala Ile Glu Glu Val Arg Gly Asp Ala His Ala Gly Val Ile Arg
35 40 45

Ile Gln Thr Ser Ala Met Phe Gly Leu Lys Thr Asp Gly Val Asp Leu
50 55 60

Ala Tyr Met Ser Phe Met Asn Gly Lys Thr Gln Lys Ser Ile Lys Ile
65 70 75 80

Asp Asn Leu His Val Arg Thr Ser Ala Pro Cys Ser Leu Val Ser His

85 90 95

His Gly Tyr Tyr Ile Leu Ala Gln Cys Pro Pro Gly Asp Thr Val Thr
100 105 110

Val Gly Phe His Asp Gly Pro Asn Arg His Thr Cys Thr Val Ala His
115 120 125

Lys Val Glu Phe Arg Pro Val Gly Arg Glu Lys Tyr Arg His Pro Pro
130 135 140

Glu His Gly Val Glu Leu Pro Cys Asn Arg Tyr Thr His Lys Arg Ala
145 150 155 160

Asp Gln Gly His Tyr Val Glu Met His Gln Pro Gly Leu Val Ala Asp
165 170 175

His Ser Leu Leu Ser Ile His Ser Ala Lys Val Lys Ile Thr Val Pro
180 185 190

Ser Gly Ala Gln Val Lys Tyr Tyr Cys Lys Cys Pro Asp Val Arg Glu
195 200 205

Gly Ile Thr Ser Ser Asp His Thr Thr Thr Cys Thr Asp Val Lys Gln
210 215 220

Cys Arg Ala Tyr Leu Ile Asp Asn Lys Lys Trp Val Tyr Asn Ser Gly
225 230 235 240

Arg Leu Pro Arg Gly Glu Gly Asp Thr Phe Lys Gly Lys Leu His Val
245 250 255

Pro Phe Val Pro Val Lys Ala Lys Cys Ile Ala Thr Leu Ala Pro Glu
260 265 270

Pro Leu Val Glu His Lys His Arg Thr Leu Ile Leu His Leu His Pro
275 280 285

Asp His Pro Thr Leu Leu Thr Thr Arg Ser Leu Gly Ser Asp Ala Asn
290 295 300

Pro Thr Arg Gln Trp Ile Glu Arg Pro Thr Thr Val Asn Phe Thr Val
305 310 315 320

Thr Gly Glu Gly Leu Glu Tyr Thr Trp Gly Asn His Pro Pro Lys Arg
325 330 335

Val Trp Ala Gln Glu Ser Gly Glu Gly Asn Pro His Gly Trp Pro His
340 345 350

Glu Val Val Val Tyr Tyr Tyr Asn Arg Tyr Pro Leu Thr Thr Ile Ile

355 360 365

Gly Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr
370 375 380

Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln
385 390 395 400

Gly Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp
405 410 415

Ala Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro
420 425 430

Leu Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu
435 440 445

Ala Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln
450 455 460

Gln Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val
465 470 475 480

Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly
485 490 495

Asn Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro
500 505 510

Val Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala
515 520 525

Val Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala
530 535 540

His Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile
545 550 555 560

Gly Ala Pro Gly Ser Leu Glu His His His His His His
565 570

<210> 77
<211> 1792
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-sE2 from Venezuelan Equine Encephalitis virus -SNAPlike-Histag for expression in S2 cells

<400> 77
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

agatcttcta ccgaggagct gtttaaggag tataagctaa cgcgccctta catggccaga 120

tgcatcagat gtgccgttgg gagctgccat agtccaatag caattgaggc agtgaagagc 180

gacgggcacg acggctatgt tagacttcag acttcctcgc agtatggcct ggattcctct 240

ggcaacttaa agggaaggac tatgcggtat gatatgcacg ggaccattga agagatacca 300

ctacatcaag tgtcactcca cacatctcgc ccgtgtcaca ttgtggatgg gcatggttat 360

tttctgcttg ctaggtgccc ggcaggggac tccatcacca tggaatttaa gaaaggttca 420

gtcacacact cctgctcagt gccgtatgaa gtgaaattta atcctgtagg cagagaactc 480

tacactcatc caccagaaca cggagcagag caagcgtgcc aagtctacgc gcacgatgca 540

cagaacagag gagcttatgt cgagatgcac ctcccgggct cagaagtgga cagcagtttg 600

atttccttga gcggcagttc agtcaccgtg acacctcctg tcgggactag cgccttggtg 660

aaatgcaagt gcggcggcac aaagatctcc gaaaccatca acaaggcaaa acagttcagc 720

cagtgcacaa agaaggagca gtgcagagca tatcgactgc agaatgacaa gtgggtgtat 780

aattctgaca aactgcccaa agcagcggga gccaccctaa aaggaaaact acacgtcccg 840

ttcttgctgg cagacggcaa atgcaccgtg cctctagcac cggaacctat gataaccttc 900

ggtttccgat cagtgtcact gaaactgcac cctaagaatc ccacatatct gaccactcgc 960

caacttgctg atgagcctca ttacacgcac gagctcatat ctgaaccagc tgttaggaat 1020

tttaccgtca ctgaaaaggg gtgggagttt gtatggggaa accatccgcc gaaaaggttt 1080

tgggcacagg aaacagcacc cggaaatcca catgggctgc cacatgaggt gataactcat 1140

tattaccaca gataccctat gtccacgcgg ccgcacggcg gaggtagcaa agactgcgaa 1200

atgaagcgca ccaccctgga tagccctctg ggcaagctgg aactgtctgg gtgcgaacag 1260

ggcctgcacg agatcaagct gctgggcaaa ggaacatctg ccgccgacgc cgtggaagtg 1320

cctgccccag ccgccgtgct gggcggacca gagccactga tgcaggccac cgcctggctc 1380

aacgcctact ttcaccagcc tgaggccatc gaggagttcc ctgtgccagc cctgcaccac 1440

ccagtgttcc agcaggagag ctttacccgc caggtgctgt ggaaactgct gaaagtggtg 1500

aagttcggag aggtcatcag ctaccagcag ctggccgccc tggccggcaa tcccgccgcc 1560

accgccgccg tgaaaaccgc cctgagcgga aatcccgtgc ccattctgat cccctgccac 1620

cgggtggtgt ctagctctgg cgccgtgggg ggctacgagg gcgggctcgc cgtgaaagag 1680

tggctgctgg cccacgaggg ccacagactg ggcaagcctg ggctgggtcc tgcaggtata 1740

ggcgcgccag ggtccctgga gcatcatcat catcatcatt gatgacgggc cc 1792

<210> 78
<211> 575
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of the fusion protein [sE2 from Venezuelan Equine Encephalitis -SNAPlike-Histag]

<400> 78

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Arg Ser Arg Ser Ser Thr Glu Glu Leu Phe Lys Glu Tyr Lys Leu Thr
1               5                  10                  15

Arg Pro Tyr Met Ala Arg Cys Ile Arg Cys Ala Val Gly Ser Cys His
            20                  25                  30

Ser Pro Ile Ala Ile Glu Ala Val Lys Ser Asp Gly His Asp Gly Tyr
        35                  40                  45

Val Arg Leu Gln Thr Ser Ser Gln Tyr Gly Leu Asp Ser Ser Gly Asn
    50                  55                  60

Leu Lys Gly Arg Thr Met Arg Tyr Asp Met His Gly Thr Ile Glu Glu
65                  70                  75                  80

Ile Pro Leu His Gln Val Ser Leu His Thr Ser Arg Pro Cys His Ile
                85                  90                  95

Val Asp Gly His Gly Tyr Phe Leu Leu Ala Arg Cys Pro Ala Gly Asp
            100                 105                 110

Ser Ile Thr Met Glu Phe Lys Lys Gly Ser Val Thr His Ser Cys Ser
        115                 120                 125

Val Pro Tyr Glu Val Lys Phe Asn Pro Val Gly Arg Glu Leu Tyr Thr
    130                 135                 140

His Pro Pro Glu His Gly Ala Glu Gln Ala Cys Gln Val Tyr Ala His
145                 150                 155                 160

Asp Ala Gln Asn Arg Gly Ala Tyr Val Glu Met His Leu Pro Gly Ser
                165                 170                 175

Glu Val Asp Ser Ser Leu Ile Ser Leu Ser Gly Ser Ser Val Thr Val
            180                 185                 190

Thr Pro Pro Val Gly Thr Ser Ala Leu Val Lys Cys Lys Cys Gly Gly
        195                 200                 205

Thr Lys Ile Ser Glu Thr Ile Asn Lys Ala Lys Gln Phe Ser Gln Cys
    210                 215                 220

Thr Lys Lys Glu Gln Cys Arg Ala Tyr Arg Leu Gln Asn Asp Lys Trp
225                 230                 235                 240

Val Tyr Asn Ser Asp Lys Leu Pro Lys Ala Ala Gly Ala Thr Leu Lys
                245                 250                 255

Gly Lys Leu His Val Pro Phe Leu Leu Ala Asp Gly Lys Cys Thr Val
            260                 265                 270
```

```
Pro Leu Ala Pro Glu Pro Met Ile Thr Phe Gly Phe Arg Ser Val Ser
        275                 280                 285

Leu Lys Leu His Pro Lys Asn Pro Thr Tyr Leu Thr Thr Arg Gln Leu
    290                 295                 300

Ala Asp Glu Pro His Tyr Thr His Glu Leu Ile Ser Glu Pro Ala Val
305                 310                 315                 320

Arg Asn Phe Thr Val Thr Glu Lys Gly Trp Glu Phe Val Trp Gly Asn
                325                 330                 335

His Pro Pro Lys Arg Phe Trp Ala Gln Glu Thr Ala Pro Gly Asn Pro
            340                 345                 350

His Gly Leu Pro His Glu Val Ile Thr His Tyr Tyr His Arg Tyr Pro
        355                 360                 365

Met Ser Thr Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys
    370                 375                 380

Arg Thr Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys
385                 390                 395                 400

Glu Gln Gly Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala
                405                 410                 415

Ala Asp Ala Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro
            420                 425                 430

Glu Pro Leu Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln
        435                 440                 445

Pro Glu Ala Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val
    450                 455                 460

Phe Gln Gln Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys
465                 470                 475                 480

Val Val Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu
                485                 490                 495

Ala Gly Asn Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly
            500                 505                 510

Asn Pro Val Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser
        515                 520                 525

Gly Ala Val Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu
    530                 535                 540
```

Leu Ala His Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala
545 550 555 560

Gly Ile Gly Ala Pro Gly Ser Leu Glu His His His His His His
565 570 575

<210> 79
<211> 1442
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike-Akabane N protein-proTEV cleavage site-Histag for expression in S2 cells

<400> 79
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga 60

tctgacaaag actgcgaaat gaaaagaact acattggatt caccacttgg gaagttggaa 120

ctgagtggat gcgagcaagg attgcatgaa attaagctac tgggaaaagg aacttctgct 180

gctgatgcag ttgaagttcc agcaccagca gctgttcttg gaggtcctga gcccctcatg 240

caagccacag cctggcttaa cgcatatttc caccagcctg aggccattga ggaatttcca 300

gtccccgccc ttcaccatcc tgtgtttcag caggagagct tcacccgcca ggtcctgtgg 360

aaattgctga aggtggtcaa gtttggtgaa gtgatttcat atcagcaact tgctgcattg 420

gccggtaacc ccgcagctac agctgccgtg aaaactgctc tcagcggaaa tcctgtgccc 480

atcctgatcc cttgtcacag agtcgtttca tcttccggag ctgtaggtgg ctatgaagga 540

ggactggcag ttaaggagtg gctgctggct catgaaggtc atagacttgg aaagcctggg 600

ctgggtcctg ctggtatagg cgcgccaggg tccctaggtg gcggatccga aaacctgtac 660

ttccagagcg atatcgcaaa tcaatttatt ttcaacgatg ttccacaacg gaatgcagct 720

acatttaatc cggatgcagg gtatgtggca tttatcagta agtatgggca gcagctcaac 780

tttactgttg ctagagtctt cttcctcaac cagaagaagg ccaagatggt cttacataag 840

acgccacaac caagtgtcga tcttactttt gcaggggtca aatttacagt ggttaataac 900

cattttcccc agtatactgc aaatccggtg tcagacactg cctttacgct ccatcgcatc 960

tcgggctact tagctcgatg ggttgctgag cagtgcaagg ctaatcagat caaacttgca 1020

gaggcagctg ctacaatcgt aatgccgctg gctgaagtga agggctgcac ctggagtgat 1080

gggtacgcaa tgtacctagg ctttgcccct ggtgctgaga tgtttctgga aacctttgag 1140

ttttacccat tggttattga catgcaccgt gtgataaagg atgggatgga tgtcaacttc 1200

atgaggaagg tcttacgcca gagatatggg cagctgactg cagaagaatg gatgacatct 1260

aagttggacg cagtcaaggc tgcatttagc tcagttgccc aaatatcctg ggccaaatct 1320

ggcttctcac ctgcagctag agctttcctg gctcaatttg gtattcagat cccgggagag 1380

aatctatatt ttcaagggcc cggcggaggt agtcaccatc atcaccatca ctaatgaccg 1440

gt 1442

<210> 80
<211> 458
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-Akabane N protein-Histag]

<400> 80

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala Asn
195 200 205

Gln Phe Ile Phe Asn Asp Val Pro Gln Arg Asn Ala Ala Thr Phe Asn
210 215 220

```
Pro Asp Ala Gly Tyr Val Ala Phe Ile Ser Lys Tyr Gly Gln Gln Leu
225                 230                 235                 240

Asn Phe Thr Val Ala Arg Val Phe Phe Leu Asn Gln Lys Lys Ala Lys
                245                 250                 255

Met Val Leu His Lys Thr Pro Gln Pro Ser Val Asp Leu Thr Phe Ala
            260                 265                 270

Gly Val Lys Phe Thr Val Val Asn Asn His Phe Pro Gln Tyr Thr Ala
        275                 280                 285

Asn Pro Val Ser Asp Thr Ala Phe Thr Leu His Arg Ile Ser Gly Tyr
    290                 295                 300

Leu Ala Arg Trp Val Ala Glu Gln Cys Lys Ala Asn Gln Ile Lys Leu
305                 310                 315                 320

Ala Glu Ala Ala Ala Thr Ile Val Met Pro Leu Ala Glu Val Lys Gly
                325                 330                 335

Cys Thr Trp Ser Asp Gly Tyr Ala Met Tyr Leu Gly Phe Ala Pro Gly
            340                 345                 350

Ala Glu Met Phe Leu Glu Thr Phe Glu Phe Tyr Pro Leu Val Ile Asp
        355                 360                 365

Met His Arg Val Ile Lys Asp Gly Met Asp Val Asn Phe Met Arg Lys
    370                 375                 380

Val Leu Arg Gln Arg Tyr Gly Gln Leu Thr Ala Glu Glu Trp Met Thr
385                 390                 395                 400

Ser Lys Leu Asp Ala Val Lys Ala Ala Phe Ser Ser Val Ala Gln Ile
                405                 410                 415

Ser Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg Ala Phe Leu Ala
            420                 425                 430

Gln Phe Gly Ile Gln Ile Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro
        435                 440                 445

Gly Gly Gly Ser His His His His His His
    450                 455
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<210> 81
<211> 1451
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike-Aino N protein-proTEV

cleavage site-Histag for expression in S2 cells

<400> 81
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga 60

tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag 120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact 180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc 240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa 300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc 360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct 420

gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct 480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat 540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag 600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac 660

ctgtacttcc agagcgatat cgcaaaccaa tttattttcc aagatgttcc tcaacggaat 720

ctcgctacat ttaacccgga ggtcgggtat gtggcattta ttgctaaaca tgggtcccaa 780

ctcaatttcg ataccgttag agtcttcttc ctcaatcaga agaaggccaa gatggtgctc 840

agtaagacgg cacaaccaag tgttgatctt acatttggtg gcatcaaatt tacactggtt 900

aataaccatt ttccccaata cacagcaaat cctgtgccag acactgccct cactctccac 960

cgtctctcag gttatctagc aaaatgggtt gcagaccaat gcaaaacaaa tcagattaaa 1020

ctggctgagg ccatggaaaa aattgtcatg ccacttgctg aagtgaaagg ttgcacctgg 1080

actgaaggac tgactatgta tctgggattt gcaccaggcg ctgaaatgtt tttagaaaca 1140

tttgagttct acccttggt tattgacatg cacagagtgc tgaaagatgg aatggatgtc 1200

aactttatga gaaaggtcct tcgccagcgc tatggcacat tgactgcaga acagtggatg 1260

actcaaaaaa tagatgctgt ccgtgcagcc ttcaatgctg ttgggcagct aagttgggct 1320

aaatcaggat tctcaccagc tgccagagcc ttccttgccc aattcggcat aaacatgatc 1380

ccgggagaga atctatattt tcaagggccc ggcggaggta gtcaccatca tcaccatcac 1440

taatgaccgg t 1451

<210> 82
<211> 459
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-Aino N protein-Histag]

<400> 82

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

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Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala Asn
        195                 200                 205

Gln Phe Ile Phe Gln Asp Val Pro Gln Arg Asn Leu Ala Thr Phe Asn
    210                 215                 220

Pro Glu Val Gly Tyr Val Ala Phe Ile Ala Lys His Gly Ser Gln Leu
225                 230                 235                 240

Asn Phe Asp Thr Val Arg Val Phe Phe Leu Asn Gln Lys Lys Ala Lys
                245                 250                 255

Met Val Leu Ser Lys Thr Ala Gln Pro Ser Val Asp Leu Thr Phe Gly
            260                 265                 270

Gly Ile Lys Phe Thr Leu Val Asn Asn His Phe Pro Gln Tyr Thr Ala
        275                 280                 285
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Asn Pro Val Pro Asp Thr Ala Leu Thr Leu His Arg Leu Ser Gly Tyr
    290                 295                 300

Leu Ala Lys Trp Val Ala Asp Gln Cys Lys Thr Asn Gln Ile Lys Leu
305                 310                 315                 320

Ala Glu Ala Met Glu Lys Ile Val Met Pro Leu Ala Glu Val Lys Gly
                325                 330                 335

Cys Thr Trp Thr Glu Gly Leu Thr Met Tyr Leu Gly Phe Ala Pro Gly
            340                 345                 350

Ala Glu Met Phe Leu Glu Thr Phe Glu Phe Tyr Pro Leu Val Ile Asp
        355                 360                 365

Met His Arg Val Leu Lys Asp Gly Met Asp Val Asn Phe Met Arg Lys
    370                 375                 380

Val Leu Arg Gln Arg Tyr Gly Thr Leu Thr Ala Glu Gln Trp Met Thr
385                 390                 395                 400

Gln Lys Ile Asp Ala Val Arg Ala Ala Phe Asn Ala Val Gly Gln Leu
                405                 410                 415

Ser Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg Ala Phe Leu Ala
            420                 425                 430

Gln Phe Gly Ile Asn Met Ile Pro Gly Glu Asn Leu Tyr Phe Gln Gly
        435                 440                 445

Pro Gly Gly Gly Ser His His His His His His
    450                 455


<210>  83
<211>  1448
<212>  DNA
<213>  artificial

<220>
<223>  DNA sequence encoding ssBiP - SNAPlike-Shamonda  N protein-proTEV
       cleavage site-Histag for expression in S2 cells

<400>  83
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga       60

tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag      120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact      180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc      240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa      300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc      360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct      420
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gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct 480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat 540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag 600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac 660

ctgtacttcc agagcgatat ctcaagccaa ttcatttttg aagatgtacc acaacggaat 720

gcagctacat ttaacccgga aggtgggtat gtggcattta ttggtaagta tgggcaacaa 780

ctcaatttcg gggttgctaa agtcttcttc ctcaaccaga agaaggccaa aatggtccta 840

cataagacgg gacaaccaag tgtcgatctt acttttggtg gggtcaaatt cacagtggtt 900

aataaccatt ttccccaata tgtctcaaat cctgtgccag acaatgccat tacacttcac 960

aggatgtcag gttatctagc acgctggatt gctgatacat gcaaggctag tgtcctcaaa 1020

ctagctgaag ctagtgctca aattgtcatg ccccttgctg aggttaaggg atgtacctgg 1080

gctgatggtt atacaatgta tcttggattt gcacctgggg ccgaaatgtt ccttgatgct 1140

tttgattttt atccgctagt tatcgaaatg catagggtcc ttaaggacaa tatggatgta 1200

aattttatga aaaaagtcct ccgccaacgc tatggaacaa tgactgctga agaatggatg 1260

actcagaaaa taccagaaat aaaggctgct ttcaattctg ttggacaact tgcctgggct 1320

aaatctggat tctctcctgc tgctagaact ttcttgcagc aatttggtat caacatcccg 1380

ggagagaatc tatattttca agggcccggc ggaggtagtc accatcatca ccatcactaa 1440

tgaccggt 1448

<210> 84
<211> 458
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-Shamonda N protein-Histag]

<400> 84

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

```
Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ser Ser
        195                 200                 205

Gln Phe Ile Phe Glu Asp Val Pro Gln Arg Asn Ala Ala Thr Phe Asn
    210                 215                 220

Pro Glu Gly Gly Tyr Val Ala Phe Ile Gly Lys Tyr Gly Gln Gln Leu
225                 230                 235                 240

Asn Phe Gly Val Ala Lys Val Phe Phe Leu Asn Gln Lys Lys Ala Lys
                245                 250                 255

Met Val Leu His Lys Thr Gly Gln Pro Ser Val Asp Leu Thr Phe Gly
            260                 265                 270

Gly Val Lys Phe Thr Val Val Asn Asn His Phe Pro Gln Tyr Val Ser
        275                 280                 285

Asn Pro Val Pro Asp Asn Ala Ile Thr Leu His Arg Met Ser Gly Tyr
    290                 295                 300

Leu Ala Arg Trp Ile Ala Asp Thr Cys Lys Ala Ser Val Leu Lys Leu
305                 310                 315                 320

Ala Glu Ala Ser Ala Gln Ile Val Met Pro Leu Ala Glu Val Lys Gly
                325                 330                 335

Cys Thr Trp Ala Asp Gly Tyr Thr Met Tyr Leu Gly Phe Ala Pro Gly
            340                 345                 350
```

```
Ala Glu Met Phe Leu Asp Ala Phe Asp Phe Tyr Pro Leu Val Ile Glu
        355                 360                 365

Met His Arg Val Leu Lys Asp Asn Met Asp Val Asn Phe Met Lys Lys
    370                 375                 380

Val Leu Arg Gln Arg Tyr Gly Thr Met Thr Ala Glu Glu Trp Met Thr
385                 390                 395                 400

Gln Lys Ile Pro Glu Ile Lys Ala Ala Phe Asn Ser Val Gly Gln Leu
                405                 410                 415

Ala Trp Ala Lys Ser Gly Phe Ser Pro Ala Ala Arg Thr Phe Leu Gln
            420                 425                 430

Gln Phe Gly Ile Asn Ile Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro
        435                 440                 445

Gly Gly Gly Ser His His His His His His
    450                 455
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<210> 85
<211> 1991
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP -SNAPlike-proTEV cleavage site-N protein from human betacoronavirus strain 2cEMC/2012- Histag for expression in S2 cells

<400> 85

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tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag      120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact      180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc      240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa      300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc      360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct      420

gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct      480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat      540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag      600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac      660

ctgtacttcc agagcgatat cgcatcccct gctgcacctc gtgctgtttc ctttgccgat      720

aacaatgata taacaaatac aaacctatct cgaggtagag gacgtaatcc aaaaccacga      780

gctgcaccaa ataacactgt ctcttggtac actgggctta cccaacacgg gaaagtccct      840
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cttacctttc cacctgggca gggtgtacct cttaatgcca attctacccc tgcgcaaaat 900

gctgggtatt ggcggagaca ggacagaaaa attaataccg ggaatggaat taagcaactg 960

gctcccaggt ggtacttcta ctacactgga actggacccg aagcagcact cccattccgg 1020

gctgttaagg atggcatcgt ttgggtccat gaagatggcg ccactgatgc tccttcaact 1080

tttgggacgc ggaaccctaa caatgattca gctattgtta cacaattcgc gcccggtact 1140

aaacttccta aaaacttcca cattgagggg actggaggca atagtcaatc atcttcaaga 1200

gcctctagct taagcagaaa ctcttccagg tctagttcac aaggttcaag atcaggaaac 1260

tctacccgcg gcacttctcc aggtccatct ggaatcggag cagtaggagg tgatctactt 1320

taccttgatc ttctgaacag actacaagcc cttgagtctg gcaaagtaaa gcaatcgcag 1380

ccaaaagtaa tcactaagaa agatgctgct gctgctaaaa ataagatgcg ccacaagcgc 1440

acttccacca aaagtttcaa catggtgcag gcttttggtc ttcgcggacc aggagacctc 1500

cagggaaact ttggtgatct tcaattgaat aaactcggca ctgaggaccc acgttggccc 1560

caaattgctg agcttgctcc tacagccagt gcttttatgg gtatgtcgca atttaaactt 1620

acccatcaga acaatgatga tcatggcaac cctgtgtact tccttcggta cagtggagcc 1680

attaaacttg acccaaagaa tcccaactac aataagtggt tggagcttct tgagcaaaat 1740

attgatgcct acaaaacctt ccctaagaag gaaaagaaac aaaaggcacc aaaagaagaa 1800

tcaacagacc aaatgtctga acctccaaag gagcagcgtg tgcaaggtag catcactcag 1860

cgcactcgca cccgtccaag tgttcagcct ggtccaatga ttgatgttaa cactgatggc 1920

ccgggagaga atctatattt tcaagggccc ggcggaggta gtcaccatca tcaccatcac 1980

taatgaccgg t 1991

<210> 86
<211> 639
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-proTEV-COV N protein-Histag]

<400> 86

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

```
Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala Ser
        195                 200                 205

Pro Ala Ala Pro Arg Ala Val Ser Phe Ala Asp Asn Asn Asp Ile Thr
    210                 215                 220

Asn Thr Asn Leu Ser Arg Gly Arg Gly Arg Asn Pro Lys Pro Arg Ala
225                 230                 235                 240

Ala Pro Asn Asn Thr Val Ser Trp Tyr Thr Gly Leu Thr Gln His Gly
                245                 250                 255

Lys Val Pro Leu Thr Phe Pro Pro Gly Gln Gly Val Pro Leu Asn Ala
            260                 265                 270

Asn Ser Thr Pro Ala Gln Asn Ala Gly Tyr Trp Arg Arg Gln Asp Arg
        275                 280                 285

Lys Ile Asn Thr Gly Asn Gly Ile Lys Gln Leu Ala Pro Arg Trp Tyr
    290                 295                 300

Phe Tyr Tyr Thr Gly Thr Gly Pro Glu Ala Ala Leu Pro Phe Arg Ala
305                 310                 315                 320

Val Lys Asp Gly Ile Val Trp Val His Glu Asp Gly Ala Thr Asp Ala
                325                 330                 335
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Pro Ser Thr Phe Gly Thr Arg Asn Pro Asn Asn Asp Ser Ala Ile Val
            340                 345                 350

Thr Gln Phe Ala Pro Gly Thr Lys Leu Pro Lys Asn Phe His Ile Glu
        355                 360                 365

Gly Thr Gly Gly Asn Ser Gln Ser Ser Ser Arg Ala Ser Ser Leu Ser
    370                 375                 380

Arg Asn Ser Ser Arg Ser Ser Ser Gln Gly Ser Arg Ser Gly Asn Ser
385                 390                 395                 400

Thr Arg Gly Thr Ser Pro Gly Pro Ser Gly Ile Gly Ala Val Gly Gly
                405                 410                 415

Asp Leu Leu Tyr Leu Asp Leu Leu Asn Arg Leu Gln Ala Leu Glu Ser
            420                 425                 430

Gly Lys Val Lys Gln Ser Gln Pro Lys Val Ile Thr Lys Lys Asp Ala
        435                 440                 445

Ala Ala Ala Lys Asn Lys Met Arg His Lys Arg Thr Ser Thr Lys Ser
    450                 455                 460

Phe Asn Met Val Gln Ala Phe Gly Leu Arg Gly Pro Gly Asp Leu Gln
465                 470                 475                 480

Gly Asn Phe Gly Asp Leu Gln Leu Asn Lys Leu Gly Thr Glu Asp Pro
                485                 490                 495

Arg Trp Pro Gln Ile Ala Glu Leu Ala Pro Thr Ala Ser Ala Phe Met
            500                 505                 510

Gly Met Ser Gln Phe Lys Leu Thr His Gln Asn Asn Asp Asp His Gly
        515                 520                 525

Asn Pro Val Tyr Phe Leu Arg Tyr Ser Gly Ala Ile Lys Leu Asp Pro
    530                 535                 540

Lys Asn Pro Asn Tyr Asn Lys Trp Leu Glu Leu Leu Glu Gln Asn Ile
545                 550                 555                 560

Asp Ala Tyr Lys Thr Phe Pro Lys Lys Glu Lys Lys Gln Lys Ala Pro
                565                 570                 575

Lys Glu Glu Ser Thr Asp Gln Met Ser Glu Pro Pro Lys Glu Gln Arg
            580                 585                 590

Val Gln Gly Ser Ile Thr Gln Arg Thr Arg Thr Arg Pro Ser Val Gln
        595                 600                 605
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Pro Gly Pro Met Ile Asp Val Asn Thr Asp Gly Pro Gly Glu Asn Leu
610 615 620

Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His His His
625 630 635

<210> 87
<211> 4465
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- S protein from human betacoronavirus strain 2cEMC/2012- SNAPlike - Histag for expression in S2 cells

<400> 87
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gctactatac gaaaaattta ccctgctttt atgctgggtt cttcagttgg taatttctca 480

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catcatcatc attgatgacg ggccc 4465

<210> 88
<211> 1463
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [huCOV.S protein -SNAPlike-Histag]

<400> 88

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20 25 30

Ser Lys Ala Asp Gly Ile Ile Tyr Pro Gln Gly Arg Thr Tyr Ser Asn
35 40 45

Ile Thr Ile Thr Tyr Gln Gly Leu Phe Pro Tyr Gln Gly Asp His Gly
50 55 60

Asp Met Tyr Val Tyr Ser Ala Gly His Ala Thr Gly Thr Thr Pro Gln
65 70 75 80

Lys Leu Phe Val Ala Asn Tyr Ser Gln Asp Val Lys Gln Phe Ala Asn
85 90 95

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Gly Phe Val Val Arg Ile Gly Ala Ala Asn Ser Thr Gly Thr Val
            100                 105                 110

Ile Ile Ser Pro Ser Thr Ser Ala Thr Ile Arg Lys Ile Tyr Pro Ala
        115                 120                 125

Phe Met Leu Gly Ser Ser Val Gly Asn Phe Ser Asp Gly Lys Met Gly
    130                 135                 140

Arg Phe Phe Asn His Thr Leu Val Leu Leu Pro Asp Gly Cys Gly Thr
145                 150                 155                 160

Leu Leu Arg Ala Phe Tyr Cys Ile Leu Glu Pro Arg Ser Gly Asn His
                165                 170                 175

Cys Pro Ala Gly Asn Ser Tyr Thr Ser Phe Ala Thr Tyr His Thr Pro
            180                 185                 190

Ala Thr Asp Cys Ser Asp Gly Asn Tyr Asn Arg Asn Ala Ser Leu Asn
        195                 200                 205

Ser Phe Lys Glu Tyr Phe Asn Leu Arg Asn Cys Thr Phe Met Tyr Thr
    210                 215                 220

Tyr Asn Ile Thr Glu Asp Glu Ile Leu Glu Trp Phe Gly Ile Thr Gln
225                 230                 235                 240

Thr Ala Gln Gly Val His Leu Phe Ser Ser Arg Tyr Val Asp Leu Tyr
                245                 250                 255

Gly Gly Asn Met Phe Gln Phe Ala Thr Leu Pro Val Tyr Asp Thr Ile
            260                 265                 270

Lys Tyr Tyr Ser Ile Ile Pro His Ser Ile Arg Ser Ile Gln Ser Asp
        275                 280                 285

Arg Lys Ala Trp Ala Ala Phe Tyr Val Tyr Lys Leu Gln Pro Leu Thr
    290                 295                 300

Phe Leu Leu Asp Phe Ser Val Asp Gly Tyr Ile Arg Arg Ala Ile Asp
305                 310                 315                 320

Cys Gly Phe Asn Asp Leu Ser Gln Leu His Cys Ser Tyr Glu Ser Phe
                325                 330                 335

Asp Val Glu Ser Gly Val Tyr Ser Val Ser Ser Phe Glu Ala Lys Pro
            340                 345                 350

Ser Gly Ser Val Val Glu Gln Ala Glu Gly Val Glu Cys Asp Phe Ser
        355                 360                 365
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Pro Leu Leu Ser Gly Thr Pro Pro Gln Val Tyr Asn Phe Lys Arg Leu
    370                 375                 380

Val Phe Thr Asn Cys Asn Tyr Asn Leu Thr Lys Leu Leu Ser Leu Phe
385                 390                 395                 400

Ser Val Asn Asp Phe Thr Cys Ser Gln Ile Ser Pro Ala Ala Ile Ala
                405                 410                 415

Ser Asn Cys Tyr Ser Ser Leu Ile Leu Asp Tyr Phe Ser Tyr Pro Leu
            420                 425                 430

Ser Met Lys Ser Asp Leu Ser Val Ser Ser Ala Gly Pro Ile Ser Gln
        435                 440                 445

Phe Asn Tyr Lys Gln Ser Phe Ser Asn Pro Thr Cys Leu Ile Leu Ala
    450                 455                 460

Thr Val Pro His Asn Leu Thr Thr Ile Thr Lys Pro Leu Lys Tyr Ser
465                 470                 475                 480

Tyr Ile Asn Lys Cys Ser Arg Leu Leu Ser Asp Asp Arg Thr Glu Val
                485                 490                 495

Pro Gln Leu Val Asn Ala Asn Gln Tyr Ser Pro Cys Val Ser Ile Val
            500                 505                 510

Pro Ser Thr Val Trp Glu Asp Gly Asp Tyr Tyr Arg Lys Gln Leu Ser
        515                 520                 525

Pro Leu Glu Gly Gly Gly Trp Leu Val Ala Ser Gly Ser Thr Val Ala
    530                 535                 540

Met Thr Glu Gln Leu Gln Met Gly Phe Gly Ile Thr Val Gln Tyr Gly
545                 550                 555                 560

Thr Asp Thr Asn Ser Val Cys Pro Lys Leu Glu Phe Ala Asn Asp Thr
                565                 570                 575

Lys Ile Ala Ser Gln Leu Gly Asn Cys Val Glu Tyr Ser Leu Tyr Gly
            580                 585                 590

Val Ser Gly Arg Gly Val Phe Gln Asn Cys Thr Ala Val Gly Val Arg
        595                 600                 605

Gln Gln Arg Phe Val Tyr Asp Ala Tyr Gln Asn Leu Val Gly Tyr Tyr
    610                 615                 620

Ser Asp Asp Gly Asn Tyr Tyr Cys Leu Arg Ala Cys Val Ser Val Pro
625                 630                 635                 640
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Val Ser Val Ile Tyr Asp Lys Glu Thr Lys Thr His Ala Thr Leu Phe
                645                 650                 655

Gly Ser Val Ala Cys Glu His Ile Ser Ser Thr Met Ser Gln Tyr Ser
            660                 665                 670

Arg Ser Thr Arg Ser Met Leu Lys Arg Arg Asp Ser Thr Tyr Gly Pro
        675                 680                 685

Leu Gln Thr Pro Val Gly Cys Val Leu Gly Leu Val Asn Ser Ser Leu
    690                 695                 700

Phe Val Glu Asp Cys Lys Leu Pro Leu Gly Gln Ser Leu Cys Ala Leu
705                 710                 715                 720

Pro Asp Thr Pro Ser Thr Leu Thr Pro Arg Ser Val Arg Ser Val Pro
                725                 730                 735

Gly Glu Met Arg Leu Ala Ser Ile Ala Phe Asn His Pro Ile Gln Val
            740                 745                 750

Asp Gln Leu Asn Ser Ser Tyr Phe Lys Leu Ser Ile Pro Thr Asn Phe
        755                 760                 765

Ser Phe Gly Val Thr Gln Glu Tyr Ile Gln Thr Thr Ile Gln Lys Val
    770                 775                 780

Thr Val Asp Cys Lys Gln Tyr Val Cys Asn Gly Phe Gln Lys Cys Glu
785                 790                 795                 800

Gln Leu Leu Arg Glu Tyr Gly Gln Phe Cys Ser Lys Ile Asn Gln Ala
                805                 810                 815

Leu His Gly Ala Asn Leu Arg Gln Asp Asp Ser Val Arg Asn Leu Phe
            820                 825                 830

Ala Ser Val Lys Ser Ser Gln Ser Ser Pro Ile Ile Pro Gly Phe Gly
        835                 840                 845

Gly Asp Phe Asn Leu Thr Leu Leu Glu Pro Val Ser Ile Ser Thr Gly
    850                 855                 860

Ser Arg Ser Ala Arg Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys Val
865                 870                 875                 880

Thr Ile Ala Asp Pro Gly Tyr Met Gln Gly Tyr Asp Asp Cys Met Gln
                885                 890                 895

Gln Gly Pro Ala Ser Ala Arg Asp Leu Ile Cys Ala Gln Tyr Val Ala
            900                 905                 910
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Gly Tyr Lys Val Leu Pro Pro Leu Met Asp Val Asn Met Glu Ala Ala
        915                 920                 925

Tyr Thr Ser Ser Leu Leu Gly Ser Ile Ala Gly Val Gly Trp Thr Ala
    930                 935                 940

Gly Leu Ser Ser Phe Ala Ala Ile Pro Phe Ala Gln Ser Ile Phe Tyr
945                 950                 955                 960

Arg Leu Asn Gly Val Gly Ile Thr Gln Gln Val Leu Ser Glu Asn Gln
                965                 970                 975

Lys Leu Ile Ala Asn Lys Phe Asn Gln Ala Leu Gly Ala Met Gln Thr
            980                 985                 990

Gly Phe Thr Thr Thr Asn Glu Ala  Phe Gln Lys Val Gln  Asp Ala Val
        995                 1000                1005

Asn Asn  Asn Ala Gln Ala Leu  Ser Lys Leu Ala Ser  Glu Leu Ser
    1010                1015                1020

Asn Thr  Phe Gly Ala Ile Ser  Ala Ser Ile Gly Asp  Ile Ile Gln
    1025                1030                1035

Arg Leu  Asp Val Leu Glu Gln  Asp Ala Gln Ile Asp  Arg Leu Ile
    1040                1045                1050

Asn Gly  Arg Leu Thr Thr Leu  Asn Ala Phe Val Ala  Gln Gln Leu
    1055                1060                1065

Val Arg  Ser Glu Ser Ala Ala  Leu Ser Ala Gln Leu  Ala Lys Asp
    1070                1075                1080

Lys Val  Asn Glu Cys Val Lys  Ala Gln Ser Lys Arg  Ser Gly Phe
    1085                1090                1095

Cys Gly  Gln Gly Thr His Ile  Val Ser Phe Val Val  Asn Ala Pro
    1100                1105                1110

Asn Gly  Leu Tyr Phe Met His  Val Gly Tyr Tyr Pro  Ser Asn His
    1115                1120                1125

Ile Glu  Val Val Ser Ala Tyr  Gly Leu Cys Asp Ala  Ala Asn Pro
    1130                1135                1140

Thr Asn  Cys Ile Ala Pro Val  Asn Gly Tyr Phe Ile  Lys Thr Asn
    1145                1150                1155

Asn Thr  Arg Ile Val Asp Glu  Trp Ser Tyr Thr Gly  Ser Ser Phe
    1160                1165                1170
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Tyr Ala  Pro Glu Pro Ile Thr  Ser Leu Asn Thr Lys  Tyr Val Ala
    1175                1180                1185

Pro Gln  Val Thr Tyr Gln Asn  Ile Ser Thr Asn Leu  Pro Pro Pro
    1190                1195                1200

Leu Leu  Gly Asn Ser Thr Gly  Ile Asp Phe Gln Asp  Glu Leu Asp
    1205                1210                1215

Glu Phe  Phe Lys Asn Val Ser  Thr Ser Ile Pro Asn  Phe Gly Ser
    1220                1225                1230

Leu Thr  Gln Ile Asn Thr Thr  Leu Leu Asp Leu Thr  Tyr Glu Met
    1235                1240                1245

Leu Ser  Leu Gln Gln Val Val  Lys Ala Leu Lys Arg  Pro His Gly
    1250                1255                1260

Gly Gly  Ser Lys Asp Cys Glu  Met Lys Arg Thr Thr  Leu Asp Ser
    1265                1270                1275

Pro Leu  Gly Lys Leu Glu Leu  Ser Gly Cys Glu Gln  Gly Leu His
    1280                1285                1290

Glu Ile  Lys Leu Leu Gly Lys  Gly Thr Ser Ala Ala  Asp Ala Val
    1295                1300                1305

Glu Val  Pro Ala Pro Ala Ala  Val Leu Gly Gly Pro  Glu Pro Leu
    1310                1315                1320

Met Gln  Ala Thr Ala Trp Leu  Asn Ala Tyr Phe His  Gln Pro Glu
    1325                1330                1335

Ala Ile  Glu Glu Phe Pro Val  Pro Ala Leu His His  Pro Val Phe
    1340                1345                1350

Gln Gln  Glu Ser Phe Thr Arg  Gln Val Leu Trp Lys  Leu Leu Lys
    1355                1360                1365

Val Val  Lys Phe Gly Glu Val  Ile Ser Tyr Gln Gln  Leu Ala Ala
    1370                1375                1380

Leu Ala  Gly Asn Pro Ala Ala  Thr Ala Ala Val Lys  Thr Ala Leu
    1385                1390                1395

Ser Gly  Asn Pro Val Pro Ile  Leu Ile Pro Cys His  Arg Val Val
    1400                1405                1410

Ser Ser  Ser Gly Ala Val Gly  Gly Tyr Glu Gly Gly  Leu Ala Val
    1415                1420                1425
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Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly Lys Pro
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Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu His
1445 1450 1455

His His His His His
1460

<210> 89
<211> 1325
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP - SNAPlike -proTEV- C protein from hepatitis C virus strain TCHM-R2/03 of genotype 1b - -proTEV - Histag for expression in S2 cells

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ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact 180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc 240

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tacctattgc cgcgaagagg tcctcgttta ggtgttcgag caactagaaa aacttctgaa 840

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ccggt 1325

<210> 90
<211> 417
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-proTEV-C protein of HCV-proTEV-Histag]

<400> 90

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Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ser Thr
        195                 200                 205

Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro
    210                 215                 220
```

```
Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr
225                 230                 235                 240

Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys
                245                 250                 255

Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys
            260                 265                 270

Ala Arg Gln Pro Glu Gly Arg Ala Trp Ala Gln Pro Gly Tyr Pro Trp
        275                 280                 285

Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp Ala Gly Trp Leu Leu Ser
    290                 295                 300

Pro Arg Gly Ser Arg Pro Ser Trp Gly Ala Asn Asp Pro Arg Arg Arg
305                 310                 315                 320

Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala
                325                 330                 335

Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala
            340                 345                 350

Ala Arg Val Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn
        355                 360                 365

Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu
    370                 375                 380

Ala Leu Leu Ser Cys Leu Thr Ile Pro Ala Ser Ala Gly Pro Gly Glu
385                 390                 395                 400

Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His His
                405                 410                 415

His
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<210> 91
<211> 1376
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike - MSP1(19) antigen from Plasmodium falciparum - proTEV - AMA-1(III) antigen from Plasmodium falciparum - Histag for expression in S2 cells

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ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact 180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc 240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa 300

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cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac 660

ctgtacttcc agagcgatat caaacaatgt ccacaaaatt ctggatgttt cagacattta 720

gatgaaagag aagaatgtaa atgtttatta aattacaaac aagaaggtga taaatgtgtt 780

gaaaatccaa atcttacttg taacgaaaat aatggtggat gtgatgcaga tgccaaatgt 840

accgaagaag attcaggcag caacggaaag aaaatcacat gtgaatgtac taaacctgat 900

tcttatccac ttttcgatgg tattttcgga ggtggctctg agaatctata ttttcaaggg 960

cccggtggag gcgaagttga aaacaatttt ccatgttcat tatataaaga tgaaataatg 1020

aaagaaatcg aaagagaatc aaaacgaatt aaattaaatg ataatgatga tgaagggaat 1080

aaaaaaatta tagctccaag aatttttatt tcagatgata aagacagttt aaaatgccca 1140

tgtgaccctg aaatggtaag taatagtaca tgtcgtttct ttgtatgtaa atgtgtagaa 1200

agaagggcag aagtaacatc aaataatgaa gttgtagtta aagaagaata taaagatgaa 1260

tatgcagata ttcctgaaca taaaccaact tatgataaaa tgctcccggg agagaatcta 1320

tattttcaag ggcccggcgg aggtagtcac catcatcacc atcactaatg accggt 1376

<210> 92
<211> 434
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-MSP1-proTEV-AMA1-Histag]

<400> 92

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

```
Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Lys Gln
        195                 200                 205

Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp Glu Arg Glu Glu
    210                 215                 220

Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val Glu
225                 230                 235                 240

Asn Pro Asn Leu Thr Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala Asp
                245                 250                 255

Ala Lys Cys Thr Glu Glu Asp Ser Gly Ser Asn Gly Lys Lys Ile Thr
            260                 265                 270

Cys Glu Cys Thr Lys Pro Asp Ser Tyr Pro Leu Phe Asp Gly Ile Phe
        275                 280                 285

Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Glu
    290                 295                 300

Val Glu Asn Asn Phe Pro Cys Ser Leu Tyr Lys Asp Glu Ile Met Lys
305                 310                 315                 320

Glu Ile Glu Arg Glu Ser Lys Arg Ile Lys Leu Asn Asp Asn Asp Asp
                325                 330                 335
```

```
Glu Gly Asn Lys Lys Ile Ile Ala Pro Arg Ile Phe Ile Ser Asp Asp
            340                 345                 350

Lys Asp Ser Leu Lys Cys Pro Cys Asp Pro Glu Met Val Ser Asn Ser
        355                 360                 365

Thr Cys Arg Phe Phe Val Cys Lys Cys Val Glu Arg Arg Ala Glu Val
    370                 375                 380

Thr Ser Asn Asn Glu Val Val Val Lys Glu Glu Tyr Lys Asp Glu Tyr
385                 390                 395                 400

Ala Asp Ile Pro Glu His Lys Pro Thr Tyr Asp Lys Met Leu Pro Gly
                405                 410                 415

Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His
            420                 425                 430

His His
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<210> 93
<211> 2195
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike -proTEV- modified short form of HbpA from Leptospira interrogans serovar Lai str.56601 - proTEV- Histag for expression in S2 cells

<400> 93

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atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga       60

tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag      120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact      180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc      240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa      300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc      360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct      420

gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct      480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat      540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag      600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac      660

ctgtacttcc agagcgatat cttcaacacc acggccaaca tgggcttcag gaacgagtac      720

gtgagcggcg cggtgtccgc aggttacaat aagaaccccg gctacaggtt ggtcccaaac      780

tctcaggcga ctactgggaa cgcctatcag gacttgaaca cgggcatcaa cctgaccttc      840
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aacccggacg gcaagttcaa ggggaagacg aggattctct accagcacag ggaccagaac 900

ggggtggacg tgacccagtc caaggccgtc ttcgaccgga acaacaagac gcacgacttc 960

ttggcgacgg ggtcgttgga gtacgggtta gggaagagga acttgatctc cttcaggggg 1020

aacatctcca agtgggagaa caagtactac aacaaccaga gggggtcgga cgagttggac 1080

gtgaagcagt tgaactcgga gttgacgtcg caggggaccg tgcagttgga catggaggcc 1140

tctgagaagc acttcatcac tgtaggtgcg gagtccttcg cgaacgagtt ggagtcggac 1200

cgcttgcaga gcaggtacgt gtacaggacg aggaaggcgg tgttcttcca ggacgagtgg 1260

accgtgtccc ggtcgccgag gattcgggtg gtgccaggag tgaggtacga cgacgactcg 1320

cagttcggga accagacgac gccgaagctg gcggcccggt acgacatatt gcagaacttg 1380

gtgtggaggg cgagctacgg gaggggatta cggccgccga gcttgcagga gttgtacctg 1440

cggttcgaga acccggccgt gggttacgtg gtggagggta acccgaactt gaagccggag 1500

cggtcgatca cgatcaactc ggacttggag tacagcccgt tcagcttctt gacgttctcc 1560

ttgagcgtgt accggaacga catcatcaac ctgatccagt acaagttcga ctcgaacaag 1620

gggagggagt tcgcggagtt ccagctgcag aacatcgcga aggcgtacac gagaggagga 1680

gagttcggcg tgcagtacag gttcttgaag tacttcacgc tggagttggg gtacaaccac 1740

acggacacga gggacctgag ctcggacagg ccgttggagg gcagggcgct gcaccaggcg 1800

tcggcgaact tcatctacaa ctcgcccgga ggattccaat tcaacctgag gggcaagcac 1860

ttggacaaga ggccgttcta cagctcgacc aacaacctgt cggcggccgg acaggactac 1920

atccccagcg aggtgaagtt gaacgagaac ccgcccgtga tctacgggaa gccgttcacg 1980

atcttgaacg tgaggatcga gcagaagttc ttcaacaagc acttcgcgct gttcttgggc 2040

gtggacaact tgctcaacca gtacgagctg gcgtacaacc ccacgcggcc gaggttctac 2100

tacggcggct tctcggccca gttcccggga gagaatctat attttcaagg gcccggcgga 2160

ggtagtcacc atcatcacca tcactaatga ccggt 2195

<210> 94
<211> 707
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV-HbPA1-proTEV-Histag]

<400> 94

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro

35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Phe Asn
195 200 205

Thr Thr Ala Asn Met Gly Phe Arg Asn Glu Tyr Val Ser Gly Ala Val
210 215 220

Ser Ala Gly Tyr Asn Lys Asn Pro Gly Tyr Arg Leu Val Pro Asn Ser
225 230 235 240

Gln Ala Thr Thr Gly Asn Ala Tyr Gln Asp Leu Asn Thr Gly Ile Asn
245 250 255

Leu Thr Phe Asn Pro Asp Gly Lys Phe Lys Gly Lys Thr Arg Ile Leu
260 265 270

Tyr Gln His Arg Asp Gln Asn Gly Val Asp Val Thr Gln Ser Lys Ala
275 280 285

Val Phe Asp Arg Asn Asn Lys Thr His Asp Phe Leu Ala Thr Gly Ser
290 295 300

Leu Glu Tyr Gly Leu Gly Lys Arg Asn Leu Ile Ser Phe Arg Gly Asn

305 310 315 320

Ile Ser Lys Trp Glu Asn Lys Tyr Tyr Asn Asn Gln Arg Gly Ser Asp
325 330 335

Glu Leu Asp Val Lys Gln Leu Asn Ser Glu Leu Thr Ser Gln Gly Thr
340 345 350

Val Gln Leu Asp Met Glu Ala Ser Glu Lys His Phe Ile Thr Val Gly
355 360 365

Ala Glu Ser Phe Ala Asn Glu Leu Glu Ser Asp Arg Leu Gln Ser Arg
370 375 380

Tyr Val Tyr Arg Thr Arg Lys Ala Val Phe Phe Gln Asp Glu Trp Thr
385 390 395 400

Val Ser Arg Ser Pro Arg Ile Arg Val Val Pro Gly Val Arg Tyr Asp
405 410 415

Asp Asp Ser Gln Phe Gly Asn Gln Thr Thr Pro Lys Leu Ala Ala Arg
420 425 430

Tyr Asp Ile Leu Gln Asn Leu Val Trp Arg Ala Ser Tyr Gly Arg Gly
435 440 445

Leu Arg Pro Pro Ser Leu Gln Glu Leu Tyr Leu Arg Phe Glu Asn Pro
450 455 460

Ala Val Gly Tyr Val Val Glu Gly Asn Pro Asn Leu Lys Pro Glu Arg
465 470 475 480

Ser Ile Thr Ile Asn Ser Asp Leu Glu Tyr Ser Pro Phe Ser Phe Leu
485 490 495

Thr Phe Ser Leu Ser Val Tyr Arg Asn Asp Ile Ile Asn Leu Ile Gln
500 505 510

Tyr Lys Phe Asp Ser Asn Lys Gly Arg Glu Phe Ala Glu Phe Gln Leu
515 520 525

Gln Asn Ile Ala Lys Ala Tyr Thr Arg Gly Gly Glu Phe Gly Val Gln
530 535 540

Tyr Arg Phe Leu Lys Tyr Phe Thr Leu Glu Leu Gly Tyr Asn His Thr
545 550 555 560

Asp Thr Arg Asp Leu Ser Ser Asp Arg Pro Leu Glu Gly Arg Ala Leu
565 570 575

His Gln Ala Ser Ala Asn Phe Ile Tyr Asn Ser Pro Gly Gly Phe Gln

580 585 590

Phe Asn Leu Arg Gly Lys His Leu Asp Lys Arg Pro Phe Tyr Ser Ser
595 600 605

Thr Asn Asn Leu Ser Ala Ala Gly Gln Asp Tyr Ile Pro Ser Glu Val
610 615 620

Lys Leu Asn Glu Asn Pro Pro Val Ile Tyr Gly Lys Pro Phe Thr Ile
625 630 635 640

Leu Asn Val Arg Ile Glu Gln Lys Phe Phe Asn Lys His Phe Ala Leu
645 650 655

Phe Leu Gly Val Asp Asn Leu Leu Asn Gln Tyr Glu Leu Ala Tyr Asn
660 665 670

Pro Thr Arg Pro Arg Phe Tyr Tyr Gly Gly Phe Ser Ala Gln Phe Pro
675 680 685

Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His
690 695 700

His His His
705

<210> 95
<211> 968
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike -proTEV- MUB40 - proTEV-Histag for expression in S2 cells

<400> 95
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga 60

tcttcgcgag ctagcaccat gaaactatgt attctacttg cagttgttgc gttcgtagga 120

ttgtccttac ctacagctct ggcaagatct gacaaagact gcgaaatgaa aagaactaca 180

ttggattcac cacttgggaa gttggaactg agtggatgcg agcaaggatt gcatgaaatt 240

aagctactgg gaaaaggaac ttctgctgct gatgcagttg aagttccagc accagcagct 300

gttcttggag gtcctgagcc cctcatgcaa gccacagcct ggcttaacgc atatttccac 360

cagcctgagg ccattgagga atttccagtc cccgcccttc accatcctgt gtttcagcag 420

gagagcttca cccgccaggt cctgtggaaa ttgctgaagg tggtcaagtt tggtgaagtg 480

atttcatatc agcaacttgc tgcattggcc ggtaaccccg cagctacagc tgccgtgaaa 540

actgctctca gcggaaatcc tgtgcccatc ctgatccctt gtcacagagt cgtttcatct 600

tccggagctg taggtggcta tgaaggagga ctggcagtta aggagtggct gctggctcat 660

gaaggtcata gacttggaaa gcctgggctg ggtcctgctg gtataggcgc gccagggtcc 720

ctaggtggcg gatccgaaaa cctgtacttc cagagcgata tcacggctga aggcatcaag 780

aagtttgaag gcgacggtta tgaactgttc aaggacaact tcccagctgg tgagaagttc 840

gataacgatg acaccaacga tcaattctac acggtaatct tcaagcacca tcgtggcccg 900

ggagagaatc tatattttca agggcccggc ggaggtagtc accatcatca ccatcactaa 960

tgaccggt 968

<210> 96
<211> 298
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV-MUB40-proTEV-Histag]

<400> 96

Ser Arg Ala Ser Thr Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala
1 5 10 15

Phe Val Gly Leu Ser Leu Pro Thr Ala Leu Ala Arg Ser Asp Lys Asp
20 25 30

Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu
35 40 45

Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu Leu Gly Lys
50 55 60

Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro Ala Ala Val
65 70 75 80

Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp Leu Asn Ala
85 90 95

Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val Pro Ala Leu
100 105 110

His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln Val Leu Trp
115 120 125

Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln
130 135 140

Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala Val Lys Thr
145 150 155 160

Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys His Arg Val
165 170 175

Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly Leu Ala Val

180 185 190

Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly Lys Pro Gly
195 200 205

Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Gly Gly Gly Ser
210 215 220

Glu Asn Leu Tyr Phe Gln Ser Asp Ile Thr Ala Glu Gly Ile Lys Lys
225 230 235 240

Phe Glu Gly Asp Gly Tyr Glu Leu Phe Lys Asp Asn Phe Pro Ala Gly
245 250 255

Glu Lys Phe Asp Asn Asp Asp Thr Asn Asp Gln Phe Tyr Thr Val Ile
260 265 270

Phe Lys His His Arg Gly Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro
275 280 285

Gly Gly Gly Ser His His His His His His
290 295

<210> 97
<211> 1238
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike -proTEV- soluble form of mouse C-type like lectin (CLEC5A) - proTEV- Histag for expression in S2 cells

<400> 97
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga 60

tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag 120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact 180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc 240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa 300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc 360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct 420

gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct 480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat 540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag 600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac 660

ctgtacttcc agagcgatat cgtttttggc aaaagtaatg atggcttcgt ccccacggag 720

agctacggaa ccactagtgt gcagaatgtc tcacaaatct ttgggagaaa tgacgaaagt 780

accatgccta caaggagcta tggaacagtc tgtcccagaa actgggattt tcaccaagga 840

aaatgctttt tcttctcctt ctccgaatca ccttggaaag acagcatgga ttattgtgca 900

acacaagggt ccacactggc aattgtcaac actccagaga aactgaagta tcttcaggac 960

atagctggta ttgagaatta ctttattggt ttggtacgtc agcctggaga gaaaaagtgg 1020

cgctggatca acaactctgt gttcaatggc aatgttacca atcaggacca gaacttcgac 1080

tgtgtcacta taggtctgac gaagacatat gatgctgcat catgtgaagt cagctatcgc 1140

tggatctgcg aaatgaatgc caaaggcccg ggagagaatc tatattttca agggcccggc 1200

ggaggtagtc accatcatca ccatcactaa tgaccggt 1238

<210> 98
<211> 388
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV-moCLEC5A-proTEV-Histag]

<400> 98

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

```
Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Val Phe
        195                 200                 205

Gly Lys Ser Asn Asp Gly Phe Val Pro Thr Glu Ser Tyr Gly Thr Thr
    210                 215                 220

Ser Val Gln Asn Val Ser Gln Ile Phe Gly Arg Asn Asp Glu Ser Thr
225                 230                 235                 240

Met Pro Thr Arg Ser Tyr Gly Thr Val Cys Pro Arg Asn Trp Asp Phe
                245                 250                 255

His Gln Gly Lys Cys Phe Phe Phe Ser Phe Ser Glu Ser Pro Trp Lys
            260                 265                 270

Asp Ser Met Asp Tyr Cys Ala Thr Gln Gly Ser Thr Leu Ala Ile Val
        275                 280                 285

Asn Thr Pro Glu Lys Leu Lys Tyr Leu Gln Asp Ile Ala Gly Ile Glu
    290                 295                 300

Asn Tyr Phe Ile Gly Leu Val Arg Gln Pro Gly Glu Lys Lys Trp Arg
305                 310                 315                 320

Trp Ile Asn Asn Ser Val Phe Asn Gly Asn Val Thr Asn Gln Asp Gln
                325                 330                 335

Asn Phe Asp Cys Val Thr Ile Gly Leu Thr Lys Thr Tyr Asp Ala Ala
            340                 345                 350

Ser Cys Glu Val Ser Tyr Arg Trp Ile Cys Glu Met Asn Ala Lys Gly
        355                 360                 365

Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His
    370                 375                 380

His His His His
385
```

<210> 99
<211> 1229
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- SNAPlike -proTEV- soluble form of human C-type like lectin (CLEC5A) - proTEV- Histag for expression

in S2 cells

<400> 99
atgaagttat gcatattact ggccgtcgtg gtggcctttg ttggcctctc gctcgggaga 60

tctagatctg acaaagactg cgaaatgaaa agaactacat tggattcacc acttgggaag 120

ttggaactga gtggatgcga gcaaggattg catgaaatta agctactggg aaaaggaact 180

tctgctgctg atgcagttga agttccagca ccagcagctg ttcttggagg tcctgagccc 240

ctcatgcaag ccacagcctg gcttaacgca tatttccacc agcctgaggc cattgaggaa 300

tttccagtcc ccgcccttca ccatcctgtg tttcagcagg agagcttcac ccgccaggtc 360

ctgtggaaat tgctgaaggt ggtcaagttt ggtgaagtga tttcatatca gcaacttgct 420

gcattggccg gtaaccccgc agctacagct gccgtgaaaa ctgctctcag cggaaatcct 480

gtgcccatcc tgatcccttg tcacagagtc gtttcatctt ccggagctgt aggtggctat 540

gaaggaggac tggcagttaa ggagtggctg ctggctcatg aaggtcatag acttggaaag 600

cctgggctgg gtcctgctgg tataggcgcg ccagggtccc taggtggcgg atccgaaaac 660

ctgtacttcc agagcgatat ctttaacaaa agtaacgatg gtttcaccac caccaggagc 720

tatggaacag tctcacagat ttttgggagc agttccccaa gtcccaacgg cttcattacc 780

acaaggagct atggaacagt ctgccccaaa gactgggaat tttatcaagc aagatgtttt 840

ttcttatcca cttctgaatc atcttggaat gaaagcaggg acttttgcaa aggaaaaggc 900

tccacattgg caattgtcaa cacgccagag aaactgaagt ttcttcagga cataactgat 960

gctgagaagt attttattgg cttaatttac catcgtgaag agaaaaggtg gcgttggatc 1020

aacaactctg tgttcaatgg caatgttacc aatcagaatc agaatttcaa ctgtgcgacc 1080

attggcctaa caaagacatt tgatgctgca tcatgtgaca tcagctaccg caggatctgt 1140

gagaagaatg ccaaaggccc gggagagaat ctatattttc aagggcccgg cggaggtagt 1200

caccatcatc accatcacta atgaccggt 1229

<210> 100
<211> 385
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV-huCLEC5A-proTEV-Histag]

<400> 100

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

```
Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Phe Asn
        195                 200                 205

Lys Ser Asn Asp Gly Phe Thr Thr Thr Arg Ser Tyr Gly Thr Val Ser
    210                 215                 220

Gln Ile Phe Gly Ser Ser Ser Pro Ser Pro Asn Gly Phe Ile Thr Thr
225                 230                 235                 240

Arg Ser Tyr Gly Thr Val Cys Pro Lys Asp Trp Glu Phe Tyr Gln Ala
                245                 250                 255

Arg Cys Phe Phe Leu Ser Thr Ser Glu Ser Ser Trp Asn Glu Ser Arg
            260                 265                 270

Asp Phe Cys Lys Gly Lys Gly Ser Thr Leu Ala Ile Val Asn Thr Pro
        275                 280                 285

Glu Lys Leu Lys Phe Leu Gln Asp Ile Thr Asp Ala Glu Lys Tyr Phe
    290                 295                 300

Ile Gly Leu Ile Tyr His Arg Glu Glu Lys Arg Trp Arg Trp Ile Asn
305                 310                 315                 320
```

Asn Ser Val Phe Asn Gly Asn Val Thr Asn Gln Asn Gln Asn Phe Asn
325 330 335

Cys Ala Thr Ile Gly Leu Thr Lys Thr Phe Asp Ala Ala Ser Cys Asp
340 345 350

Ile Ser Tyr Arg Arg Ile Cys Glu Lys Asn Ala Lys Gly Pro Gly Glu
355 360 365

Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His His
370 375 380

His
385

<210> 101
<211> 962
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding BiPlike- SNAPlike- cxVAGO protein from Culex quinquefasciatus - Histag for expression in S2 cells

<400> 101
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac 60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt 120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat 180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc 240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctga agccgttcta 660

caaaatgccg agcatccaga ttaccctgga aagtgttacg acgaaggtac gcagaccgtt 720

gtagctcccc tagaaagtgc gaagctacca aaatcgtgta caaaggtatt ctgctcgact 780

aacctttcac tgacctatac tacgtgtggg tcagtacttg tcaatgaccc gcactgcgag 840

aagatcgaac aagacctgac taaagacttc ccagagtgct gtcacaagta taaatgtgaa 900

ctggagggag tagtcacgta ccacggaggt ggccatcacc atcaccatca ctgatgaccg 960

gt 962

<210> 102
<211> 300

<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-cxVAGO-Histag]

<400> 102

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

Leu Glu Gly Gly Gly Gly Ser Glu Ala Val Leu Gln Asn Ala Glu His
195 200 205

Pro Asp Tyr Pro Gly Lys Cys Tyr Asp Glu Gly Thr Gln Thr Val Val
210 215 220

Ala Pro Leu Glu Ser Ala Lys Leu Pro Lys Ser Cys Thr Lys Val Phe
225 230 235 240

```
Cys Ser Thr Asn Leu Ser Leu Thr Tyr Thr Thr Cys Gly Ser Val Leu
                245                 250                 255

Val Asn Asp Pro His Cys Glu Lys Ile Glu Gln Asp Leu Thr Lys Asp
            260                 265                 270

Phe Pro Glu Cys Cys His Lys Tyr Lys Cys Glu Leu Glu Gly Val Val
        275                 280                 285

Thr Tyr His Gly Gly Gly His His His His His His
    290                 295                 300
```

<210> 103
<211> 962
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding BiPlike- SNAPlike- aaVAGO protein from Aedes albopictus - Histag for expression in S2 cells

<400> 103

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atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc      300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg      360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt      420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg      480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg      540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt      600

cctgctggta taggcgcgcc agggtccctg gagggaggtg gcgggtctac ggctatcttc      660

ccaaattcgg agaacaaaga tttcccaggc gaatgctatg acacggagac taagattcat      720

ttcaagccag gggaaaatcg tcaacgacct ggcaactgtg aagagatgtc atgcggaact      780

gacttctcga ttcacttttt cggatgcgga ctagctatac tagacgatga cccggattgc      840

gagatcccag ttcaggattt cacaaaggac acgcagtgtt gccataagta caagtgtgtg      900

cgtaacggtg aagtcaatta cattggaggt ggccatcacc atcaccatca ctgatgaccg      960

gt                                                                     962
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<210> 104
<211> 300
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-aaVAGO-Histag]

<400> 104

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Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1               5                  10                  15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Glu Gly Gly Gly Gly Ser Thr Ala Ile Phe Pro Asn Ser Glu Asn
        195                 200                 205

Lys Asp Phe Pro Gly Glu Cys Tyr Asp Thr Glu Thr Lys Ile His Phe
    210                 215                 220

Lys Pro Gly Glu Asn Arg Gln Arg Pro Gly Asn Cys Glu Glu Met Ser
225                 230                 235                 240

Cys Gly Thr Asp Phe Ser Ile His Phe Phe Gly Cys Gly Leu Ala Ile
                245                 250                 255

Leu Asp Asp Asp Pro Asp Cys Glu Ile Pro Val Gln Asp Phe Thr Lys
```

260 265 270

Asp Thr Gln Cys Cys His Lys Tyr Lys Cys Val Arg Asn Gly Glu Val
275 280 285

Asn Tyr Ile Gly Gly Gly His His His His His His
290 295 300

<210> 105
<211> 4108
<212> DNA
<213> artificial

<220>
<223> DNA sequence of OpIE2SP-SNAP cloned into pUC57 for constitutive expression of secreted chimeric SNAP-target protein in invertebrate cells

<400> 105
tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtggaat tctcatgatg ataaacaatg 420

tatggtgcta atgttgcttc aacaacaatt ctgttgaact gtgttttcat gtttgccaac 480

aagcaccttt atactcggtg gcctccccac caccaacttt tttgcactgc aaaaaaacac 540

gcttttgcac gcgggcccat acatagtaca aactctacgt ttcgtagact attttacata 600

aatagtctac accgttgtat acgctccaaa tacactacca cacattgaac ctttttgcag 660

tgcaaaaaag tacgtgtcgg cagtcacgta ggccggcctt atcgggtcgc gtcctgtcac 720

gtacgaatca cattatcgga ccggacgagt gttgtcttat cgtgacagga cgccagcttc 780

ctgtgttgct aaccgcagcc ggacgcaact ccttatcgga acaggacgcg cctccatatc 840

agccgcgcgt tatctcatgc gcgtgaccgg acacgaggcg cccgtcccgc ttatcgcgcc 900

tataaataca gcccgcaacg atctggtaaa cacagttgaa cagcatctgt tcgaaggatc 960

cttgatcgag ctagcatgaa actatgtatt ctacttgcag ttgttgcgtt cgtaggattg 1020

tccttaagat ctgacaaaga ctgcgaaatg aaaagaacta cattggattc accacttggg 1080

aagttggaac tgagtggatg cgagcaagga ttgcatgaaa ttaagctact gggaaaagga 1140

acttctgctg ctgatgcagt tgaagttcca gcaccagcag ctgttcttgg aggtcctgag 1200

cccctcatgc aagccacagc ctggcttaac gcatatttcc accagcctga ggccattgag 1260

gaatttccag tccccgccct tcaccatcct gtgtttcagc aggagagctt cacccgccag 1320

gtcctgtgga aattgctgaa ggtggtcaag tttggtgaag tgatttcata tcagcaactt 1380

gctgcattgg ccggtaaccc cgcagctaca gctgccgtga aaactgctct cagcggaaat 1440

cctgtgccca tcctgatccc ttgtcacaga gtcgtttcat cttccggagc tgtaggtggc 1500

tatgaaggag gactggcagt taaggagtgg ctgctggctc atgaaggtca tagacttgga 1560

aagcctgggc tgggtcctgc tggtataggc gcgccagggt ccctaggtgg cggatccgaa 1620

aacctgtact tccagagcga tatcggaggt ggaggcccgg gaggtggcgg aagtgactat 1680

aaagatgacg acgataagtg ataagcggcc gcaaaaccgg ttgagtttat ctgactaaat 1740

cttagtttgt attgtcatgt tttaatacaa tatgttatgt ttaaatatgt ttttaataaa 1800

ttttataaaa taatttcaac ttttattgta acaacattgt ccatttacac actcctttca 1860

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ctcacaattc cacacaacat acgagccgga agcataaagt gtaaagcctg gggtgcctaa 1980

tgagtgagct aactcacatt aattgcgttg cgctcactgc ccgctttcca gtcgggaaac 2040

ctgtcgtgcc agctgcatta atgaatcggc caacgcgcgg ggagaggcgg tttgcgtatt 2100

gggcgctctt ccgcttcctc gctcactgac tcgctgcgct cggtcgttcg gctgcggcga 2160

gcggtatcag ctcactcaaa ggcggtaata cggttatcca cagaatcagg ggataacgca 2220

ggaaagaaca tgtgagcaaa aggccagcaa aaggccagga accgtaaaaa ggccgcgttg 2280

ctggcgtttt tccataggct ccgcccccct gacgagcatc acaaaaatcg acgctcaagt 2340

cagaggtggc gaaacccgac aggactataa agataccagg cgtttccccc tggaagctcc 2400

ctcgtgcgct ctcctgttcc gaccctgccg cttaccggat acctgtccgc ctttctccct 2460

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agcggtggtt tttttgtttg caagcagcag attacgcgca gaaaaaaagg atctcaagaa 2880

gatcctttga tcttttctac ggggtctgac gctcagtgga acgaaaactc acgttaaggg 2940

attttggtca tgagattatc aaaaaggatc ttcacctaga tccttttaaa ttaaaaatga 3000

agttttaaat caatctaaag tatatatgag taaacttggt ctgacagtta ccaatgctta 3060

atcagtgagg cacctatctc agcgatctgt ctatttcgtt catccatagt tgcctgactc 3120

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caacgatcaa ggcgagttac atgatccccc atgttgtgca aaaaagcggt tagctccttc 3480

ggtcctccga tcgttgtcag aagtaagttg gccgcagtgt tatcactcat ggttatggca 3540

gcactgcata attctcttac tgtcatgcca tccgtaagat gcttttctgt gactggtgag 3600

tactcaacca agtcattctg agaatagtgt atgcggcgac cgagttgctc ttgcccggcg 3660

tcaatacggg ataataccgc gccacatagc agaactttaa aagtgctcat cattggaaaa 3720

cgttcttcgg ggcgaaaact ctcaaggatc ttaccgctgt tgagatccag ttcgatgtaa 3780

cccactcgtg cacccaactg atcttcagca tcttttactt tcaccagcgt ttctgggtga 3840

gcaaaaacag gaaggcaaaa tgccgcaaaa aagggaataa gggcgacacg gaaatgttga 3900

atactcatac tcttcctttt tcaatattat tgaagcattt atcagggtta ttgtctcatg 3960

agcggataca tatttgaatg tatttagaaa aataaacaaa taggggttcc gcgcacattt 4020

ccccgaaaag tgccacctga cgtctaagaa accattatta tcatgacatt aacctataaa 4080

aataggcgta tcacgaggcc ctttcgtc 4108

<210> 106
<211> 24
<212> DNA
<213> artificial

<220>
<223> DNA sequence of C-term peptide tag of OpIE2SP-SNAP

<400> 106
gactataaag atgacgacga taag 24

<210> 107
<211> 8
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of C-term peptide tag of OpIE2SP-SNAP

<400> 107

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 108
<211> 2200
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Crimean-Congo hemorragic fever virus-proTEV2-Histag for expression in S2 cells

<400> 108
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gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

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ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgaaaacaa gatcgaggtg aataacaaag atgagatgaa caggtggttt 720

gaagagttca aaaaaggaaa tggacttgtg gacaccttca caaactccta ttccttttgc 780

gagagtgttc ccaatttgga caggtttgtg tttcagatgg ccagtgccac cgatgatgca 840

cagaaggact ccatctacgc atctgctctg gtggaggcaa caaagttttg tgcacctata 900

tatgagtgcg catgggttag ctccactggc attgtaaaaa agggacttga atggttcgag 960

aaaaatgcag gaaccattaa gtcctgggat gaaagttata ctgagctaaa ggtcgacgtc 1020

ccgaaaatag agcagcttac gggttaccaa caagctgcct tgaagtggag aaaagacata 1080

ggtttccgtg tcaatgccaa cacagcagct ctgagcaaca aagtcctcgc agaatacaaa 1140

gtccctggtg agattgtgat gtctgtcaaa gagatgctgt cagacatgat taggagaagg 1200

aacctgattc taaacagggg tggtgatgag aacccacgtg gcccagtgag ccatgagcat 1260

gtagactggt gcagggagtt tgtcaaaggc aaatacatca tggccttcaa cccaccatgg 1320

ggggacatca acaagtcagg ccgttcagga atagcacttg ttgcaacagg ccttgctaag 1380

cttgcagaga ctgaaggaaa gggaatattt gatgaagcca aaaagactgt ggaggccctc 1440

aacgggtatc tggacaagca taaggacgaa gttgatagag caagcgccga cagcatgata 1500

acaaaccttc ttaagcatat tgccaaggca caggagctct ataaaaattc atctgcactt 1560

cgtgcacaaa gcgcacagat tgacactgct ttcagctcat actattggct ttacaaggct 1620

ggcgtgactc ctgaaacctt cccgacggtg tcacagttcc tctttgagct agggaaacag 1680

ccaagaggta ccaagaaaat gaagaaggct cttctgagca ccccaatgaa gtgggggaag 1740

aagctttatg agctctttgc cgatgattct ttccagcaga acaggattta catgcatcct 1800

gccgtgctta cagctggtag aatcagtgaa atgggagtct gctttgggac aatccctgtg 1860

gccaatcctg atgatgctgc ccaaggatct ggacacacta agtctattct caacctccgt 1920

accaacactg agaccaataa tccgtgtgcc aaaaccatcg tcaagctatt tgaagttcaa 1980

aaaacagggt tcaacattca ggacatggac atagtggcct ctgagcactt gctacaccaa 2040

tcccttgttg gcaagcaatc cccattccag aacgcctaca acgtcaaggg caatgccacc 2100

agtgctaaca tcatcccggg agagaatcta tattttcaag ggcccggcgg aggtagtcac 2160

catcatcacc atcactaatg accggtgcgg ccgcaagctt 2200

<210> 109
<211> 707
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-CCHF.N-proTEV2-Histag]

<400> 109

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Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1               5                   10                  15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Glu Asn
        195                 200                 205

Lys Ile Glu Val Asn Asn Lys Asp Glu Met Asn Arg Trp Phe Glu Glu
    210                 215                 220
```

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Phe Lys Lys Gly Asn Gly Leu Val Asp Thr Phe Thr Asn Ser Tyr Ser
225                 230                 235                 240

Phe Cys Glu Ser Val Pro Asn Leu Asp Arg Phe Val Phe Gln Met Ala
                245                 250                 255

Ser Ala Thr Asp Asp Ala Gln Lys Asp Ser Ile Tyr Ala Ser Ala Leu
            260                 265                 270

Val Glu Ala Thr Lys Phe Cys Ala Pro Ile Tyr Glu Cys Ala Trp Val
        275                 280                 285

Ser Ser Thr Gly Ile Val Lys Lys Gly Leu Glu Trp Phe Glu Lys Asn
    290                 295                 300

Ala Gly Thr Ile Lys Ser Trp Asp Glu Ser Tyr Thr Glu Leu Lys Val
305                 310                 315                 320

Asp Val Pro Lys Ile Glu Gln Leu Thr Gly Tyr Gln Gln Ala Ala Leu
                325                 330                 335

Lys Trp Arg Lys Asp Ile Gly Phe Arg Val Asn Ala Asn Thr Ala Ala
            340                 345                 350

Leu Ser Asn Lys Val Leu Ala Glu Tyr Lys Val Pro Gly Glu Ile Val
        355                 360                 365

Met Ser Val Lys Glu Met Leu Ser Asp Met Ile Arg Arg Arg Asn Leu
    370                 375                 380

Ile Leu Asn Arg Gly Gly Asp Glu Asn Pro Arg Gly Pro Val Ser His
385                 390                 395                 400

Glu His Val Asp Trp Cys Arg Glu Phe Val Lys Gly Lys Tyr Ile Met
                405                 410                 415

Ala Phe Asn Pro Pro Trp Gly Asp Ile Asn Lys Ser Gly Arg Ser Gly
            420                 425                 430

Ile Ala Leu Val Ala Thr Gly Leu Ala Lys Leu Ala Glu Thr Glu Gly
        435                 440                 445

Lys Gly Ile Phe Asp Glu Ala Lys Lys Thr Val Glu Ala Leu Asn Gly
    450                 455                 460

Tyr Leu Asp Lys His Lys Asp Glu Val Asp Arg Ala Ser Ala Asp Ser
465                 470                 475                 480

Met Ile Thr Asn Leu Leu Lys His Ile Ala Lys Ala Gln Glu Leu Tyr
                485                 490                 495
```

```
Lys Asn Ser Ser Ala Leu Arg Ala Gln Ser Ala Gln Ile Asp Thr Ala
            500                 505                 510

Phe Ser Ser Tyr Tyr Trp Leu Tyr Lys Ala Gly Val Thr Pro Glu Thr
        515                 520                 525

Phe Pro Thr Val Ser Gln Phe Leu Phe Glu Leu Gly Lys Gln Pro Arg
    530                 535                 540

Gly Thr Lys Lys Met Lys Lys Ala Leu Leu Ser Thr Pro Met Lys Trp
545                 550                 555                 560

Gly Lys Lys Leu Tyr Glu Leu Phe Ala Asp Asp Ser Phe Gln Gln Asn
                565                 570                 575

Arg Ile Tyr Met His Pro Ala Val Leu Thr Ala Gly Arg Ile Ser Glu
            580                 585                 590

Met Gly Val Cys Phe Gly Thr Ile Pro Val Ala Asn Pro Asp Asp Ala
        595                 600                 605

Ala Gln Gly Ser Gly His Thr Lys Ser Ile Leu Asn Leu Arg Thr Asn
    610                 615                 620

Thr Glu Thr Asn Asn Pro Cys Ala Lys Thr Ile Val Lys Leu Phe Glu
625                 630                 635                 640

Val Gln Lys Thr Gly Phe Asn Ile Gln Asp Met Asp Ile Val Ala Ser
                645                 650                 655

Glu His Leu Leu His Gln Ser Leu Val Gly Lys Gln Ser Pro Phe Gln
            660                 665                 670

Asn Ala Tyr Asn Val Lys Gly Asn Ala Thr Ser Ala Asn Ile Ile Pro
        675                 680                 685

Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His
    690                 695                 700

His His His
705


<210>  110
<211>  2974
<212>  DNA
<213>  artificial

<220>
<223>  DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of
       the Ebola virus-proTEV2-Histag for expression in S2 cells

<400>  110
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agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgattctcg tcctcagaaa atctggatgg cgccgagtct cactgaatct 720

gacatggatt accacaaaat cttgacagca ggtctgtccg ttcaacaggg gattgttcgg 780

caaagagtca tcccagtgta tcaagtaaac aatcttgaag aaatttgcca acttatcata 840

caggcctttg aagcaggtgt tgattttcaa gagagtgcgg acagtttcct tctcatgctt 900

tgtcttcatc atgcgtacca gggagattac aaacttttct tggaaagtgg cgcagtcaag 960

tatttggaag ggcacgggtt ccgttttgaa gtcaagaagc gtgatggagt gaagcgcctt 1020

gaggaattgc tgccagcagt atctagtgga aaaaacatta agagaacact tgctgccatg 1080

ccggaagagg agacaactga agctaatgcc ggtcagtttc tctcctttgc aagtctattc 1140

cttccgaaat tggtagtagg agaaaaggct tgccttgaga aggttcaaag gcaaattcaa 1200

gtacatgcag agcaaggact gatacaatat ccaacagctt ggcaatcagt aggacacatg 1260

atggtgattt tccgtttgat gcgaacaaat tttctgatca aatttctcct aatacaccaa 1320

gggatgcaca tggttgccgg gcatgatgcc aacgatgctg tgatttcaaa ttcagtggct 1380

caagctcgtt tttcaggctt attgattgtc aaaacagtac ttgatcatat cctacaaaag 1440

acagaacgag gagttcgtct ccatcctctt gcaaggaccg ccaaggtaaa aaatgaggtg 1500

aactccttta aggctgcact cagctccctg gccaagcatg gagagtatgc tcctttcgcc 1560

cgacttttga acctttctgg agtaaataat cttgagcatg gtcttttccc tcaactatcg 1620

gcaattgcac tcggagtcgc cacagcacac gggagtaccc tcgcaggagt aaatgttgga 1680

gaacagtatc aacaactcag agaggctgcc actgaggctg agaagcaact ccaacaatat 1740

gcagagtctc gcgaacttga ccatcttgga cttgatgatc aggaaaagaa aattcttatg 1800

aacttccatc agaaaaagaa cgaaatcagc ttccagcaaa caaacgctat ggtaactcta 1860

agaaaagagc gcctggccaa gctgacagaa gctatcactg ctgcgtcact gcccaaaaca 1920

agtggacatt acgatgatga tgacgacatt ccctttccag gacccatcaa tgatgacgac 1980

aatcctggcc atcaagatga tgatccgact gactcacagg atacgaccat tcccgatgtg 2040

gtggttgatc ccgatgatgg aagctacggc gaataccaga gttactcgga aaacggcatg 2100

aatgcaccag atgacttggt cctattcgat ctggacgagg acgacgagga cactaagcca 2160

gtgcctaata gatcgaccaa gggtggacaa cagaagaaca gtcaaaaggg ccagcatata 2220

gagggcagac agacacaatc caggccaatt caaaatgtcc caggccctca cagaacaatc 2280

caccacgcca gtgcgccact cacggacaat gacagaagaa atgaaccctc cggctcaacc 2340

agccctcgca tgctgacacc aattaacgaa gaggcagacc cactggacga tgccgacgac 2400

gagacgtcta gccttccgcc cttggagtca gatgatgaag agcaggacag ggacggaact 2460

tccaaccgca cacccactgt cgccccaccg gctcccgtat acagagatca ctctgaaaag 2520

aaagaactcc cgcaagacga gcaacaagat caggaccaca ctcaagaggc caggaaccag 2580

gacagtgaca acacccagtc agaacactct tttgaggaga tgtatcgcca cattctaaga 2640

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ttcagtacca gtgatggcaa agagtacacg tatccagact cccttgaaga ggaatatcca 2760

ccatggctca ctgaaaaaga ggctatgaat gaagagaata gatttgttac attggatggt 2820

caacaatttt attggccggt gatgaatcac aagaataaat tcatggcaat cctgcaacat 2880

catcagggcc cgggagagaa tctatatttt caagggcccg gcggaggtag tcaccatcat 2940

caccatcact aatgaccggt gcggccgcaa gctt 2974

<210> 111
<211> 965
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-EBO.N-proTEV2-Histag]

<400> 111

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

```
Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Asp Ser
        195                 200                 205

Arg Pro Gln Lys Ile Trp Met Ala Pro Ser Leu Thr Glu Ser Asp Met
    210                 215                 220

Asp Tyr His Lys Ile Leu Thr Ala Gly Leu Ser Val Gln Gln Gly Ile
225                 230                 235                 240

Val Arg Gln Arg Val Ile Pro Val Tyr Gln Val Asn Asn Leu Glu Glu
                245                 250                 255

Ile Cys Gln Leu Ile Ile Gln Ala Phe Glu Ala Gly Val Asp Phe Gln
            260                 265                 270

Glu Ser Ala Asp Ser Phe Leu Leu Met Leu Cys Leu His His Ala Tyr
        275                 280                 285

Gln Gly Asp Tyr Lys Leu Phe Leu Glu Ser Gly Ala Val Lys Tyr Leu
    290                 295                 300

Glu Gly His Gly Phe Arg Phe Glu Val Lys Lys Arg Asp Gly Val Lys
305                 310                 315                 320

Arg Leu Glu Glu Leu Leu Pro Ala Val Ser Ser Gly Lys Asn Ile Lys
                325                 330                 335

Arg Thr Leu Ala Ala Met Pro Glu Glu Glu Thr Thr Glu Ala Asn Ala
            340                 345                 350

Gly Gln Phe Leu Ser Phe Ala Ser Leu Phe Leu Pro Lys Leu Val Val
        355                 360                 365

Gly Glu Lys Ala Cys Leu Glu Lys Val Gln Arg Gln Ile Gln Val His
    370                 375                 380
```

```
Ala Glu Gln Gly Leu Ile Gln Tyr Pro Thr Ala Trp Gln Ser Val Gly
385                 390                 395                 400

His Met Met Val Ile Phe Arg Leu Met Arg Thr Asn Phe Leu Ile Lys
                405                 410                 415

Phe Leu Leu Ile His Gln Gly Met His Met Val Ala Gly His Asp Ala
            420                 425                 430

Asn Asp Ala Val Ile Ser Asn Ser Val Ala Gln Ala Arg Phe Ser Gly
        435                 440                 445

Leu Leu Ile Val Lys Thr Val Leu Asp His Ile Leu Gln Lys Thr Glu
    450                 455                 460

Arg Gly Val Arg Leu His Pro Leu Ala Arg Thr Ala Lys Val Lys Asn
465                 470                 475                 480

Glu Val Asn Ser Phe Lys Ala Ala Leu Ser Ser Leu Ala Lys His Gly
                485                 490                 495

Glu Tyr Ala Pro Phe Ala Arg Leu Leu Asn Leu Ser Gly Val Asn Asn
            500                 505                 510

Leu Glu His Gly Leu Phe Pro Gln Leu Ser Ala Ile Ala Leu Gly Val
        515                 520                 525

Ala Thr Ala His Gly Ser Thr Leu Ala Gly Val Asn Val Gly Glu Gln
    530                 535                 540

Tyr Gln Gln Leu Arg Glu Ala Ala Thr Glu Ala Glu Lys Gln Leu Gln
545                 550                 555                 560

Gln Tyr Ala Glu Ser Arg Glu Leu Asp His Leu Gly Leu Asp Asp Gln
                565                 570                 575

Glu Lys Lys Ile Leu Met Asn Phe His Gln Lys Lys Asn Glu Ile Ser
            580                 585                 590

Phe Gln Gln Thr Asn Ala Met Val Thr Leu Arg Lys Glu Arg Leu Ala
        595                 600                 605

Lys Leu Thr Glu Ala Ile Thr Ala Ala Ser Leu Pro Lys Thr Ser Gly
    610                 615                 620

His Tyr Asp Asp Asp Asp Asp Ile Pro Phe Pro Gly Pro Ile Asn Asp
625                 630                 635                 640

Asp Asp Asn Pro Gly His Gln Asp Asp Asp Pro Thr Asp Ser Gln Asp
                645                 650                 655
```

```
Thr Thr Ile Pro Asp Val Val Val Asp Pro Asp Asp Gly Ser Tyr Gly
            660                 665                 670

Glu Tyr Gln Ser Tyr Ser Glu Asn Gly Met Asn Ala Pro Asp Asp Leu
        675                 680                 685

Val Leu Phe Asp Leu Asp Glu Asp Asp Glu Asp Thr Lys Pro Val Pro
    690                 695                 700

Asn Arg Ser Thr Lys Gly Gly Gln Gln Lys Asn Ser Gln Lys Gly Gln
705                 710                 715                 720

His Ile Glu Gly Arg Gln Thr Gln Ser Arg Pro Ile Gln Asn Val Pro
                725                 730                 735

Gly Pro His Arg Thr Ile His His Ala Ser Ala Pro Leu Thr Asp Asn
            740                 745                 750

Asp Arg Arg Asn Glu Pro Ser Gly Ser Thr Ser Pro Arg Met Leu Thr
        755                 760                 765

Pro Ile Asn Glu Glu Ala Asp Pro Leu Asp Asp Ala Asp Asp Glu Thr
    770                 775                 780

Ser Ser Leu Pro Pro Leu Glu Ser Asp Asp Glu Glu Gln Asp Arg Asp
785                 790                 795                 800

Gly Thr Ser Asn Arg Thr Pro Thr Val Ala Pro Pro Ala Pro Val Tyr
                805                 810                 815

Arg Asp His Ser Glu Lys Lys Glu Leu Pro Gln Asp Glu Gln Gln Asp
            820                 825                 830

Gln Asp His Thr Gln Glu Ala Arg Asn Gln Asp Ser Asp Asn Thr Gln
        835                 840                 845

Ser Glu His Ser Phe Glu Glu Met Tyr Arg His Ile Leu Arg Ser Gln
    850                 855                 860

Gly Pro Phe Asp Ala Val Leu Tyr Tyr His Met Met Lys Asp Glu Pro
865                 870                 875                 880

Val Val Phe Ser Thr Ser Asp Gly Lys Glu Tyr Thr Tyr Pro Asp Ser
                885                 890                 895

Leu Glu Glu Glu Tyr Pro Pro Trp Leu Thr Glu Lys Glu Ala Met Asn
            900                 905                 910

Glu Glu Asn Arg Phe Val Thr Leu Asp Gly Gln Gln Phe Tyr Trp Pro
        915                 920                 925
```

Val Met Asn His Lys Asn Lys Phe Met Ala Ile Leu Gln His His Gln
930 935 940

Gly Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His
945 950 955 960

His His His His His
965

<210> 112
<211> 2842
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Marburg virus-proTEV2-Histag for expression in S2 cells

<400> 112
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gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgatttaca cagtttgttg gagttgggta caaaacccac tgcccctcat 720

gttcgtaata agaaagtgat attatttgac acaaatcatc aggttagtat ctgtaatcag 780

ataatagatg caataaactc agggattgat cttggtgatc tcctagaagg gggtttgctg 840

acgttgtgtg ttgagcatta ctataattct gataaggata aattcaacac aagtcctatc 900

gcgaagtact tacgtgatgc gggctatgaa tttgatgtca tcaagaatgc agatgcaacc 960

cgctttctgg atgtgattcc taatgaacct cattacagcc ctttaattct agcccttaag 1020

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caaaaaactg attcaggggt gacactacat cctttggtgc ggacctccaa agtaaaaaat 1440

gaagttgcta gtttcaagca ggcgttgagc aacctagccc gacatgggga atacgcacca 1500

tttgcacggg ttctgaattt atcagggatt aacaacctcg aacatggact ctatcctcag 1560

ctttcagcaa ttgcgctggg tgtggcaaca gcacacggca gtacattggc tggtgtcaat 1620

gttggcgaac aatatcaaca actacgagag gcggcacatg atgcggaagt aaaactacaa 1680

aggcgacatg aacatcagga aattcaagct attgccgagg atgacgagga aaggaagata 1740

ttagaacaat tccaccttca gaaaactgaa atcacacaca gtcagacact agccgtcctc 1800

agccagaaac gagaaaaatt agctcgtctc gctgcagaaa ttgaaaacaa tattgtggaa 1860

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gttgacgaca agattgagca tgaatctaca gaagatagct cttcttcaag tagctttgtt 2040

gacttgaatg atccatttgc actgctgaat gaggacgagg atactcttga tgacagtgtc 2100

atgatccccg gcacaacatc gagagaattt caagggattc ctgaaccgcc aagacaatcc 2160

caagacctca ataacagcca aggaaagcag gaagatgaat ccacaaatcc gattaagaaa 2220

cagtttctga gataccaaga attgcctcct gttcaagagg atgatgaatc ggaatacaca 2280

actgactctc aagaaagcat cgaccaacca ggttccgaca atgaacaagg agttgatctt 2340

ccacctcctc cgttgtacgc tcaggaaaaa agacaggacc caatacagca cccagcagca 2400

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tcacttataa cagccctcgt tgaggaatac caaaatcctg tctcagctaa ggagcttcaa 2700

gcagattggc ccgacatgtc atttgatgaa aggagacatg ttgcgatgaa cttgggcccg 2760

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tgaccggtgc ggccgcaagc tt 2842

<210> 113
<211> 921
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-EBO.N-proTEV2-Histag]

<400> 113

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
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Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
            20                  25                  30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Asp Leu
        195                 200                 205

His Ser Leu Leu Glu Leu Gly Thr Lys Pro Thr Ala Pro His Val Arg
    210                 215                 220

Asn Lys Lys Val Ile Leu Phe Asp Thr Asn His Gln Val Ser Ile Cys
225                 230                 235                 240

Asn Gln Ile Ile Asp Ala Ile Asn Ser Gly Ile Asp Leu Gly Asp Leu
                245                 250                 255

Leu Glu Gly Gly Leu Leu Thr Leu Cys Val Glu His Tyr Tyr Asn Ser
            260                 265                 270

Asp Lys Asp Lys Phe Asn Thr Ser Pro Ile Ala Lys Tyr Leu Arg Asp
        275                 280                 285
```

```
Ala Gly Tyr Glu Phe Asp Val Ile Lys Asn Ala Asp Ala Thr Arg Phe
    290                 295                 300

Leu Asp Val Ile Pro Asn Glu Pro His Tyr Ser Pro Leu Ile Leu Ala
305                 310                 315                 320

Leu Lys Thr Leu Glu Ser Thr Glu Ser Gln Arg Gly Arg Ile Gly Leu
                325                 330                 335

Phe Leu Ser Phe Cys Ser Leu Phe Leu Pro Lys Leu Val Val Gly Asp
            340                 345                 350

Arg Ala Ser Ile Glu Lys Ala Leu Arg Gln Val Thr Val His Gln Glu
        355                 360                 365

Gln Gly Ile Val Thr Tyr Pro Asn His Trp Leu Thr Thr Gly His Met
    370                 375                 380

Lys Val Ile Phe Gly Ile Leu Arg Ser Ser Phe Ile Leu Lys Phe Val
385                 390                 395                 400

Leu Ile His Gln Gly Val Asn Leu Val Thr Gly His Asp Ala Tyr Asp
                405                 410                 415

Ser Ile Ile Ser Asn Ser Val Gly Gln Thr Arg Phe Ser Gly Leu Leu
            420                 425                 430

Ile Val Lys Thr Val Leu Glu Phe Ile Leu Gln Lys Thr Asp Ser Gly
        435                 440                 445

Val Thr Leu His Pro Leu Val Arg Thr Ser Lys Val Lys Asn Glu Val
    450                 455                 460

Ala Ser Phe Lys Gln Ala Leu Ser Asn Leu Ala Arg His Gly Glu Tyr
465                 470                 475                 480

Ala Pro Phe Ala Arg Val Leu Asn Leu Ser Gly Ile Asn Asn Leu Glu
                485                 490                 495

His Gly Leu Tyr Pro Gln Leu Ser Ala Ile Ala Leu Gly Val Ala Thr
            500                 505                 510

Ala His Gly Ser Thr Leu Ala Gly Val Asn Val Gly Glu Gln Tyr Gln
        515                 520                 525

Gln Leu Arg Glu Ala Ala His Asp Ala Glu Val Lys Leu Gln Arg Arg
    530                 535                 540

His Glu His Gln Glu Ile Gln Ala Ile Ala Glu Asp Asp Glu Glu Arg
545                 550                 555                 560
```

```
Lys Ile Leu Glu Gln Phe His Leu Gln Lys Thr Glu Ile Thr His Ser
                565                 570                 575

Gln Thr Leu Ala Val Leu Ser Gln Lys Arg Glu Lys Leu Ala Arg Leu
            580                 585                 590

Ala Ala Glu Ile Glu Asn Asn Ile Val Glu Asp Gln Gly Phe Lys Gln
        595                 600                 605

Ser Gln Asn Arg Val Ser Gln Ser Phe Leu Asn Asp Pro Thr Pro Val
    610                 615                 620

Glu Val Thr Val Gln Ala Arg Pro Met Asn Arg Pro Thr Ala Leu Pro
625                 630                 635                 640

Pro Pro Val Asp Asp Lys Ile Glu His Glu Ser Thr Glu Asp Ser Ser
                645                 650                 655

Ser Ser Ser Ser Phe Val Asp Leu Asn Asp Pro Phe Ala Leu Leu Asn
            660                 665                 670

Glu Asp Glu Asp Thr Leu Asp Asp Ser Val Met Ile Pro Gly Thr Thr
        675                 680                 685

Ser Arg Glu Phe Gln Gly Ile Pro Glu Pro Pro Arg Gln Ser Gln Asp
    690                 695                 700

Leu Asn Asn Ser Gln Gly Lys Gln Glu Asp Glu Ser Thr Asn Pro Ile
705                 710                 715                 720

Lys Lys Gln Phe Leu Arg Tyr Gln Glu Leu Pro Pro Val Gln Glu Asp
                725                 730                 735

Asp Glu Ser Glu Tyr Thr Thr Asp Ser Gln Glu Ser Ile Asp Gln Pro
            740                 745                 750

Gly Ser Asp Asn Glu Gln Gly Val Asp Leu Pro Pro Pro Pro Leu Tyr
        755                 760                 765

Ala Gln Glu Lys Arg Gln Asp Pro Ile Gln His Pro Ala Ala Asn Pro
    770                 775                 780

Gln Asp Pro Phe Gly Ser Ile Gly Asp Val Asn Gly Asp Ile Leu Glu
785                 790                 795                 800

Pro Ile Arg Ser Pro Ser Ser Pro Ser Ala Pro Gln Glu Asp Thr Arg
                805                 810                 815

Met Arg Glu Ala Tyr Glu Leu Ser Pro Asp Phe Thr Asn Asp Glu Asp
            820                 825                 830
```

```
Asn Gln Gln Asn Trp Pro Gln Arg Val Val Thr Lys Lys Gly Arg Thr
        835                 840                 845

Phe Leu Tyr Pro Asn Asp Leu Leu Gln Thr Asn Pro Pro Glu Ser Leu
    850                 855                 860

Ile Thr Ala Leu Val Glu Glu Tyr Gln Asn Pro Val Ser Ala Lys Glu
865                 870                 875                 880

Leu Gln Ala Asp Trp Pro Asp Met Ser Phe Asp Glu Arg Arg His Val
                885                 890                 895

Ala Met Asn Leu Gly Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly
            900                 905                 910

Gly Gly Ser His His His His His His
        915                 920
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<210> 114
<211> 2461
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Lassa virus-proTEV2-Histag for expression in S2 cells

<400> 114

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agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct      180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa      240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc      300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa      360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc      420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc      480

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agggaattat ctggttactg ctccaacatc aaactacagg tggtgaaaga tgcccaggct      780

cttttacatg gacttgactt ctccgaagtc agtaatgttc aacggttgat gcgcaaggag      840

agaagggatg acaatgattt gaaacggttg agggacctaa atcaagcggt caacaatctt      900

gttgaattaa aatcaactca acaaaagagt atactgagag ttgggactct aacctcagat      960

gacttattaa tcttagccgc tgacctagag aagttaaagt caaaggtgat cagaacagaa     1020
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aggccattaa gtgcaggtgt ctatatgggc aacctaagct cacagcaact tgaccaaaga 1080

agagctctcc tgaatatgat aggaatgagt ggtggtaatc aaggggctcg ggctgggaga 1140

gatggagtgg tgagagtttg ggatgtgaaa aatgcagagt tgctcaataa tcagttcggg 1200

accatgccaa gtctgacact ggcatgtctg acaaaacagg ggcaggttga cttgaatgat 1260

gcagtacaag cattgacaga tttgggtttg atctacacag caaagtatcc caacacttca 1320

gacttagaca ggctgactca aagtcatccc atcctaaata tgattgacac caagaaaagc 1380

tctttgaata tctcaggtta taattttagc ttgggtgcag ctgtgaaggc aggagcttgc 1440

atgctggatg gtggcaatat gttggagaca atcaaggtgt cacctcagac aatggatggt 1500

atcctcaaat ccattttaaa ggtcaagaag gctcttggaa tgttcatttc agacacccct 1560

ggtgaaagga atccttatga aaacatactc tacaagattt gtttgtcagg agatggatgg 1620

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tcggcagggt ttaccgctgg gcttacctat tctcagctga tgaccctcaa ggatgcaatg 1800

ctgcaacttg acccaaatgc taagacctgg atggacattg aaggaagacc tgaagatcca 1860

gtggaaattg ccctctatca accaagttca ggctgctaca tacacttctt ccgtgaacct 1920

actgatttaa agcagttcaa gcaggatgct aagtactcac atgggattga tgtcacagac 1980

ctcttcgcta cacaaccggg cttgaccagt gctgtcattg atgcactccc ccggaatatg 2040

gtcattacct gtcaggggtc cgatgacata aggaaactcc ttgaatcaca aggaagaaaa 2100

gacattaaac taattgatat tgccctcagc aaaactgatt ccaggaagta tgaaaatgca 2160

gtctgggacc agtataaaga cttatgccac atgcacacag gtgtcgttgt tgaaaagaag 2220

aaaagaggcg gtaaagagga aataacccct cactgtgcac taatggactg catcatgttt 2280

gatgcagcag tgtcaggagg actgaacaca tcggttttga gagcagtgct gcccagagat 2340

atggtgttca gaacatcgac acctagagtc gttctcccgg gagagaatct atattttcaa 2400

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t 2461

<210> 115
<211> 794
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-LAS.N-proTEV2-Histag]

<400> 115

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

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Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
        35                  40                  45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ser Ala
        195                 200                 205

Ser Lys Glu Ile Lys Ser Phe Leu Trp Thr Gln Ser Leu Arg Arg Glu
    210                 215                 220

Leu Ser Gly Tyr Cys Ser Asn Ile Lys Leu Gln Val Val Lys Asp Ala
225                 230                 235                 240

Gln Ala Leu Leu His Gly Leu Asp Phe Ser Glu Val Ser Asn Val Gln
                245                 250                 255

Arg Leu Met Arg Lys Glu Arg Arg Asp Asp Asn Asp Leu Lys Arg Leu
            260                 265                 270

Arg Asp Leu Asn Gln Ala Val Asn Asn Leu Val Glu Leu Lys Ser Thr
        275                 280                 285

Gln Gln Lys Ser Ile Leu Arg Val Gly Thr Leu Thr Ser Asp Asp Leu
    290                 295                 300
```

```
Leu Ile Leu Ala Ala Asp Leu Glu Lys Leu Lys Ser Lys Val Ile Arg
305                 310                 315                 320

Thr Glu Arg Pro Leu Ser Ala Gly Val Tyr Met Gly Asn Leu Ser Ser
                325                 330                 335

Gln Gln Leu Asp Gln Arg Arg Ala Leu Leu Asn Met Ile Gly Met Ser
            340                 345                 350

Gly Gly Asn Gln Gly Ala Arg Ala Gly Arg Asp Gly Val Val Arg Val
        355                 360                 365

Trp Asp Val Lys Asn Ala Glu Leu Leu Asn Asn Gln Phe Gly Thr Met
    370                 375                 380

Pro Ser Leu Thr Leu Ala Cys Leu Thr Lys Gln Gly Gln Val Asp Leu
385                 390                 395                 400

Asn Asp Ala Val Gln Ala Leu Thr Asp Leu Gly Leu Ile Tyr Thr Ala
                405                 410                 415

Lys Tyr Pro Asn Thr Ser Asp Leu Asp Arg Leu Thr Gln Ser His Pro
            420                 425                 430

Ile Leu Asn Met Ile Asp Thr Lys Lys Ser Ser Leu Asn Ile Ser Gly
        435                 440                 445

Tyr Asn Phe Ser Leu Gly Ala Ala Val Lys Ala Gly Ala Cys Met Leu
    450                 455                 460

Asp Gly Gly Asn Met Leu Glu Thr Ile Lys Val Ser Pro Gln Thr Met
465                 470                 475                 480

Asp Gly Ile Leu Lys Ser Ile Leu Lys Val Lys Lys Ala Leu Gly Met
                485                 490                 495

Phe Ile Ser Asp Thr Pro Gly Glu Arg Asn Pro Tyr Glu Asn Ile Leu
            500                 505                 510

Tyr Lys Ile Cys Leu Ser Gly Asp Gly Trp Pro Tyr Ile Ala Ser Arg
        515                 520                 525

Thr Ser Ile Thr Gly Arg Ala Trp Glu Asn Thr Val Val Asp Leu Glu
    530                 535                 540

Ser Asp Gly Lys Pro Gln Lys Ala Asp Ser Asn Asn Ser Ser Lys Ser
545                 550                 555                 560

Leu Gln Ser Ala Gly Phe Thr Ala Gly Leu Thr Tyr Ser Gln Leu Met
                565                 570                 575
```

```
Thr Leu Lys Asp Ala Met Leu Gln Leu Asp Pro Asn Ala Lys Thr Trp
            580                 585                 590

Met Asp Ile Glu Gly Arg Pro Glu Asp Pro Val Glu Ile Ala Leu Tyr
        595                 600                 605

Gln Pro Ser Ser Gly Cys Tyr Ile His Phe Phe Arg Glu Pro Thr Asp
    610                 615                 620

Leu Lys Gln Phe Lys Gln Asp Ala Lys Tyr Ser His Gly Ile Asp Val
625                 630                 635                 640

Thr Asp Leu Phe Ala Thr Gln Pro Gly Leu Thr Ser Ala Val Ile Asp
                645                 650                 655

Ala Leu Pro Arg Asn Met Val Ile Thr Cys Gln Gly Ser Asp Asp Ile
            660                 665                 670

Arg Lys Leu Leu Glu Ser Gln Gly Arg Lys Asp Ile Lys Leu Ile Asp
        675                 680                 685

Ile Ala Leu Ser Lys Thr Asp Ser Arg Lys Tyr Glu Asn Ala Val Trp
    690                 695                 700

Asp Gln Tyr Lys Asp Leu Cys His Met His Thr Gly Val Val Val Glu
705                 710                 715                 720

Lys Lys Lys Arg Gly Gly Lys Glu Glu Ile Thr Pro His Cys Ala Leu
                725                 730                 735

Met Asp Cys Ile Met Phe Asp Ala Ala Val Ser Gly Gly Leu Asn Thr
            740                 745                 750

Ser Val Leu Arg Ala Val Leu Pro Arg Asp Met Val Phe Arg Thr Ser
        755                 760                 765

Thr Pro Arg Val Val Leu Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro
    770                 775                 780

Gly Gly Gly Ser His His His His His His
785                 790
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<210> 116
<211> 2446
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Junin virus-proTEV2-Histag for expression in S2 cells

<400> 116

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agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgcacactc caaggaggtt cctagcttta gatggactca gtccttaagg 720

agaggtttga gccaattcac tcagactgtc aagtcagatg ttttgaagga cgccaagcta 780

attgctgaca gcatcgactt caaccaagtg gcacaggtgc agcgggcact cagaaagact 840

aaaaaggggg aagaagacct caataagttg agggacctga ataaagaggt tgacagactc 900

atgtccatga ggagtgttca acgaaacaca gttttcaagg tgggtgatct ggggagggat 960

gaactgatgg agttggcgtc tgaccttgag aaattaaaaa acaagataag aagagcagag 1020

acaggctctc agggggttta catgggtaac ttgtcccagt cacaacttgc taaaagatca 1080

gagatattga gaacactggg atttcaacag caagggactg ggggaaatgg tgtggtgagg 1140

atatgggatg ttaaagaccc ttcaaagcta aacaatcagt ttggctctgt tcctgcattg 1200

acaattgcat gcatgactgt tcaaggaggt gagacaatga acagtgtcat acaggcttta 1260

acctcacttg ggcttctata cactgtgaag tatccaaact taagtgacct tgacagactg 1320

actcaggaac atgactgcct tcagattgtg actaaagatg aaagctccat caatatttct 1380

ggttacaact tcagtctttc agctgcagta aaggctggag catctattct tgatggtgga 1440

aacatgttgg aaacaatcag agtcacccca gaaaacttct cttccctcat aaaatcaacc 1500

attcaggtta aacgaagaga aggcatgttt attgatgaga aaccaggcaa tagaaatcct 1560

tatgaaaacc ttttgtacaa actttgtctt tctggcgatg gttggcctta tattggttca 1620

agatcacaaa tcacaggcag gtcatgggac aacacaagta ttgatctgac aaggaaacca 1680

gttgctggtc ctagacagcc ggaaaaaaac ggtcagaatt tgagattggc taacttgaca 1740

gagatacaag aagctgtcat cagagaggca gtggggaaac tcgaccccac caacaccctt 1800

tggctcgaca ttgaaggacc agccactgac cctgttgaga tggcattatt ccaacctgca 1860

ggtaagcagt acattcactg cttcagaaaa ccacatgatg agaaagggtt taaaaatggt 1920

agcagacact ctcacggcat cttaatgaag gacatagaag atgcaatgcc aggagttctt 1980

agttacgtga tcggcttgct gcctccagac atggttgtga ctactcaagg ttccgatgac 2040

atcaggaagt tgtttgacct ccatggaaga agggatctta aactggttga tgttaagctc 2100

acatctgaac aagccaggca gtttgatcaa caggtctggg agaaatatgg tcacttatgc 2160

aaatatcaca atggagtggt tgtcaataag aaaaagaggg aaaaggatac tcccttcaag 2220

ttggcctcca gtgaaccaca ctgtgctctg ctagactgca taatgtttca gtcagtgcta 2280

gatgggaagc tctatgagga ggaacctaca cctctattac caccgagctt gctgttcctc 2340

ccgaaggcag cctatgcact cccgggagag aatctatatt ttcaagggcc cggcggaggt 2400

agtcaccatc atcaccatca ctaatgaccg gtgcggccgc aagctt 2446

<210> 117
<211> 789
<212> PRT
<213> artificial

<220>
<223> Aa sequence of fusion protein
[SNAPlike-proTEV1-JUN.N-proTEV2-Histag]

<400> 117

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

```
Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala His
        195                 200                 205

Ser Lys Glu Val Pro Ser Phe Arg Trp Thr Gln Ser Leu Arg Arg Gly
    210                 215                 220

Leu Ser Gln Phe Thr Gln Thr Val Lys Ser Asp Val Leu Lys Asp Ala
225                 230                 235                 240

Lys Leu Ile Ala Asp Ser Ile Asp Phe Asn Gln Val Ala Gln Val Gln
                245                 250                 255

Arg Ala Leu Arg Lys Thr Lys Lys Gly Glu Glu Asp Leu Asn Lys Leu
            260                 265                 270

Arg Asp Leu Asn Lys Glu Val Asp Arg Leu Met Ser Met Arg Ser Val
        275                 280                 285

Gln Arg Asn Thr Val Phe Lys Val Gly Asp Leu Gly Arg Asp Glu Leu
    290                 295                 300

Met Glu Leu Ala Ser Asp Leu Glu Lys Leu Lys Asn Lys Ile Arg Arg
305                 310                 315                 320

Ala Glu Thr Gly Ser Gln Gly Val Tyr Met Gly Asn Leu Ser Gln Ser
                325                 330                 335

Gln Leu Ala Lys Arg Ser Glu Ile Leu Arg Thr Leu Gly Phe Gln Gln
            340                 345                 350

Gln Gly Thr Gly Gly Asn Gly Val Val Arg Ile Trp Asp Val Lys Asp
        355                 360                 365

Pro Ser Lys Leu Asn Asn Gln Phe Gly Ser Val Pro Ala Leu Thr Ile
    370                 375                 380

Ala Cys Met Thr Val Gln Gly Gly Glu Thr Met Asn Ser Val Ile Gln
385                 390                 395                 400

Ala Leu Thr Ser Leu Gly Leu Leu Tyr Thr Val Lys Tyr Pro Asn Leu
                405                 410                 415

Ser Asp Leu Asp Arg Leu Thr Gln Glu His Asp Cys Leu Gln Ile Val
            420                 425                 430

Thr Lys Asp Glu Ser Ser Ile Asn Ile Ser Gly Tyr Asn Phe Ser Leu
        435                 440                 445
```

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Ser Ala Ala Val Lys Ala Gly Ala Ser Ile Leu Asp Gly Gly Asn Met
    450                 455                 460

Leu Glu Thr Ile Arg Val Thr Pro Glu Asn Phe Ser Ser Leu Ile Lys
465                 470                 475                 480

Ser Thr Ile Gln Val Lys Arg Arg Glu Gly Met Phe Ile Asp Glu Lys
                485                 490                 495

Pro Gly Asn Arg Asn Pro Tyr Glu Asn Leu Leu Tyr Lys Leu Cys Leu
            500                 505                 510

Ser Gly Asp Gly Trp Pro Tyr Ile Gly Ser Arg Ser Gln Ile Thr Gly
        515                 520                 525

Arg Ser Trp Asp Asn Thr Ser Ile Asp Leu Thr Arg Lys Pro Val Ala
    530                 535                 540

Gly Pro Arg Gln Pro Glu Lys Asn Gly Gln Asn Leu Arg Leu Ala Asn
545                 550                 555                 560

Leu Thr Glu Ile Gln Glu Ala Val Ile Arg Glu Ala Val Gly Lys Leu
                565                 570                 575

Asp Pro Thr Asn Thr Leu Trp Leu Asp Ile Glu Gly Pro Ala Thr Asp
            580                 585                 590

Pro Val Glu Met Ala Leu Phe Gln Pro Ala Gly Lys Gln Tyr Ile His
        595                 600                 605

Cys Phe Arg Lys Pro His Asp Glu Lys Gly Phe Lys Asn Gly Ser Arg
    610                 615                 620

His Ser His Gly Ile Leu Met Lys Asp Ile Glu Asp Ala Met Pro Gly
625                 630                 635                 640

Val Leu Ser Tyr Val Ile Gly Leu Leu Pro Pro Asp Met Val Val Thr
                645                 650                 655

Thr Gln Gly Ser Asp Asp Ile Arg Lys Leu Phe Asp Leu His Gly Arg
            660                 665                 670

Arg Asp Leu Lys Leu Val Asp Val Lys Leu Thr Ser Glu Gln Ala Arg
        675                 680                 685

Gln Phe Asp Gln Gln Val Trp Glu Lys Tyr Gly His Leu Cys Lys Tyr
    690                 695                 700

His Asn Gly Val Val Val Asn Lys Lys Lys Arg Glu Lys Asp Thr Pro
705                 710                 715                 720
```

Phe Lys Leu Ala Ser Ser Glu Pro His Cys Ala Leu Leu Asp Cys Ile
725 730 735

Met Phe Gln Ser Val Leu Asp Gly Lys Leu Tyr Glu Glu Glu Pro Thr
740 745 750

Pro Leu Leu Pro Pro Ser Leu Leu Phe Leu Pro Lys Ala Ala Tyr Ala
755 760 765

Leu Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His
770 775 780

His His His His His
785

<210> 118
<211> 2446
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Machupo virus-proTEV2-Histag for expression in S2 cells

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gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

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ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgctcactc caaggaaatt cccagctttc ggtggactca gtcactgaga 720

agaggcctga gtcagttcac ccacactgtg aaaacagatg tgttgaaaga tgccaagctc 780

atagctgaca gcatcgactt caaccaggtt tcacaagtgc agagggctct cagaaagaac 840

aaaaggggtg aagaggatct gaacaagctg agggatttaa acaaagaagt ggataggctc 900

atgtctatga aaagcatcca gaaaaacacc atattcaaga ttggtgatct ggggagagat 960

gaattgatgg agcttgcatc agacttggaa aaactgaaga acaagataaa gaggactgag 1020

tcaggtcccc aagggctgta catgggtaat ttgtcacagc tgcaactgac aaagaggtca 1080

gaaatcttga agaccctggg attccaacag cagagaggtg ctggaaatgg tgtggttaga 1140

atctgggatg tctcagatcc atcaaaactg aataatcagt ttggttccat gccagctctc 1200

acaatcgctt gtatgactgt ccagggtgga gaaacgatga atagtgtggt ccaagcgttg 1260

acatctctgg gcctgttata cactgttaaa tatccgaatt taaatgacct tgacaagcta 1320

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aacatgctgg aaacaattag ggtcactcct gataatttct ccagcttgat taaatcaact 1500

ctgcaagtca agcgaaaaga ggggatgttt atagacgaga aacctgggaa tcgaaatcct 1560

tatgagaacc ttctgtataa attgtgtctc tcaggtgacg ggtggcctta cattggttcc 1620

agatcacaaa ttcttgggag gtcttgggac aacacaagtg ttgatctaac aaagaaacct 1680

caagttggac cgagacaacc cgagaaaaac ggtcagaatc taagactagc aaacctgact 1740

gaaatgcaag aagcagtgat taaagaggct gtaaagaagt tagaccccac taatacactg 1800

tggcttgaca ttgaagggcc tccaacagac cctgtggaat tggcactata tcagccagcc 1860

aacaagcatt atattcattg ttttagaaag ccacatgatg agaagggctt caaaaatggc 1920

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agttatgtaa taggtttact accacaagat atggtgatta caactcaagg ttctgacgac 2040

ataaggaaac ttttagacat tcatggacgg aaggatttaa agctggtaga tgtgaaactc 2100

acatctgatc aagcaagact ctatgatcag caaatttggg agaagtttgg acatctttgc 2160

aaacatcata atggagttgt tgtcaacaag aaaaagagag aaaaagactc tccattcaaa 2220

ttgagttctg gtgaacctca ctgtgctctg ttggattgta tcatgtatca atcagtgatg 2280

gatggcaaaa tggtagatga agaaccagtg gcacttttac ctctcagcct tctatttcta 2340

cccaaggcag cctttgcact cccgggagag aatctatatt ttcaagggcc cggcggaggt 2400

agtcaccatc atcaccatca ctaatgaccg gtgcggccgc aagctt 2446

<210> 119
<211> 789
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-MAC.N-proTEV2-Histag]

<400> 119

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

```
Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
    50                  55                  60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala His
        195                 200                 205

Ser Lys Glu Ile Pro Ser Phe Arg Trp Thr Gln Ser Leu Arg Arg Gly
    210                 215                 220

Leu Ser Gln Phe Thr His Thr Val Lys Thr Asp Val Leu Lys Asp Ala
225                 230                 235                 240

Lys Leu Ile Ala Asp Ser Ile Asp Phe Asn Gln Val Ser Gln Val Gln
                245                 250                 255

Arg Ala Leu Arg Lys Asn Lys Arg Gly Glu Glu Asp Leu Asn Lys Leu
            260                 265                 270

Arg Asp Leu Asn Lys Glu Val Asp Arg Leu Met Ser Met Lys Ser Ile
        275                 280                 285

Gln Lys Asn Thr Ile Phe Lys Ile Gly Asp Leu Gly Arg Asp Glu Leu
    290                 295                 300

Met Glu Leu Ala Ser Asp Leu Glu Lys Leu Lys Asn Lys Ile Lys Arg
305                 310                 315                 320
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```
Thr Glu Ser Gly Pro Gln Gly Leu Tyr Met Gly Asn Leu Ser Gln Leu
                325                 330                 335

Gln Leu Thr Lys Arg Ser Glu Ile Leu Lys Thr Leu Gly Phe Gln Gln
            340                 345                 350

Gln Arg Gly Ala Gly Asn Gly Val Val Arg Ile Trp Asp Val Ser Asp
        355                 360                 365

Pro Ser Lys Leu Asn Asn Gln Phe Gly Ser Met Pro Ala Leu Thr Ile
    370                 375                 380

Ala Cys Met Thr Val Gln Gly Gly Glu Thr Met Asn Ser Val Val Gln
385                 390                 395                 400

Ala Leu Thr Ser Leu Gly Leu Leu Tyr Thr Val Lys Tyr Pro Asn Leu
                405                 410                 415

Asn Asp Leu Asp Lys Leu Thr Leu Glu His Glu Cys Leu Gln Ile Val
            420                 425                 430

Thr Lys Asp Glu Ser Ser Ile Asn Ile Ser Gly Tyr Asn Phe Ser Leu
        435                 440                 445

Ser Ala Ala Val Lys Ala Gly Ala Ser Ile Leu Asp Gly Gly Asn Met
    450                 455                 460

Leu Glu Thr Ile Arg Val Thr Pro Asp Asn Phe Ser Ser Leu Ile Lys
465                 470                 475                 480

Ser Thr Leu Gln Val Lys Arg Lys Glu Gly Met Phe Ile Asp Glu Lys
                485                 490                 495

Pro Gly Asn Arg Asn Pro Tyr Glu Asn Leu Leu Tyr Lys Leu Cys Leu
            500                 505                 510

Ser Gly Asp Gly Trp Pro Tyr Ile Gly Ser Arg Ser Gln Ile Leu Gly
        515                 520                 525

Arg Ser Trp Asp Asn Thr Ser Val Asp Leu Thr Lys Lys Pro Gln Val
    530                 535                 540

Gly Pro Arg Gln Pro Glu Lys Asn Gly Gln Asn Leu Arg Leu Ala Asn
545                 550                 555                 560

Leu Thr Glu Met Gln Glu Ala Val Ile Lys Glu Ala Val Lys Lys Leu
                565                 570                 575

Asp Pro Thr Asn Thr Leu Trp Leu Asp Ile Glu Gly Pro Pro Thr Asp
            580                 585                 590
```

```
Pro Val Glu Leu Ala Leu Tyr Gln Pro Ala Asn Lys His Tyr Ile His
        595                 600                 605

Cys Phe Arg Lys Pro His Asp Glu Lys Gly Phe Lys Asn Gly Ser Arg
    610                 615                 620

His Ser His Gly Ile Leu Met Gln Asp Ile Glu Asp Ala Met Pro Gly
625                 630                 635                 640

Val Leu Ser Tyr Val Ile Gly Leu Leu Pro Gln Asp Met Val Ile Thr
                645                 650                 655

Thr Gln Gly Ser Asp Asp Ile Arg Lys Leu Leu Asp Ile His Gly Arg
            660                 665                 670

Lys Asp Leu Lys Leu Val Asp Val Lys Leu Thr Ser Asp Gln Ala Arg
        675                 680                 685

Leu Tyr Asp Gln Gln Ile Trp Glu Lys Phe Gly His Leu Cys Lys His
    690                 695                 700

His Asn Gly Val Val Val Asn Lys Lys Lys Arg Glu Lys Asp Ser Pro
705                 710                 715                 720

Phe Lys Leu Ser Ser Gly Glu Pro His Cys Ala Leu Leu Asp Cys Ile
                725                 730                 735

Met Tyr Gln Ser Val Met Asp Gly Lys Met Val Asp Glu Glu Pro Val
            740                 745                 750

Ala Leu Leu Pro Leu Ser Leu Leu Phe Leu Pro Lys Ala Ala Phe Ala
        755                 760                 765

Leu Pro Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His
    770                 775                 780

His His His His His
785
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<210> 120
<211> 2434
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Guanarito virus-proTEV2-Histag for expression in S2 cells

<400> 120
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcgctcactc caaagaaatc cccagcttcc gctggactca atctctaagg 720

agagaactag ggatgttcac agaaccaacc aaatcaagtg ttcttaatga tgcaaagctc 780

attgcagact cccttgattt cacacaagtt tctcaagttc aaagactcct acgtaagtcc 840

aaacgaggag acactgatct tgataaactt agggacttaa ataaagaagt tgatagactg 900

atgagcatga aaagtgttca gaacaacaca gttctgaaag ttggtgattt gggcaaagat 960

gaattaatgg acttggcctc tgacctagaa aaactaaaga agaagattgg agatagggaa 1020

agcaatagtc caaggatgta catgggaaac ttgacgcagt cacaattgga aaaaagagca 1080

gggattctta gaaccctggg attccaacaa caaaggggg ctgcaggtgg ggttgtcagg 1140

ttgtgggatg tatctgatcc ctccaaactg aataaccaat ttggttcaat gccagctctt 1200

accattgcct gcatgacagt tcagggagga gaaacaatga acaatgttgt gcaggcacta 1260

acatcacttg gtcttctcta cactgtcaaa tatcccaatc ttgatgacct ggaaaaacta 1320

actttagaac acgactgcct acagattata acaaaggatg agagtgcact caacatatct 1380

ggttataact tcagtctttc agctgctgta aaagctggtg catcacttat agatggtggc 1440

aacatgctgg agacaataaa agtcacaccc aacaacttct cttctattgt caaggccgca 1500

ttgaacgtca aaagaagaga aggcatgttc atagatgaga gaccgggcaa tagaaaccct 1560

tatgagaacc ttctctacaa gctgtgtttg tctggggagg gttggccata tattggatca 1620

aggtcacaaa tactcgggag gtcttgggac aacacaagtg tcgatttaaa tgcaagacct 1680

gtaacaggtc cccgagctcc tgaaaagaat ggacaaaata tcagactatc aaatctttct 1740

gaaatgcaag aagcgatcgt aaaggaagca atgaggaaat tagattcatc agacacaatc 1800

tggatggaca ttgaaggccc gccaactgat cctgtggagt tggcagtttt ccaaccttct 1860

tcaggaaact atgtacactg tttcagaaaa cctcatgatg agaaaggttt taaaaatgga 1920

agtaggcact cacacggcat actattaaag gaccttgaag atgctcaacc tggtctattg 1980

agttacgtca ttggcttatt gccacagggt tcagttatca ctgttcaagg ggcagatgac 2040

atcaaaaagc tattcgacat acatggaagg aaagatttaa aacttgttga tgtcagactg 2100

actggggaac agtccagaat ttttgaacag gaagtttggg aaaaatttgg ccacctctgc 2160

agagcacaca atggtgtcat tgttcctaag aagaaaaaca aggaggctaa ctccacgaag 2220

gagccacact gtgctcttct cgattgcatc atgtttcagt ctgttcttga tggtcatctt 2280

cccgacacca ttcccattca actgctacca aacacattag tgttccaagc caagagcgca 2340

tttgtgatcc cgggagagaa tctatatttt caagggcccg gcggaggtag tcaccatcat 2400

caccatcact aatgaccggt gcggccgcaa gctt 2434

<210> 121
<211> 785
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-GUA.N-proTEV2-Histag]

<400> 121

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

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Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ala His
        195                 200                 205

Ser Lys Glu Ile Pro Ser Phe Arg Trp Thr Gln Ser Leu Arg Arg Glu
    210                 215                 220

Leu Gly Met Phe Thr Glu Pro Thr Lys Ser Ser Val Leu Asn Asp Ala
225                 230                 235                 240

Lys Leu Ile Ala Asp Ser Leu Asp Phe Thr Gln Val Ser Gln Val Gln
                245                 250                 255

Arg Leu Leu Arg Lys Ser Lys Arg Gly Asp Thr Asp Leu Asp Lys Leu
            260                 265                 270

Arg Asp Leu Asn Lys Glu Val Asp Arg Leu Met Ser Met Lys Ser Val
        275                 280                 285

Gln Asn Asn Thr Val Leu Lys Val Gly Asp Leu Gly Lys Asp Glu Leu
    290                 295                 300

Met Asp Leu Ala Ser Asp Leu Glu Lys Leu Lys Lys Lys Ile Gly Asp
305                 310                 315                 320

Arg Glu Ser Asn Ser Pro Arg Met Tyr Met Gly Asn Leu Thr Gln Ser
                325                 330                 335

Gln Leu Glu Lys Arg Ala Gly Ile Leu Arg Thr Leu Gly Phe Gln Gln
            340                 345                 350

Gln Arg Gly Ala Ala Gly Gly Val Val Arg Leu Trp Asp Val Ser Asp
        355                 360                 365

Pro Ser Lys Leu Asn Asn Gln Phe Gly Ser Met Pro Ala Leu Thr Ile
    370                 375                 380

Ala Cys Met Thr Val Gln Gly Gly Glu Thr Met Asn Asn Val Val Gln
385                 390                 395                 400

Ala Leu Thr Ser Leu Gly Leu Leu Tyr Thr Val Lys Tyr Pro Asn Leu
                405                 410                 415

Asp Asp Leu Glu Lys Leu Thr Leu Glu His Asp Cys Leu Gln Ile Ile
            420                 425                 430

Thr Lys Asp Glu Ser Ala Leu Asn Ile Ser Gly Tyr Asn Phe Ser Leu
        435                 440                 445

Ser Ala Ala Val Lys Ala Gly Ala Ser Leu Ile Asp Gly Gly Asn Met
    450                 455                 460
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Leu Glu Thr Ile Lys Val Thr Pro Asn Asn Phe Ser Ser Ile Val Lys
465                 470                 475                 480

Ala Ala Leu Asn Val Lys Arg Arg Glu Gly Met Phe Ile Asp Glu Arg
                485                 490                 495

Pro Gly Asn Arg Asn Pro Tyr Glu Asn Leu Leu Tyr Lys Leu Cys Leu
            500                 505                 510

Ser Gly Glu Gly Trp Pro Tyr Ile Gly Ser Arg Ser Gln Ile Leu Gly
        515                 520                 525

Arg Ser Trp Asp Asn Thr Ser Val Asp Leu Asn Ala Arg Pro Val Thr
    530                 535                 540

Gly Pro Arg Ala Pro Glu Lys Asn Gly Gln Asn Ile Arg Leu Ser Asn
545                 550                 555                 560

Leu Ser Glu Met Gln Glu Ala Ile Val Lys Glu Ala Met Arg Lys Leu
                565                 570                 575

Asp Ser Ser Asp Thr Ile Trp Met Asp Ile Glu Gly Pro Pro Thr Asp
            580                 585                 590

Pro Val Glu Leu Ala Val Phe Gln Pro Ser Ser Gly Asn Tyr Val His
        595                 600                 605

Cys Phe Arg Lys Pro His Asp Glu Lys Gly Phe Lys Asn Gly Ser Arg
    610                 615                 620

His Ser His Gly Ile Leu Leu Lys Asp Leu Glu Asp Ala Gln Pro Gly
625                 630                 635                 640

Leu Leu Ser Tyr Val Ile Gly Leu Leu Pro Gln Gly Ser Val Ile Thr
                645                 650                 655

Val Gln Gly Ala Asp Asp Ile Lys Lys Leu Phe Asp Ile His Gly Arg
            660                 665                 670

Lys Asp Leu Lys Leu Val Asp Val Arg Leu Thr Gly Glu Gln Ser Arg
        675                 680                 685

Ile Phe Glu Gln Glu Val Trp Glu Lys Phe Gly His Leu Cys Arg Ala
    690                 695                 700

His Asn Gly Val Ile Val Pro Lys Lys Lys Asn Lys Glu Ala Asn Ser
705                 710                 715                 720

Thr Lys Glu Pro His Cys Ala Leu Leu Asp Cys Ile Met Phe Gln Ser
                725                 730                 735
```

Val Leu Asp Gly His Leu Pro Asp Thr Ile Pro Ile Gln Leu Leu Pro
740 745 750

Asn Thr Leu Val Phe Gln Ala Lys Ser Ala Phe Val Ile Pro Gly Glu
755 760 765

Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His His His
770 775 780

His
785

<210> 122
<211> 2440
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-SNAPlike-proTEV1-N nucleoprotein of the Sabia virus-proTEV2-Histag for expression in S2 cells

<400> 122
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

gacaaagact gcgaaatgaa aagaactaca ttggattcac cacttgggaa gttggaactg 120

agtggatgcg agcaaggatt gcatgaaatt aagctactgg gaaaaggaac ttctgctgct 180

gatgcagttg aagttccagc accagcagct gttcttggag gtcctgagcc cctcatgcaa 240

gccacagcct ggcttaacgc atatttccac cagcctgagg ccattgagga atttccagtc 300

cccgcccttc accatcctgt gtttcagcag gagagcttca cccgccaggt cctgtggaaa 360

ttgctgaagg tggtcaagtt tggtgaagtg atttcatatc agcaacttgc tgcattggcc 420

ggtaaccccg cagctacagc tgccgtgaaa actgctctca gcggaaatcc tgtgcccatc 480

ctgatccctt gtcacagagt cgtttcatct tccggagctg taggtggcta tgaaggagga 540

ctggcagtta aggagtggct gctggctcat gaaggtcata gacttggaaa gcctgggctg 600

ggtcctgctg gtataggcgc gccagggtcc ctaggtggcg gatccgaaaa cctgtacttc 660

cagagcgata tcagcaactc aaaggaaatc cccagcttca gatggactca atccctgaga 720

agagggctca gtgagttcac aacacccgtg aagaccgatg ttctgaggga tgccaaaatg 780

atacttgatg gtcttgattt caatcaagtc tctcttgttc aaagaatcct tagaaagtct 840

aaaaggaatg atggtgatct tgataaactg agagacctaa ataaagaagt ggacaacctg 900

atgagcatga agagttccca aagagacaca atcttaaaac ttggtgatct caacaaatct 960

gaactgatgg atcttgcatc agacctggag aaactgaaaa gaaaagttgg acaaacagaa 1020

agatcagcct caggaggtgt gtacctggga aacctttccc aatcacagct caccaaaagg 1080

tctgatcttt taaggaaact tggttttcaa cagcagcaag tgaggtctcc aggggttgta 1140

aggatttggg acgtagctga tccgaacagg ctgaataatc aatttggatc tgtccctgca 1200

ctgacaatcg cttgtatgac taaacaaagt gacaatacca tgggggatgt tgttcaggca 1260

ctaacatctt tgggacttct ttatacagtt aagttcccca acctgattga cctagaaaaa 1320

cttacagcag aacatgactg tcttcaaata gtgactaaag atgagagcgg cttgaacatc 1380

tcaggatata actatagtct ttctgcagct gttaaagctg gtgcaacgct tctggatggt 1440

ggtaacatgc tggaaaccat aaggatcact cctgacaact tttctcagat cataaagaca 1500

accctatcca taaagaaaaa ggaaggcatg tttgtagatg agaaacctgg aaatagaaac 1560

ccttatgaaa accttctgta caaaatctgc ctttcaggag aaggttggcc ttacattggc 1620

tccagatccc agatcaaggg taggtcatgg gaaaacacca ctgttgattt aagcacaaag 1680

ccccaacaag ggccgagaac accagaaaag gcaggtcaga acattagact ctcccacttg 1740

actgagttgc aagagtcagt tgtgagagag gcaatgggta agattgaccc aactctgaca 1800

acatggattg acattgaggg taccagtaat gatccggttg aattagcatt gtaccaacca 1860

gacacaggta attatatcct ctgttatagg aaaccacatg atgagaaggg gttcaaaaat 1920

ggtagcaggc attcacatgg gatgttgcta aaggacctag aatctgcaca gccaggcttg 1980

ctcagctatg ttatagggct ccttcctcaa aacatggtcc tcaccaccca aggttcagat 2040

gatataaggc gcttagtaga tacacacggt cgcaaagact taaagattgt cgacattaaa 2100

ttggcatctg aacaggcgag aaagtttgag gagccaatct ggtcagattt tggtcacctc 2160

tgtaagaaac acaatggagt tattgtgcca aagaaaaaga aagacaaaga catcccacag 2220

tcctcagagc cacactgtgc cctacttgat tgtctaatgt ttcagtcagc catagcaggc 2280

caaccacctc aaaccaaact ggaaggttta ttgcctgatg cattgctctt cacactggag 2340

gcagcattca ccatcccggg agagaatcta tattttcaag ggcccggcgg aggtagtcac 2400

catcatcacc atcactaatg accggtgcgg ccgcaagctt 2440

<210> 123
<211> 787
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-proTEV1-SAB.N-proTEV2-Histag]

<400> 123

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

```
Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65                  70                  75                  80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
                85                  90                  95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
            100                 105                 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
        115                 120                 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
            180                 185                 190

Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Ser Asn
        195                 200                 205

Ser Lys Glu Ile Pro Ser Phe Arg Trp Thr Gln Ser Leu Arg Arg Gly
    210                 215                 220

Leu Ser Glu Phe Thr Thr Pro Val Lys Thr Asp Val Leu Arg Asp Ala
225                 230                 235                 240

Lys Met Ile Leu Asp Gly Leu Asp Phe Asn Gln Val Ser Leu Val Gln
                245                 250                 255

Arg Ile Leu Arg Lys Ser Lys Arg Asn Asp Gly Asp Leu Asp Lys Leu
            260                 265                 270

Arg Asp Leu Asn Lys Glu Val Asp Asn Leu Met Ser Met Lys Ser Ser
        275                 280                 285

Gln Arg Asp Thr Ile Leu Lys Leu Gly Asp Leu Asn Lys Ser Glu Leu
    290                 295                 300

Met Asp Leu Ala Ser Asp Leu Glu Lys Leu Lys Arg Lys Val Gly Gln
305                 310                 315                 320

Thr Glu Arg Ser Ala Ser Gly Gly Val Tyr Leu Gly Asn Leu Ser Gln
                325                 330                 335
```

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Ser Gln Leu Thr Lys Arg Ser Asp Leu Leu Arg Lys Leu Gly Phe Gln
            340                 345                 350

Gln Gln Gln Val Arg Ser Pro Gly Val Val Arg Ile Trp Asp Val Ala
        355                 360                 365

Asp Pro Asn Arg Leu Asn Asn Gln Phe Gly Ser Val Pro Ala Leu Thr
    370                 375                 380

Ile Ala Cys Met Thr Lys Gln Ser Asp Asn Thr Met Gly Asp Val Val
385                 390                 395                 400

Gln Ala Leu Thr Ser Leu Gly Leu Leu Tyr Thr Val Lys Phe Pro Asn
                405                 410                 415

Leu Ile Asp Leu Glu Lys Leu Thr Ala Glu His Asp Cys Leu Gln Ile
            420                 425                 430

Val Thr Lys Asp Glu Ser Gly Leu Asn Ile Ser Gly Tyr Asn Tyr Ser
        435                 440                 445

Leu Ser Ala Ala Val Lys Ala Gly Ala Thr Leu Leu Asp Gly Gly Asn
    450                 455                 460

Met Leu Glu Thr Ile Arg Ile Thr Pro Asp Asn Phe Ser Gln Ile Ile
465                 470                 475                 480

Lys Thr Thr Leu Ser Ile Lys Lys Lys Glu Gly Met Phe Val Asp Glu
                485                 490                 495

Lys Pro Gly Asn Arg Asn Pro Tyr Glu Asn Leu Leu Tyr Lys Ile Cys
            500                 505                 510

Leu Ser Gly Glu Gly Trp Pro Tyr Ile Gly Ser Arg Ser Gln Ile Lys
        515                 520                 525

Gly Arg Ser Trp Glu Asn Thr Thr Val Asp Leu Ser Thr Lys Pro Gln
    530                 535                 540

Gln Gly Pro Arg Thr Pro Glu Lys Ala Gly Gln Asn Ile Arg Leu Ser
545                 550                 555                 560

His Leu Thr Glu Leu Gln Glu Ser Val Val Arg Glu Ala Met Gly Lys
                565                 570                 575

Ile Asp Pro Thr Leu Thr Thr Trp Ile Asp Ile Glu Gly Thr Ser Asn
            580                 585                 590

Asp Pro Val Glu Leu Ala Leu Tyr Gln Pro Asp Thr Gly Asn Tyr Ile
        595                 600                 605
```

```
Leu Cys Tyr Arg Lys Pro His Asp Glu Lys Gly Phe Lys Asn Gly Ser
    610                 615                 620

Arg His Ser His Gly Met Leu Leu Lys Asp Leu Glu Ser Ala Gln Pro
625                 630                 635                 640

Gly Leu Leu Ser Tyr Val Ile Gly Leu Leu Pro Gln Asn Met Val Leu
                645                 650                 655

Thr Thr Gln Gly Ser Asp Asp Ile Arg Arg Leu Val Asp Thr His Gly
            660                 665                 670

Arg Lys Asp Leu Lys Ile Val Asp Ile Lys Leu Ala Ser Glu Gln Ala
        675                 680                 685

Arg Lys Phe Glu Glu Pro Ile Trp Ser Asp Phe Gly His Leu Cys Lys
    690                 695                 700

Lys His Asn Gly Val Ile Val Pro Lys Lys Lys Lys Asp Lys Asp Ile
705                 710                 715                 720

Pro Gln Ser Ser Glu Pro His Cys Ala Leu Leu Asp Cys Leu Met Phe
                725                 730                 735

Gln Ser Ala Ile Ala Gly Gln Pro Pro Gln Thr Lys Leu Glu Gly Leu
            740                 745                 750

Leu Pro Asp Ala Leu Leu Phe Thr Leu Glu Ala Ala Phe Thr Ile Pro
        755                 760                 765

Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His
    770                 775                 780

His His His
785
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<210> 124
<211> 992
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding BiPlike-SNAPlike- EDIII protein of the Omsk virus-Histag for expression in S2 cells

<400> 124

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aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240
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acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc aggaggtggc gggctggaaa aactcaagat gaagggtctt 660

acctatacaa tgtgcgacaa ggcaaagttc acgtggaaaa gagctcccac agacagtggg 720

cacgacacag ttgtcatgga agtcgctttc tctggaacaa agccttgcag aatacccgtc 780

agggctgtgg cacatggttc cccagatgtg gatgtggcca tgctcataac gccaaatcca 840

acaatcgaaa acaatggagg tggctttata gagatgcagc tccccccagg agacaacatc 900

atctatgttg gggaactaaa acaccagtgg ttccagaagg ggagtagcat tggcggaggt 960

ggccatcacc atcaccatca ctgatgaccg gt 992

<210> 125
<211> 310
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein
[SNAPlike-OMSK.EDIII-Histag]

<400> 125

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

```
Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Gly
            180                 185                 190

Gly Gly Leu Glu Lys Leu Lys Met Lys Gly Leu Thr Tyr Thr Met Cys
        195                 200                 205

Asp Lys Ala Lys Phe Thr Trp Lys Arg Ala Pro Thr Asp Ser Gly His
    210                 215                 220

Asp Thr Val Val Met Glu Val Ala Phe Ser Gly Thr Lys Pro Cys Arg
225                 230                 235                 240

Ile Pro Val Arg Ala Val Ala His Gly Ser Pro Asp Val Asp Val Ala
                245                 250                 255

Met Leu Ile Thr Pro Asn Pro Thr Ile Glu Asn Asn Gly Gly Gly Phe
            260                 265                 270

Ile Glu Met Gln Leu Pro Pro Gly Asp Asn Ile Ile Tyr Val Gly Glu
        275                 280                 285

Leu Lys His Gln Trp Phe Gln Lys Gly Ser Ser Ile Gly Gly Gly Gly
    290                 295                 300

His His His His His His
305                 310
```

<210> 126
<211> 992
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding BiPlike-SNAPlike- EDIII protein of the Kyasanur Forest Disease virus-Histag for expression in S2 cells

<400> 126

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atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240
```

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc 300

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc aggaggtggc gggcttgaaa aacttaagat gaaagggatg 660

acatacacgg tttgtgaggg atcaaaattt gcttggaaaa ggccgccaac cgacagtgga 720

catgataccg tagtcatgga ggtgacttac accgggagca agccatgcag aataccagtg 780

agagccgtgg cccatggaga acccaatgtt aacgtggcaa gtctaataac cccaaaccca 840

tccatggaaa caactggagg agggttcgtt gagctacagc taccaccagg agacaacatc 900

atctatgttg gtgagctgag ccaccagtgg tttcagaagg gcagcacaat tggcggaggt 960

ggccatcacc atcaccatca ctgatgaccg gt 992

<210> 127
<211> 310
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-KYA.EDIII-Histag]

<400> 127

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

```
Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Gly
            180                 185                 190

Gly Gly Leu Glu Lys Leu Lys Met Lys Gly Met Thr Tyr Thr Val Cys
        195                 200                 205

Glu Gly Ser Lys Phe Ala Trp Lys Arg Pro Pro Thr Asp Ser Gly His
    210                 215                 220

Asp Thr Val Val Met Glu Val Thr Tyr Thr Gly Ser Lys Pro Cys Arg
225                 230                 235                 240

Ile Pro Val Arg Ala Val Ala His Gly Glu Pro Asn Val Asn Val Ala
                245                 250                 255

Ser Leu Ile Thr Pro Asn Pro Ser Met Glu Thr Thr Gly Gly Gly Phe
            260                 265                 270

Val Glu Leu Gln Leu Pro Pro Gly Asp Asn Ile Ile Tyr Val Gly Glu
        275                 280                 285

Leu Ser His Gln Trp Phe Gln Lys Gly Ser Thr Ile Gly Gly Gly Gly
    290                 295                 300

His His His His His His
305                 310


<210>  128
<211>  992
<212>  DNA
<213>  artificial

<220>
<223>  DNA sequence encoding BiPlike-SNAPlike- EDIII protein of the
       Alkhumra virus-Histag for expression in S2 cells

<400>  128
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt aagatctgac       60

aaagactgcg aaatgaaaag aactacattg gattcaccac ttgggaagtt ggaactgagt      120

ggatgcgagc aaggattgca tgaaattaag ctactgggaa aaggaacttc tgctgctgat      180

gcagttgaag ttccagcacc agcagctgtt cttggaggtc ctgagcccct catgcaagcc      240

acagcctggc ttaacgcata tttccaccag cctgaggcca ttgaggaatt tccagtcccc      300
```

gcccttcacc atcctgtgtt tcagcaggag agcttcaccc gccaggtcct gtggaaattg 360

ctgaaggtgg tcaagtttgg tgaagtgatt tcatatcagc aacttgctgc attggccggt 420

aaccccgcag ctacagctgc cgtgaaaact gctctcagcg gaaatcctgt gcccatcctg 480

atcccttgtc acagagtcgt ttcatcttcc ggagctgtag gtggctatga aggaggactg 540

gcagttaagg agtggctgct ggctcatgaa ggtcatagac ttggaaagcc tgggctgggt 600

cctgctggta taggcgcgcc aggaggtggc gggcttgaaa aactcaagat gaaaggaatg 660

acatacacgg tctgtgaggg atcaaagttt gcttggaaga ggccgccaac cgacagtggg 720

catgacactg tggtcatgga agtgacttac actgggagca agccatgcag aataccagtg 780

agagccgtgg cccatggaga acctaatgtc aatgtagcta gcctgataac tccaaatcca 840

tccatggaga caactggagg aggtttcgtt gaactgcagc tgccaccagg agacaacatc 900

atctatgttg gtgagctgag tcaccagtgg tttcagaagg gtagtacaat aggcggaggt 960

ggccatcacc atcaccatca ctgatgaccg gt 992

<210> 129
<211> 310
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SNAPlike-ALK.EDIII-Histag]

<400> 129

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

```
Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
    130                 135                 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145                 150                 155                 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
                165                 170                 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Gly
            180                 185                 190

Gly Gly Leu Glu Lys Leu Lys Met Lys Gly Met Thr Tyr Thr Val Cys
        195                 200                 205

Glu Gly Ser Lys Phe Ala Trp Lys Arg Pro Pro Thr Asp Ser Gly His
    210                 215                 220

Asp Thr Val Val Met Glu Val Thr Tyr Thr Gly Ser Lys Pro Cys Arg
225                 230                 235                 240

Ile Pro Val Arg Ala Val Ala His Gly Glu Pro Asn Val Asn Val Ala
                245                 250                 255

Ser Leu Ile Thr Pro Asn Pro Ser Met Glu Thr Thr Gly Gly Gly Phe
            260                 265                 270

Val Glu Leu Gln Leu Pro Pro Gly Asp Asn Ile Ile Tyr Val Gly Glu
        275                 280                 285

Leu Ser His Gln Trp Phe Gln Lys Gly Ser Thr Ile Gly Gly Gly Gly
    290                 295                 300

His His His His His His
305                 310
```

<210> 130
<211> 1288
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP1 from Lassa virus-SNAPlike-Histag for expression in S2 cells

<400> 130

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atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct       60

accagtctgt acaaggggtg gtacgagctt cagactctgg aactgaacat ggagacactc      120

aacatgacca tgcctctctc ctgcacaaag aacaacagtc atcattacat aatggtgggc      180

aatgagacag gactggaact gaccttgacc aacacgagca tcatcaatca caagttctgc      240

aacctgtctg atgcccacaa gaagaacctc tacgaccacg ctctgatgag cataatctca      300
```

actttccact tgtccatccc caacttcaac cagtacgagg caatgagctg cgatttcaat 360

gggggcaaga tcagtgtgca gtacaacctg agtcacagct acgctgggga tgcagccaac 420

cattgtggca ctgtggcaaa cggtgtgttg cagactttca tgaggatggc ttggggcggg 480

agctacatcg ctcttgactc aggccgtggc aactgggact gtatcatgac tagttaccaa 540

tacctgataa tccagaacac aacctgggaa gatcactgcc aattctccag accatctccc 600

atcggctacc tcgggctcct ctcacaaagg actagagata tttacatcag tagaagattg 660

ctgcggccgc acggcggagg tagcaaagac tgcgaaatga agcgcaccac cctggatagc 720

cctctgggca agctggaact gtctgggtgc gaacagggcc tgcacgagat caagctgctg 780

ggcaaaggaa catctgccgc cgacgccgtg gaagtgcctg ccccagccgc cgtgctgggc 840

ggaccagagc cactgatgca ggccaccgcc tggctcaacg cctactttca ccagcctgag 900

gccatcgagg agttccctgt gccagccctg caccacccag tgttccagca ggagagcttt 960

acccgccagg tgctgtggaa actgctgaaa gtggtgaagt tcggagaggt catcagctac 1020

cagcagctgg ccgccctggc cggcaatccc gccgccaccg ccgccgtgaa aaccgccctg 1080

agcggaaatc ccgtgcccat tctgatcccc tgccaccggg tggtgtctag ctctggcgcc 1140

gtgggggct acgagggcgg gctcgccgtg aaagagtggc tgctggccca cgagggccac 1200

agactgggca agcctgggct gggtcctgca ggtataggcg cgccagggtc cctggagcat 1260

catcatcatc atcattgatg acgggccc 1288

<210> 131
<211> 407
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [LAS.ectoGP1-SNAPlike-Histag]

<400> 131

Arg Ser Thr Ser Leu Tyr Lys Gly Val Tyr Glu Leu Gln Thr Leu Glu
1 5 10 15

Leu Asn Met Glu Thr Leu Asn Met Thr Met Pro Leu Ser Cys Thr Lys
20 25 30

Asn Asn Ser His His Tyr Ile Met Val Gly Asn Glu Thr Gly Leu Glu
35 40 45

Leu Thr Leu Thr Asn Thr Ser Ile Ile Asn His Lys Phe Cys Asn Leu
50 55 60

Ser Asp Ala His Lys Lys Asn Leu Tyr Asp His Ala Leu Met Ser Ile
65 70 75 80

Ile Ser Thr Phe His Leu Ser Ile Pro Asn Phe Asn Gln Tyr Glu Ala
85 90 95

```
Met Ser Cys Asp Phe Asn Gly Gly Lys Ile Ser Val Gln Tyr Asn Leu
            100                 105                 110

Ser His Ser Tyr Ala Gly Asp Ala Ala Asn His Cys Gly Thr Val Ala
        115                 120                 125

Asn Gly Val Leu Gln Thr Phe Met Arg Met Ala Trp Gly Gly Ser Tyr
    130                 135                 140

Ile Ala Leu Asp Ser Gly Arg Gly Asn Trp Asp Cys Ile Met Thr Ser
145                 150                 155                 160

Tyr Gln Tyr Leu Ile Ile Gln Asn Thr Thr Trp Glu Asp His Cys Gln
                165                 170                 175

Phe Ser Arg Pro Ser Pro Ile Gly Tyr Leu Gly Leu Leu Ser Gln Arg
            180                 185                 190

Thr Arg Asp Ile Tyr Ile Ser Arg Arg Leu Leu Arg Pro His Gly Gly
        195                 200                 205

Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu
    210                 215                 220

Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys
225                 230                 235                 240

Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala
                245                 250                 255

Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala
            260                 265                 270

Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro
        275                 280                 285

Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg
    290                 295                 300

Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile
305                 310                 315                 320

Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala
                325                 330                 335

Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro
            340                 345                 350

Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly
        355                 360                 365
```

Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu
370 375 380

Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu
385 390 395 400

Glu His His His His His His
405

<210> 132
<211> 1264
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP1 from Junin virus-SNAPlike-Histag for expression in S2 cells

<400> 132
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

gaggaggctt tcaagatcgg cctgcacacc gagttccaga cggtgtcctt ctcgatggtg 120

ggcctcttct ccaacaaccc acacgacctg cctttgttgt gtaccttgaa caagagccat 180

ctgtacatta agggggggcaa cgcttcattc cagatcagct tcgacgacat cgcggtgttg 240

ttgccacagt acgacgttat catccagcac ccagcagaca tgagctggtg ctccaagagt 300

gatgatcaga tttggttgtc tcagtggttc atgaatgctg tgggacatga ttggcaccta 360

gacccaccat tcctgtgtag gaaccgtaca aagacagaag gcttcatctt ccaagtcaac 420

acctccaaga ctggtgttaa tgaaaattat gctaagaagt tcaagactgg catgcaccac 480

ttgtatagag agtaccctga ctcttgcccg aacggcaagc tgtgcttaat gaaggcacaa 540

cctaccagtt ggcctctcca atgtccactc gaccacgtca acacattaca cttccttaca 600

agaggcaaga acattcagct tccaaggagg tccttgaagc ggccgcacgg cggaggtagc 660

aaagactgcg aaatgaagcg caccaccctg gatagccctc tgggcaagct ggaactgtct 720

gggtgcgaac agggcctgca cgagatcaag ctgctgggca aaggaacatc tgccgccgac 780

gccgtggaag tgcctgcccc agccgccgtg ctgggcggac cagagccact gatgcaggcc 840

accgcctggc tcaacgccta ctttcaccag cctgaggcca tcgaggagtt ccctgtgcca 900

gccctgcacc acccagtgtt ccagcaggag agctttaccc gccaggtgct gtggaaactg 960

ctgaaagtgg tgaagttcgg agaggtcatc agctaccagc agctggccgc cctggccggc 1020

aatcccgccg ccaccgccgc cgtgaaaacc gccctgagcg gaaatcccgt gcccattctg 1080

atcccctgcc accgggtggt gtctagctct ggcgccgtgg ggggctacga gggcgggctc 1140

gccgtgaaag agtggctgct ggcccacgag ggccacagac tgggcaagcc tgggctgggt 1200

cctgcaggta taggcgcgcc agggtccctg gagcatcatc atcatcatca ttgatgacgg 1260

gccc 1264

<210> 133
<211> 399
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [JUN.ectoGP1-SNAPlike-Histag]

<400> 133

```
Arg Ser Glu Glu Ala Phe Lys Ile Gly Leu His Thr Glu Phe Gln Thr
1               5                   10                  15

Val Ser Phe Ser Met Val Gly Leu Phe Ser Asn Asn Pro His Asp Leu
            20                  25                  30

Pro Leu Leu Cys Thr Leu Asn Lys Ser His Leu Tyr Ile Lys Gly Gly
        35                  40                  45

Asn Ala Ser Phe Gln Ile Ser Phe Asp Asp Ile Ala Val Leu Leu Pro
    50                  55                  60

Gln Tyr Asp Val Ile Ile Gln His Pro Ala Asp Met Ser Trp Cys Ser
65                  70                  75                  80

Lys Ser Asp Asp Gln Ile Trp Leu Ser Gln Trp Phe Met Asn Ala Val
                85                  90                  95

Gly His Asp Trp His Leu Asp Pro Pro Phe Leu Cys Arg Asn Arg Thr
            100                 105                 110

Lys Thr Glu Gly Phe Ile Phe Gln Val Asn Thr Ser Lys Thr Gly Val
        115                 120                 125

Asn Glu Asn Tyr Ala Lys Lys Phe Lys Thr Gly Met His His Leu Tyr
    130                 135                 140

Arg Glu Tyr Pro Asp Ser Cys Pro Asn Gly Lys Leu Cys Leu Met Lys
145                 150                 155                 160

Ala Gln Pro Thr Ser Trp Pro Leu Gln Cys Pro Leu Asp His Val Asn
                165                 170                 175

Thr Leu His Phe Leu Thr Arg Gly Lys Asn Ile Gln Leu Pro Arg Arg
            180                 185                 190

Ser Leu Lys Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys
        195                 200                 205

Arg Thr Thr Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys
    210                 215                 220
```

Glu Gln Gly Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala
225 230 235 240

Ala Asp Ala Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro
245 250 255

Glu Pro Leu Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln
260 265 270

Pro Glu Ala Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val
275 280 285

Phe Gln Gln Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys
290 295 300

Val Val Lys Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu
305 310 315 320

Ala Gly Asn Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly
325 330 335

Asn Pro Val Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser
340 345 350

Gly Ala Val Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu
355 360 365

Leu Ala His Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala
370 375 380

Gly Ile Gly Ala Pro Gly Ser Leu Glu His His His His His His
385 390 395

<210> 134
<211> 1297
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP1 from Machupo virus-SNAPlike-Histag for expression in S2 cells

<400> 134
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

gacggcacat tcaagatcgg cctgcacacg gagttccagt cagtcaccct caccatgcag 120

agacttttgg ctaaccattc aaacgagctc ccgtctctct gcatgctgaa caacagtttc 180

tattatatga ggggaggtgt gaacaccttc ctgatccgtg tttctgatat ttcagtcctc 240

atgaaggagt acgatgtatc aatctacgag ccagaggacc tcggaaactg tctgaacaag 300

tctgactcaa gctgggctat ccattggttc tcaaacgctt tgggacatga ctggctgatg 360

gaccctccaa tgctctgtag aaacaagaca aagaaggagg gatctaacat ccaattcaac 420

atcagcaagg ctgatgatgc cagagtgtat ggaaagaaga tcagaaacgg tatgaggcat 480

ctcttcaggg gcttccatga cccgtgtgag gaagggaagg tgtgctacct gaccatcaac 540

cagtgtggtg accccagttc cttcgactac tgtggcgtga accatctgtc caagtgtcag 600

ttcgaccatg tgaacaccct gcatttcctg gtgagaagta agacacatct caacttcgag 660

aggtctttga agcggccgca cggcggaggt agcaaagact gcgaaatgaa gcgcaccacc 720

ctggatagcc ctctgggcaa gctggaactg tctgggtgcg aacagggcct gcacgagatc 780

aagctgctgg gcaaaggaac atctgccgcc gacgccgtgg aagtgcctgc cccagccgcc 840

gtgctgggcg gaccagagcc actgatgcag gccaccgcct ggctcaacgc ctactttcac 900

cagcctgagg ccatcgagga gttccctgtg ccagccctgc accacccagt gttccagcag 960

gagagcttta cccgccaggt gctgtggaaa ctgctgaaag tggtgaagtt cggagaggtc 1020

atcagctacc agcagctggc cgccctggcc ggcaatcccg ccgccaccgc cgccgtgaaa 1080

accgccctga gcggaaatcc cgtgcccatt ctgatcccct gccaccgggt ggtgtctagc 1140

tctggcgccg tgggggggcta cgagggcggg ctcgccgtga aagagtggct gctggcccac 1200

gagggccaca gactgggcaa gcctgggctg ggtcctgcag gtataggcgc gccagggtcc 1260

ctggagcatc atcatcatca tcattgatga cgggccc 1297

<210> 135
<211> 410
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [MAC.ectoGP1-SNAPlike-Histag]

<400> 135

Arg Ser Asp Gly Thr Phe Lys Ile Gly Leu His Thr Glu Phe Gln Ser
1 5 10 15

Val Thr Leu Thr Met Gln Arg Leu Leu Ala Asn His Ser Asn Glu Leu
20 25 30

Pro Ser Leu Cys Met Leu Asn Asn Ser Phe Tyr Tyr Met Arg Gly Gly
35 40 45

Val Asn Thr Phe Leu Ile Arg Val Ser Asp Ile Ser Val Leu Met Lys
50 55 60

Glu Tyr Asp Val Ser Ile Tyr Glu Pro Glu Asp Leu Gly Asn Cys Leu
65 70 75 80

Asn Lys Ser Asp Ser Ser Trp Ala Ile His Trp Phe Ser Asn Ala Leu
85 90 95

Gly His Asp Trp Leu Met Asp Pro Pro Met Leu Cys Arg Asn Lys Thr
100 105 110

```
Lys Lys Glu Gly Ser Asn Ile Gln Phe Asn Ile Ser Lys Ala Asp Asp
        115                 120                 125

Ala Arg Val Tyr Gly Lys Lys Ile Arg Asn Gly Met Arg His Leu Phe
    130                 135                 140

Arg Gly Phe His Asp Pro Cys Glu Glu Gly Lys Val Cys Tyr Leu Thr
145                 150                 155                 160

Ile Asn Gln Cys Gly Asp Pro Ser Ser Phe Asp Tyr Cys Gly Val Asn
                165                 170                 175

His Leu Ser Lys Cys Gln Phe Asp His Val Asn Thr Leu His Phe Leu
            180                 185                 190

Val Arg Ser Lys Thr His Leu Asn Phe Glu Arg Ser Leu Lys Arg Pro
        195                 200                 205

His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp
    210                 215                 220

Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His
225                 230                 235                 240

Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu
                245                 250                 255

Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln
            260                 265                 270

Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu
        275                 280                 285

Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser
    290                 295                 300

Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly
305                 310                 315                 320

Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala
                325                 330                 335

Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile
            340                 345                 350

Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly
        355                 360                 365

Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly
    370                 375                 380
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His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro
385 390 395 400

Gly Ser Leu Glu His His His His His His
405 410

<210> 136
<211> 1246
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP1 from Guanarito virus- SNAPlike-Histag for expression in S2 cells

<400> 136
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ttcaaggttg gtcatcatac gaacttcgag tcgttcacgg ttaagctggg aggtgtcttc 120

catgaattgc cttcgctgtg tagggtcaac aactcctaca gtctgatcag gctctcccat 180

aacagtaacc aggcattgtc ggttgagtac gtggatgtgc accctgtcct ctgttcgtcc 240

agtccaacca tcctcgacaa ctacacgcaa tgtatcaagg gctcgccaga gttcgattgg 300

attctcgggt ggacgatcaa gggattggga catgacttct tgagagatcc aagaatctgc 360

tgtgagccta agaagacgac taacgctgag ttcacgttcc aattgaactt gacggatagt 420

cctgagaccc atcactacag gagcaagatt gaggtaggca tccgacactt gttcgggaac 480

tacatcacca acgatagcta ctcgaagatg tccgtggtta tgaggaacac cacctgggaa 540

ggtcaatgct cgaacagtca tgtgaacacg ctgagattcc tcgttaagaa cgcaggttac 600

ctcgttggaa ggaagccact gcggccgcac ggcggaggta gcaaagactg cgaaatgaag 660

cgcaccaccc tggatagccc tctgggcaag ctggaactgt ctgggtgcga acagggcctg 720

cacgagatca agctgctggg caaaggaaca tctgccgccg acgccgtgga agtgcctgcc 780

ccagccgccg tgctgggcgg accagagcca ctgatgcagg ccaccgcctg gctcaacgcc 840

tactttcacc agcctgaggc catcgaggag ttccctgtgc cagccctgca ccacccagtg 900

ttccagcagg agagctttac ccgccaggtg ctgtggaaac tgctgaaagt ggtgaagttc 960

ggagaggtca tcagctacca gcagctggcc gccctggccg gcaatcccgc cgccaccgcc 1020

gccgtgaaaa ccgccctgag cggaaatccc gtgcccattc tgatcccctg ccaccgggtg 1080

gtgtctagct ctggcgccgt ggggggctac gagggcgggc tcgccgtgaa agagtggctg 1140

ctggcccacg agggccacag actgggcaag cctgggctgg gtcctgcagg tataggcgcg 1200

ccagggtccc tggagcatca tcatcatcat cattgatgac gggccc 1246

<210> 137
<211> 393
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [GUA.ectoGP1-SNAPlike-Histag]

<400> 137

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Arg Ser Phe Lys Val Gly His His Thr Asn Phe Glu Ser Phe Thr Val
1               5                  10                  15

Lys Leu Gly Gly Val Phe His Glu Leu Pro Ser Leu Cys Arg Val Asn
            20                  25                  30

Asn Ser Tyr Ser Leu Ile Arg Leu Ser His Asn Ser Asn Gln Ala Leu
        35                  40                  45

Ser Val Glu Tyr Val Asp Val His Pro Val Leu Cys Ser Ser Ser Pro
    50                  55                  60

Thr Ile Leu Asp Asn Tyr Thr Gln Cys Ile Lys Gly Ser Pro Glu Phe
65                  70                  75                  80

Asp Trp Ile Leu Gly Trp Thr Ile Lys Gly Leu Gly His Asp Phe Leu
                85                  90                  95

Arg Asp Pro Arg Ile Cys Cys Glu Pro Lys Lys Thr Thr Asn Ala Glu
            100                 105                 110

Phe Thr Phe Gln Leu Asn Leu Thr Asp Ser Pro Glu Thr His His Tyr
        115                 120                 125

Arg Ser Lys Ile Glu Val Gly Ile Arg His Leu Phe Gly Asn Tyr Ile
    130                 135                 140

Thr Asn Asp Ser Tyr Ser Lys Met Ser Val Val Met Arg Asn Thr Thr
145                 150                 155                 160

Trp Glu Gly Gln Cys Ser Asn Ser His Val Asn Thr Leu Arg Phe Leu
                165                 170                 175

Val Lys Asn Ala Gly Tyr Leu Val Gly Arg Lys Pro Leu Arg Pro His
            180                 185                 190

Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser
        195                 200                 205

Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu
    210                 215                 220

Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val
225                 230                 235                 240

Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala
                245                 250                 255
```

```
Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu
            260                 265                 270

Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe
        275                 280                 285

Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu
    290                 295                 300

Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala
305                 310                 315                 320

Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu
                325                 330                 335

Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr
            340                 345                 350

Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His
        355                 360                 365

Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly
    370                 375                 380

Ser Leu Glu His His His His His His
385                 390


<210>  138
<211>  1273
<212>  DNA
<213>  artificial

<220>
<223>  DNA sequence encoding ssBiP- Glycoprotein GP1 from Sabia virus-
       SNAPlike-Histag for expression in S2 cells

<400>  138
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct       60

ttcagaatcg gaaggagcac agaattgcag aacatcacgt tcgatatgtt gaaggtgttc      120

gaggaccacc ccacatcctg catggtgaac cattccacct actacgtcca tgagaacaag      180

aacgccactt ggtgtctgga ggtgtccgtg actgatgtta ccctgctcat ggctgaacat      240

gatcgtcaag tcctcaacaa cctgtcgaac tgtgtgcacc ctgcagtcga gcacagaagc      300

aggatggttg gcttgctgga gtggatcttc agagccctga agtacgactt caaccatgat      360

ccaacaccgt tgtgtcagaa gcagacttcg acagtgaacg agacacgtgt gcagatcaac      420

atcactgagg ggttcgggtc ccacgggttc gaagatacca tcctccagag actcggggtt      480

ctgttcggtt cgagaattgc attctcgaac atccaggact tgggtaagaa gaggttcttg      540

ttgatcagga actcgacttg gaagaaccaa tgcgagatga accatgtgaa ctccatgcac      600
```

ttgatgttgg cgaacgctgg tcgctcgtcc ggttcgagaa gaccactgcg gccgcacggc 660

ggaggtagca aagactgcga aatgaagcgc accaccctgg atagccctct gggcaagctg 720

gaactgtctg ggtgcgaaca gggcctgcac gagatcaagc tgctgggcaa aggaacatct 780

gccgccgacg ccgtggaagt gcctgcccca gccgccgtgc tgggcggacc agagccactg 840

atgcaggcca ccgcctggct caacgcctac tttcaccagc ctgaggccat cgaggagttc 900

cctgtgccag ccctgcacca cccagtgttc cagcaggaga gctttacccg ccaggtgctg 960

tggaaactgc tgaaagtggt gaagttcgga gaggtcatca gctaccagca gctggccgcc 1020

ctggccggca atcccgccgc caccgccgcc gtgaaaaccg ccctgagcgg aaatcccgtg 1080

cccattctga tcccctgcca ccgggtggtg tctagctctg gcgccgtggg gggctacgag 1140

ggcgggctcg ccgtgaaaga gtggctgctg gcccacgagg gccacagact gggcaagcct 1200

gggctgggtc ctgcaggtat aggcgcgcca gggtccctgg agcatcatca tcatcatcat 1260

tgatgacggg ccc 1273

<210> 139
<211> 396
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SAB.ectoGP1-SNAPlike-Histag]

<400> 139

Arg Ser Thr Glu Leu Gln Asn Ile Thr Phe Asp Met Leu Lys Val Phe
1 5 10 15

Glu Asp His Pro Thr Ser Cys Met Val Asn His Ser Thr Tyr Tyr Val
20 25 30

His Glu Asn Lys Asn Ala Thr Trp Cys Leu Glu Val Ser Val Thr Asp
35 40 45

Val Thr Leu Leu Met Ala Glu His Asp Arg Gln Val Leu Asn Asn Leu
50 55 60

Ser Asn Cys Val His Pro Ala Val Glu His Arg Ser Arg Met Val Gly
65 70 75 80

Leu Leu Glu Trp Ile Phe Arg Ala Leu Lys Tyr Asp Phe Asn His Asp
85 90 95

Pro Thr Pro Leu Cys Gln Lys Gln Thr Ser Thr Val Asn Glu Thr Arg
100 105 110

Val Gln Ile Asn Ile Thr Glu Gly Phe Gly Ser His Gly Phe Glu Asp
115 120 125

```
Thr Ile Leu Gln Arg Leu Gly Val Leu Phe Gly Ser Arg Ile Ala Phe
    130                 135                 140

Ser Asn Ile Gln Asp Leu Gly Lys Lys Arg Phe Leu Leu Ile Arg Asn
145                 150                 155                 160

Ser Thr Trp Lys Asn Gln Cys Glu Met Asn His Val Asn Ser Met His
                165                 170                 175

Leu Met Leu Ala Asn Ala Gly Arg Ser Ser Gly Ser Arg Arg Pro Leu
            180                 185                 190

Arg Pro His Gly Gly Gly Ser Lys Asp Cys Glu Met Lys Arg Thr Thr
        195                 200                 205

Leu Asp Ser Pro Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly
    210                 215                 220

Leu His Glu Ile Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala
225                 230                 235                 240

Val Glu Val Pro Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu
                245                 250                 255

Met Gln Ala Thr Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala
            260                 265                 270

Ile Glu Glu Phe Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln
        275                 280                 285

Glu Ser Phe Thr Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys
    290                 295                 300

Phe Gly Glu Val Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn
305                 310                 315                 320

Pro Ala Ala Thr Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val
                325                 330                 335

Pro Ile Leu Ile Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val
            340                 345                 350

Gly Gly Tyr Glu Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His
        355                 360                 365

Glu Gly His Arg Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly
    370                 375                 380

Ala Pro Gly Ser Leu Glu His His His His His His
385                 390                 395
```

<210> 140
<211> 1189
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP2 from Lassa virus-SNAPlike-Histag for expression in S2 cells

<400> 140
atgaagttat gcatattact ggccgtcgtg gcctttgttg gcctctcgct cgggagatct 60

ggcacattca catggacact gtcggattct gaaggtaagg acacaccagg gggatactgt 120

ctgaccaggt ggatgctgat cgaggctgaa ctgaagtgct tcgggaacac agctgtggcg 180

aagtgtaacg agaagcatga tgaggagttc tgtgacatgc tgaggctgtt cgacttcaac 240

aagcaagcca tccagaggtt gaaggctgaa gcacagatga gcatccagtt gatcaacaag 300

gcagtgaatg ccttgatcaa cgaccaactg atcatgaaga accatctgcg ggacatcatg 360

ggtatcccat actgtaacta cagcaagtac tggtacctca accacacaac tactgggaga 420

acatcgctgc ccaagtgttg gctggtgtcg aacggttcgt acttgaacga gacccacttc 480

tccgatgaca tcgaacaaca agctgacaac atgatcactg agatgttgca gaaggagtac 540

atggagaggc aggggaagac accgcggccg cacggcggag gtagcaaaga ctgcgaaatg 600

aagcgcacca ccctggatag ccctctgggc aagctggaac tgtctgggtg cgaacagggc 660

ctgcacgaga tcaagctgct gggcaaagga acatctgccg ccgacgccgt ggaagtgcct 720

gccccagccg ccgtgctggg cggaccagag ccactgatgc aggccaccgc ctggctcaac 780

gcctactttc accagcctga ggccatcgag gagttccctg tgccagccct gcaccaccca 840

gtgttccagc aggagagctt tacccgccag gtgctgtgga aactgctgaa agtggtgaag 900

ttcggagagg tcatcagcta ccagcagctg gccgccctgg ccggcaatcc cgccgccacc 960

gccgccgtga aaaccgccct gagcggaaat cccgtgccca ttctgatccc ctgccaccgg 1020

gtggtgtcta gctctggcgc cgtgggggggc tacgagggcg ggctcgccgt gaaagagtgg 1080

ctgctggccc acgagggcca cagactgggc aagcctgggc tgggtcctgc aggtataggc 1140

gcgccagggt ccctggagca tcatcatcat catcattgat gacgggccc 1189

<210> 141
<211> 374
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [LAS.ectoGP2-SNAPlike-Histag]

<400> 141

Arg Ser Gly Thr Phe Thr Trp Thr Leu Ser Asp Ser Glu Gly Lys Asp
1 5 10 15

Thr Pro Gly Gly Tyr Cys Leu Thr Arg Trp Met Leu Ile Glu Ala Glu
20 25 30

```
Leu Lys Cys Phe Gly Asn Thr Ala Val Ala Lys Cys Asn Glu Lys His
        35                  40                  45

Asp Glu Glu Phe Cys Asp Met Leu Arg Leu Phe Asp Phe Asn Lys Gln
    50                  55                  60

Ala Ile Gln Arg Leu Lys Ala Glu Ala Gln Met Ser Ile Gln Leu Ile
65                  70                  75                  80

Asn Lys Ala Val Asn Ala Leu Ile Asn Asp Gln Leu Ile Met Lys Asn
                85                  90                  95

His Leu Arg Asp Ile Met Gly Ile Pro Tyr Cys Asn Tyr Ser Lys Tyr
            100                 105                 110

Trp Tyr Leu Asn His Thr Thr Thr Gly Arg Thr Ser Leu Pro Lys Cys
        115                 120                 125

Trp Leu Val Ser Asn Gly Ser Tyr Leu Asn Glu Thr His Phe Ser Asp
    130                 135                 140

Asp Ile Glu Gln Gln Ala Asp Asn Met Ile Thr Glu Met Leu Gln Lys
145                 150                 155                 160

Glu Tyr Met Glu Arg Gln Gly Lys Thr Pro Arg Pro His Gly Gly Gly
                165                 170                 175

Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
            180                 185                 190

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
        195                 200                 205

Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
    210                 215                 220

Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp
225                 230                 235                 240

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
                245                 250                 255

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
            260                 265                 270

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
        275                 280                 285

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala
    290                 295                 300
```

```
Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
305                 310                 315                 320

His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly
                325                 330                 335

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
            340                 345                 350

Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu
        355                 360                 365

His His His His His His
    370
```

<210> 142
<211> 1189
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP- Glycoprotein GP2 from Junin virus-SNAPlike-Histag for expression in S2 cells

<400> 142

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gcattcttct cctggtcgtt gacagactca tccggcaagg ataccctgg aggctactgc      120

ctggaagagt ggatgctcgt ggcagccaag atgaagtgct tcggcaacac tgctgtggcc      180

aagtgcaact tgaaccatga ctcggagttc tgtgacatgt tgaggctgtt cgattacaac      240

aagaacgcta tcaagaccct gaacgatgag actaagaagc aagtgaacct gatggggcag      300

acaatcaacg ccctgatctc ggacaacttg ttgatgaaga acaagatcag ggaactgatg      360

agtgtccctt actgcaacta cacgaagttc tggtacgtca accacacact ctccggacaa      420

cactcgttgc caaggtgctg gttgatcaag aacaacagct acttgaacat ctccgacttc      480

cgtaacgact ggatcttgga gagtgacttc ttgatctccg agatgctgag caaggagtac      540

tcggacaggc agggtaagac tccgcggccg cacggcggag gtagcaaaga ctgcgaaatg      600

aagcgcacca ccctggatag ccctctgggc aagctggaac tgtctgggtg cgaacagggc      660

ctgcacgaga tcaagctgct gggcaaagga acatctgccg ccgacgccgt ggaagtgcct      720

gccccagccg ccgtgctggg cggaccagag ccactgatgc aggccaccgc ctggctcaac      780

gcctactttc accagcctga ggccatcgag gagttccctg tgccagccct gcaccaccca      840

gtgttccagc aggagagctt tacccgccag gtgctgtgga aactgctgaa agtggtgaag      900

ttcggagagg tcatcagcta ccagcagctg gccgccctgg ccggcaatcc cgccgccacc      960

gccgccgtga aaaccgccct gagcggaaat cccgtgccca ttctgatccc ctgccaccgg     1020

gtggtgtcta gctctggcgc cgtgggggc tacgagggcg ggctcgccgt gaaagagtgg     1080
```

ctgctggccc acgagggcca cagactgggc aagcctgggc tgggtcctgc aggtataggc 1140

gcgccagggt ccctggagca tcatcatcat catcattgat gacgggccc 1189

<210> 143
<211> 374
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [JUN.ectoGP2-SNAPlike-Histag]

<400> 143

Arg Ser Gly Thr Phe Thr Trp Thr Leu Ser Asp Ser Glu Gly Lys Asp
1 5 10 15

Thr Pro Gly Gly Tyr Cys Leu Thr Arg Trp Met Leu Ile Glu Ala Glu
20 25 30

Leu Lys Cys Phe Gly Asn Thr Ala Val Ala Lys Cys Asn Glu Lys His
35 40 45

Asp Glu Glu Phe Cys Asp Met Leu Arg Leu Phe Asp Phe Asn Lys Gln
50 55 60

Ala Ile Gln Arg Leu Lys Ala Glu Ala Gln Met Ser Ile Gln Leu Ile
65 70 75 80

Asn Lys Ala Val Asn Ala Leu Ile Asn Asp Gln Leu Ile Met Lys Asn
85 90 95

His Leu Arg Asp Ile Met Gly Ile Pro Tyr Cys Asn Tyr Ser Lys Tyr
100 105 110

Trp Tyr Leu Asn His Thr Thr Thr Gly Arg Thr Ser Leu Pro Lys Cys
115 120 125

Trp Leu Val Ser Asn Gly Ser Tyr Leu Asn Glu Thr His Phe Ser Asp
130 135 140

Asp Ile Glu Gln Gln Ala Asp Asn Met Ile Thr Glu Met Leu Gln Lys
145 150 155 160

Glu Tyr Met Glu Arg Gln Gly Lys Thr Pro Arg Pro His Gly Gly Gly
165 170 175

Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
180 185 190

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
195 200 205

```
Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
    210                 215                 220

Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp
225                 230                 235                 240

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
                245                 250                 255

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
            260                 265                 270

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
        275                 280                 285

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala
    290                 295                 300

Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
305                 310                 315                 320

His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly
                325                 330                 335

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
            340                 345                 350

Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu
        355                 360                 365

His His His His His His
    370
```

<210> 144
<211> 1189
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-Glycoprotein GP2 from Machupo virus-SNAPlike-Histag for expression in S2 cells

<400> 144

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gcattcttct catggtcgct gaccgactcc tccggcaagg acatgccagg aggttactgt      120

ctggaggaat ggatgttgat cgcagccaag atgaagtgct tcggcaacac cgctgtcgct      180

aagtgtaacc agaaccatga ctcagagttc tgtgatatgc tgaggctatt cgactacaac      240

aagaacgcaa tcaagaccct caacgatgaa tcgaagaagg agatcaacct gctaagccag      300

accgtgaacg ccttgatctc ggataacttg ttaatgaaga acaagatcaa ggagctaatg      360

agcatccctt actgtaatta cacgaagttc tggtacgtca accataccct gacagggcag      420
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cacacgctgc caaggtgttg gttgatcagg aacggaagtt acctcaacac ctcggagttc 480

aggaacgact ggatcttgga gagtgatcac ctcatctcgg agatgttgag taaggaatac 540

gctgagaggc aaggcaagac cccgcggccg cacggcggag gtagcaaaga ctgcgaaatg 600

aagcgcacca ccctggatag ccctctgggc aagctggaac tgtctgggtg cgaacagggc 660

ctgcacgaga tcaagctgct gggcaaagga acatctgccg ccgacgccgt ggaagtgcct 720

gccccagccg ccgtgctggg cggaccagag ccactgatgc aggccaccgc ctggctcaac 780

gcctactttc accagcctga ggccatcgag gagttccctg tgccagccct gcaccaccca 840

gtgttccagc aggagagctt tacccgccag gtgctgtgga aactgctgaa agtggtgaag 900

ttcggagagg tcatcagcta ccagcagctg gccgccctgg ccggcaatcc cgccgccacc 960

gccgccgtga aaaccgccct gagcggaaat cccgtgccca ttctgatccc ctgccaccgg 1020

gtggtgtcta gctctggcgc cgtgggggggc tacgagggcg ggctcgccgt gaaagagtgg 1080

ctgctggccc acgagggcca cagactgggc aagcctgggc tgggtcctgc aggtataggc 1140

gcgccagggt ccctggagca tcatcatcat catcattgat gacgggccc 1189

<210> 145
<211> 374
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [MAC.ectoGP2-SNAPlike-Histag]

<400> 145

Arg Ser Ala Phe Phe Ser Trp Ser Leu Thr Asp Ser Ser Gly Lys Asp
1 5 10 15

Met Pro Gly Gly Tyr Cys Leu Glu Glu Trp Met Leu Ile Ala Ala Lys
20 25 30

Met Lys Cys Phe Gly Asn Thr Ala Val Ala Lys Cys Asn Gln Asn His
35 40 45

Asp Ser Glu Phe Cys Asp Met Leu Arg Leu Phe Asp Tyr Asn Lys Asn
50 55 60

Ala Ile Lys Thr Leu Asn Asp Glu Ser Lys Lys Glu Ile Asn Leu Leu
65 70 75 80

Ser Gln Thr Val Asn Ala Leu Ile Ser Asp Asn Leu Leu Met Lys Asn
85 90 95

Lys Ile Lys Glu Leu Met Ser Ile Pro Tyr Cys Asn Tyr Thr Lys Phe
100 105 110

Trp Tyr Val Asn His Thr Leu Thr Gly Gln His Thr Leu Pro Arg Cys
115 120 125

```
Trp Leu Ile Arg Asn Gly Ser Tyr Leu Asn Thr Ser Glu Phe Arg Asn
    130                 135                 140

Asp Trp Ile Leu Glu Ser Asp His Leu Ile Ser Glu Met Leu Ser Lys
145                 150                 155                 160

Glu Tyr Ala Glu Arg Gln Gly Lys Thr Pro Arg Pro His Gly Gly Gly
                165                 170                 175

Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
            180                 185                 190

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
        195                 200                 205

Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
    210                 215                 220

Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp
225                 230                 235                 240

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
                245                 250                 255

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
            260                 265                 270

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
        275                 280                 285

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala
    290                 295                 300

Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
305                 310                 315                 320

His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly
                325                 330                 335

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
            340                 345                 350

Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu
        355                 360                 365

His His His His His His
    370
```

<210> 146
<211> 1189

<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-Glycoprotein GP2 from Guanarito virus- SNAPlike-Histag for expression in S2 cells

<400> 146
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gcattcttca gttggtcgct gtctgacccg aagggtaatg acatgccagg tggttactgt 120

ctggagaggt ggatgttggt cgctggggac ttgaagtgct tcggcaacac agctgtcgcc 180

aagtgtaact tgaaccatga ttccgagttc tgtgacatgt tgaggctgtt cgacttcaac 240

aagaacgcca tcgagaagct gaacaaccag actaagactg ctgtcaacat gttgactcac 300

tcgatcaaca gtctgatctc cgataacttg ttgatgagga acaagctgaa ggagattttg 360

aaggtcccat actgcaacta cacaagattc tggtacatca accacacgaa gtccggcgag 420

cactcgctgc ctcggtgttg gctggtcagt aacggttcct acttgaacga gagtgacttc 480

aggaacgagt ggatcttgga gagtgatcac ctgatcgcag agatgttgag caaggaatac 540

caagataggc aggggaagac tccgcggccg cacggcggag gtagcaaaga ctgcgaaatg 600

aagcgcacca ccctggatag ccctctgggc aagctggaac tgtctgggtg cgaacagggc 660

ctgcacgaga tcaagctgct gggcaaagga acatctgccg ccgacgccgt ggaagtgcct 720

gccccagccg ccgtgctggg cggaccagag ccactgatgc aggccaccgc ctggctcaac 780

gcctactttc accagcctga ggccatcgag gagttccctg tgccagccct gcaccaccca 840

gtgttccagc aggagagctt tacccgccag gtgctgtgga aactgctgaa agtggtgaag 900

ttcggagagg tcatcagcta ccagcagctg gccgccctgg ccggcaatcc cgccgccacc 960

gccgccgtga aaaccgccct gagcggaaat cccgtgccca ttctgatccc ctgccaccgg 1020

gtggtgtcta gctctggcgc cgtgggggc tacgagggcg ggctcgccgt gaaagagtgg 1080

ctgctggccc acgagggcca cagactgggc aagcctgggc tgggtcctgc aggtataggc 1140

gcgccagggt ccctggagca tcatcatcat catcattgat gacgggccc 1189

<210> 147
<211> 374
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [GUA.ectoGP2-SNAPlike-Histag]

<400> 147

Arg Ser Ala Phe Phe Ser Trp Ser Leu Ser Asp Pro Lys Gly Asn Asp
1 5 10 15

Met Pro Gly Gly Tyr Cys Leu Glu Arg Trp Met Leu Val Ala Gly Asp
20 25 30

```
Leu Lys Cys Phe Gly Asn Thr Ala Val Ala Lys Cys Asn Leu Asn His
        35                  40                  45

Asp Ser Glu Phe Cys Asp Met Leu Arg Leu Phe Asp Phe Asn Lys Asn
    50                  55                  60

Ala Ile Glu Lys Leu Asn Asn Gln Thr Lys Thr Ala Val Asn Met Leu
65                  70                  75                  80

Thr His Ser Ile Asn Ser Leu Ile Ser Asp Asn Leu Leu Met Arg Asn
                85                  90                  95

Lys Leu Lys Glu Ile Leu Lys Val Pro Tyr Cys Asn Tyr Thr Arg Phe
            100                 105                 110

Trp Tyr Ile Asn His Thr Lys Ser Gly Glu His Ser Leu Pro Arg Cys
        115                 120                 125

Trp Leu Val Ser Asn Gly Ser Tyr Leu Asn Glu Ser Asp Phe Arg Asn
    130                 135                 140

Glu Trp Ile Leu Glu Ser Asp His Leu Ile Ala Glu Met Leu Ser Lys
145                 150                 155                 160

Glu Tyr Gln Asp Arg Gln Gly Lys Thr Pro Arg Pro His Gly Gly Gly
                165                 170                 175

Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
            180                 185                 190

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
        195                 200                 205

Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
    210                 215                 220

Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp
225                 230                 235                 240

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
                245                 250                 255

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
            260                 265                 270

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
        275                 280                 285

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala
    290                 295                 300
```

```
Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
305                 310                 315                 320

His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly
                325                 330                 335

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
            340                 345                 350

Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu
        355                 360                 365

His His His His His His
    370
```

<210> 148
<211> 1189
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding ssBiP-Glycoprotein GP2 from Sabia virus-SNAPlike-Histag for expression in S2 cells

<400> 148

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ggcatcttct cctggacgat cacggatgca gtgggcaacg acatgcctgg tggttactgt      120

ctggagagat ggatgctggt gacgtcggat cttaagtgct tcggcaacac ggcactggcg      180

aagtgtaacc tcgaccacga ttcggagttc tgtgacatgt tgaagttgtt cgagttcaac      240

aagaaggcga tcgagacatt gaacgacaac acgaagaaca aggtgaactt gctgacccac      300

tcgatcaacg cattgatctc cgacaacttg ctgatgaaga accgactcaa ggaattgttg      360

aacacgcctt actgtaacta caccaagttc tggtatgtca accacacggc atccggggag      420

cactcattgc cacggtgctg gctggttagg aacaatagct acttgaacga gagtgagttc      480

aggaatgatt ggatcatcga gagtgatcac ttgttgtccg agatgctcaa caaggaatac      540

atcgatagac agggcaagac gccgcggccg cacggcggag gtagcaaaga ctgcgaaatg      600

aagcgcacca ccctggatag ccctctgggc aagctggaac tgtctgggtg cgaacagggc      660

ctgcacgaga tcaagctgct gggcaaagga acatctgccg ccgacgccgt ggaagtgcct      720

gccccagccg ccgtgctggg cggaccagag ccactgatgc aggccaccgc ctggctcaac      780

gcctactttc accagcctga ggccatcgag gagttccctg tgccagccct gcaccaccca      840

gtgttccagc aggagagctt tacccgccag gtgctgtgga aactgctgaa agtggtgaag      900

ttcggagagg tcatcagcta ccagcagctg gccgccctgg ccggcaatcc cgccgccacc      960

gccgccgtga aaaccgccct gagcggaaat cccgtgccca ttctgatccc ctgccaccgg     1020

gtggtgtcta gctctggcgc cgtgggggggc tacgagggcg ggctcgccgt gaaagagtgg     1080

ctgctggccc acgagggcca cagactgggc aagcctgggc tgggtcctgc aggtataggc     1140
```

gcgccagggt ccctggagca tcatcatcat catcattgat gacgggccc 1189

<210> 149
<211> 374
<212> PRT
<213> artificial

<220>
<223> Amino acid sequence of fusion protein [SAB.ectoGP2-SNAPlike-Histag]

<400> 149

Arg Ser Gly Ile Phe Ser Trp Thr Ile Thr Asp Ala Val Gly Asn Asp
1 5 10 15

Met Pro Gly Gly Tyr Cys Leu Glu Arg Trp Met Leu Val Thr Ser Asp
20 25 30

Leu Lys Cys Phe Gly Asn Thr Ala Leu Ala Lys Cys Asn Leu Asp His
35 40 45

Asp Ser Glu Phe Cys Asp Met Leu Lys Leu Phe Glu Phe Asn Lys Lys
50 55 60

Ala Ile Glu Thr Leu Asn Asp Asn Thr Lys Asn Lys Val Asn Leu Leu
65 70 75 80

Thr His Ser Ile Asn Ala Leu Ile Ser Asp Asn Leu Leu Met Lys Asn
85 90 95

Arg Leu Lys Glu Leu Leu Asn Thr Pro Tyr Cys Asn Tyr Thr Lys Phe
100 105 110

Trp Tyr Val Asn His Thr Ala Ser Gly Glu His Ser Leu Pro Arg Cys
115 120 125

Trp Leu Val Arg Asn Asn Ser Tyr Leu Asn Glu Ser Glu Phe Arg Asn
130 135 140

Asp Trp Ile Ile Glu Ser Asp His Leu Leu Ser Glu Met Leu Asn Lys
145 150 155 160

Glu Tyr Ile Asp Arg Gln Gly Lys Thr Pro Arg Pro His Gly Gly Gly
165 170 175

Ser Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro Leu Gly
180 185 190

Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile Lys Leu
195 200 205

Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro Ala Pro
210 215 220

```
Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr Ala Trp
225                 230                 235                 240

Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe Pro Val
                245                 250                 255

Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr Arg Gln
            260                 265                 270

Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val Ile Ser
        275                 280                 285

Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr Ala Ala
    290                 295                 300

Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile Pro Cys
305                 310                 315                 320

His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu Gly Gly
                325                 330                 335

Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg Leu Gly
            340                 345                 350

Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser Leu Glu
        355                 360                 365

His His His His His His
    370
```

<210> 150
<211> 2774
<212> DNA
<213> artificial

<220>
<223> DNA sequence encoding BiPlike- SNAPlike-proTEV1-C protein of Hepatitis E virus- proTEV2-Histag for expression in S2 cells

<400> 150

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ctggcaagat ctgacaaaga ctgcgaaatg aaaagaacta cattggattc accacttggg      120

aagttggaac tgagtggatg cgagcaagga ttgcatgaaa ttaagctact gggaaaagga      180

acttctgctg ctgatgcagt tgaagttcca gcaccagcag ctgttcttgg aggtcctgag      240

cccctcatgc aagccacagc ctggcttaac gcatatttcc accagcctga ggccattgag      300

gaatttccag tccccgccct tcaccatcct gtgtttcagc aggagagctt cacccgccag      360

gtcctgtgga aattgctgaa ggtggtcaag tttggtgaag tgatttcata tcagcaactt      420

gctgcattgg ccggtaaccc cgcagctaca gctgccgtga aaactgctct cagcggaaat      480
```

cctgtgccca tcctgatccc ttgtcacaga gtcgtttcat cttccggagc tgtaggtggc 540

tatgaaggag gactggcagt taaggagtgg ctgctggctc atgaaggtca tagacttgga 600

aagcctgggc tgggtcctgc tggtataggc gcgccagggt ccctaggtgg cggatccgaa 660

aacctgtact tccagagcga tatcaataac atgttctttt gctctgtgca tggagatgcc 720

accatgcgct ctcgggcttt tctgtttttg ttcctcgtgt ttctgcctat gctgcccgcg 780

ccaccggccg gtcagccgtc tggccgccgc cgcgggcggc gcagcggcgg tgccggcggt 840

ggtttctggg gtgaccggat tgattctcag cccttcgccc tcccctatat tcatccaacc 900

aacccccttcg cacctgacat tccagccgca gccggggctg gagctcgccc tcggcagcca 960

gcccgcccac tcggctccgc ttggcgtgac caatcccagc gccccgccac ttccgcccgt 1020

cgtcgatctg ccccagctgg ggcttcgccg ctgactgctg tggccccggc ccccgatact 1080

gttcctgttc ccgatgtcga ttctcgcggc gctatattac gccgccagta taatttatca 1140

acatccccgc taacatctac tattgccact ggtactaacc ttgttctata tgctgctccg 1200

ctgagccctt tgcttccgct ccaagatgga actaacactc acattatggc cactgaagca 1260

tcaaattatg cccagtaccg tgttgtccgc gctaccatcc ggtaccgtcc gcttgtgccg 1320

aacgctgtcg gcggatacgc tatatctatc tctttctggc ctcagacaac tactaccccg 1380

acatctgtgg acatgaactc tatcacctcc acggatgtcc gaatccttgt ccagcctggt 1440

attgcttcag aacttgtgat ccccagtgag cgcctgcatt atcgtaacca aggctggcgc 1500

tctgttgaga cctctggtgt tgcggaggag gaggcgacct ccggccttgt catgctttgc 1560

atccacggat cacctgtaaa ttcttacacc aatacgcctt atactggtgc ccttggcttg 1620

cttgatttcg cactcgagct cgagttccgc aatttgacac ctggtaacac gaacacacgt 1680

gtttcccgct actcgagtag tgcgcgccac aagctacgcc gagggcctga tggcactgct 1740

gagttaacta cgactgctgc tacacgcttt atgaaggacc ttcattttac agggactaat 1800

ggagttggtg aagtcggtcg tggtatagcg ctaactctgt tcaaccttgc tgatacgctt 1860

ctcggcgggc tcccgacaga attgatttcg tcggctggtg gtcagctatt ttattctcgc 1920

cccgtcgtct cagccaatgg cgagccgacg gtgaagctct acacttcagt cgagaacgct 1980

cagcaggata agggtatagc tatcccacat gatattgatc ttggtgagtc ccgtgttgtc 2040

attcaggatt atgataacca acatgagcag gatcgtccca ccccttctcc tgctccctct 2100

cggccttttt ctgtccttcg tgctaatgat gtgctatggc tttcacttac agcagctgag 2160

tatgatcaga ctacctatgg ctcctctact aatcccatgt atgtctctga taccgtgaca 2220

tttgtcaatg ttgctactgg tgcccagggg gtatctcgct ctctggactg gtctaaagtc 2280

acccttgatg ggcgcccact tatgactatc cagcagtatt ctaagacttt ctttgttctg 2340

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aattataata ctactgccag tgaccagatt ttaattgaga atgcagctgg tcaccgtgta 2460

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gctcacactt ttgatgattt ctgccctgag tgccgtacac tcggccttca gggttgtgct 2640

ttccaatcaa ctgttgctga gctacagcgt cttaaaatga aggtgggtaa aactcgggag 2700

tacccgggag agaatctata ttttcaaggg cccggcggag gtagtcacca tcatcaccat 2760

cactaatgac cggt 2774

<210> 151
<211> 899
<212> PRT
<213> artificial

<220>
<223> Aa sequence of fusion protein
[SNAPlike-proTEV1-HEV.C-proTEV2-Histag]

<400> 151

Arg Ser Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro
1 5 10 15

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile
20 25 30

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro
35 40 45

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr
50 55 60

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe
65 70 75 80

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr
85 90 95

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val
100 105 110

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr
115 120 125

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile
130 135 140

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu
145 150 155 160

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg
165 170 175

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly Ala Pro Gly Ser
180 185 190

```
Leu Gly Gly Gly Ser Glu Asn Leu Tyr Phe Gln Ser Asp Ile Asn Asn
        195                 200                 205

Met Phe Phe Cys Ser Val His Gly Asp Ala Thr Met Arg Ser Arg Ala
    210                 215                 220

Phe Leu Phe Leu Phe Leu Val Phe Leu Pro Met Leu Pro Ala Pro Pro
225                 230                 235                 240

Ala Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg Arg Ser Gly Gly Ala
                245                 250                 255

Gly Gly Gly Phe Trp Gly Asp Arg Ile Asp Ser Gln Pro Phe Ala Leu
            260                 265                 270

Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro Asp Ile Pro Ala Ala
        275                 280                 285

Ala Gly Ala Gly Ala Arg Pro Arg Gln Pro Ala Arg Pro Leu Gly Ser
    290                 295                 300

Ala Trp Arg Asp Gln Ser Gln Arg Pro Ala Thr Ser Ala Arg Arg Arg
305                 310                 315                 320

Ser Ala Pro Ala Gly Ala Ser Pro Leu Thr Ala Val Ala Pro Ala Pro
                325                 330                 335

Asp Thr Val Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg
            340                 345                 350

Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser Thr Ile Ala Thr
        355                 360                 365

Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Ser Pro Leu Leu Pro
    370                 375                 380

Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu Ala Ser Asn
385                 390                 395                 400

Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile Arg Tyr Arg Pro Leu
                405                 410                 415

Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser Phe Trp Pro
            420                 425                 430

Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser Ile Thr Ser
        435                 440                 445

Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser Glu Leu Val
    450                 455                 460
```

```
Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp Arg Ser Val
465                 470                 475                 480

Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr Ser Gly Leu Val Met
                485                 490                 495

Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn Thr Pro Tyr
            500                 505                 510

Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu Glu Phe Arg
        515                 520                 525

Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg Tyr Ser Ser
    530                 535                 540

Ser Ala Arg His Lys Leu Arg Arg Gly Pro Asp Gly Thr Ala Glu Leu
545                 550                 555                 560

Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu His Phe Thr Gly
                565                 570                 575

Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala Leu Thr Leu Phe
            580                 585                 590

Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr Glu Leu Ile Ser
        595                 600                 605

Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn
    610                 615                 620

Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln
625                 630                 635                 640

Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp Leu Gly Glu Ser Arg
                645                 650                 655

Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr
            660                 665                 670

Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp
        675                 680                 685

Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp Gln Thr Thr Tyr
    690                 695                 700

Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr Val Thr Phe Val
705                 710                 715                 720

Asn Val Ala Thr Gly Ala Gln Gly Val Ser Arg Ser Leu Asp Trp Ser
                725                 730                 735
```

```
Lys Val Thr Leu Asp Gly Arg Pro Leu Met Thr Ile Gln Gln Tyr Ser
            740                 745                 750

Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu
        755                 760                 765

Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala
    770                 775                 780

Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His Arg Val Cys Ile
785                 790                 795                 800

Ser Thr Tyr Thr Thr Asn Leu Gly Ser Gly Pro Val Ser Ile Ser Ala
                805                 810                 815

Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Ala Leu Glu Asp Thr
            820                 825                 830

Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu
        835                 840                 845

Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Val Ala
    850                 855                 860

Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Tyr Pro
865                 870                 875                 880

Gly Glu Asn Leu Tyr Phe Gln Gly Pro Gly Gly Gly Ser His His His
                885                 890                 895

His His His
```

<210> 152
<211> 51
<212> DNA
<213> artificial

<220>
<223> DNA encoding biPlike sequence

<400> 152
atgaaactat gtattctact tgcagttgtt gcgttcgtag gattgtcctt a 51

<210> 153
<211> 17
<212> PRT
<213> artificial

<220>
<223> Amino acid of BIPlike sequence

<400> 153

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1   5   10   15

Leu