

RECOMBINANT NUCLEOCAPSID PROTEIN AND G GLYCOPROTEIN OF NIPAH VIRUS

Technical Field of the Invention

5 The present invention relates to the construction of recombinant expression vectors harbouring the nucleocapsid (N) and glycoprotein G genes of Nipah virus (NiV), and the expression of the corresponding proteins in *Escherichia coli* and baculovirus, respectively.

Background of the Invention

10 Nipah virus (NiV) is the causative agent for the outbreak of fatal viral encephalitis, which causes respiratory and neurological syndromes and deaths in swine (Harcourt *et al.*, 2000). A recent outbreak in Malaysia which claimed 105 human lives was controlled only after more than 1 million swine in the affected areas were culled (Chua *et al.*, 2000); this nearly abolished the swine industry in the country.

15 NiV has a nonsegmented, single-stranded negative sense RNA genome, which encodes six main structural proteins. The virion is enveloped by a lipid bilayer containing two transmembrane glycoproteins: a cell receptor binding protein known as G, and a fusion (F) protein (Chua *et al.*, 2000). These glycoproteins are implicated in an immunoreaction. Lining the interior surface of the viral envelope is the matrix (M) protein, which is believed to link the glycoproteins to the viral nucleocapsid or
20 ribonucleoprotein (RNP) complex. The latter is made up of many copies of nucleoprotein (N) that encapsidates the RNA genome. Associated with the RNP are the phosphoprotein (P) and the large protein or RNA polymerase (L). These three components are believed to be involved in replication as well as transcription of viral RNA (vRNA) (Lamb & Kolakofsky, 1996). The N protein is the most abundant
25 structural protein and the essential component of the helical nucleocapsid (Yu *et al.*, 1998).

Currently the viral proteins have been prepared by directly culturing the virus on animal cell cultures. However, NiV has been classified by the Center for Disease Control in Atlanta, United States into Biosafety Level 4, putting it in the class known

as "the most dangerous organisms known to man", an "elite" group of bugs which includes the Ebola virus. Hence, it is absolutely dangerous to culture the live virus. Furthermore, it is very difficult to purify the proteins from the intact virion. Hence, recombinant DNA technology provides an alternative means to produce the viral proteins.

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The inventors have now successfully developed a novel expression system capable of producing the NiV N protein intact or in a fused form either as a full length protein (N_{fus}) or truncated forms in *E. coli*. The inventors had discovered that these fusion proteins bearing the *myc* epitope and six residues of His at their carboxyl terminal end could be successfully produced in *E. coli* by means of recombinant DNA technologies. The N_{fus} protein and its derivatives were expressed to a substantial level in the bacteria as recognised by swine anti-NiV serum indicating the potential of these products as diagnostic reagents. As the G glycoprotein is highly glycosylated, the inventors have developed a novel baculovirus expression system capable of producing truncated G glycoprotein. The recombinant G protein was detected by the swine anti-NiV serum and also the anti-G monoclonal antibody, implying the potential of the recombinant glycoprotein as a diagnostic reagent.

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Summary of the Invention

Accordingly, it is the primary object of the present invention to provide a recombinant expression plasmid containing the nucleocapsid (N) gene of Nipah virus (NiV) having the following nucleotide sequence:

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61  GATGGGAGGGCTAGTGCAGCAACTGCTACTTTGACAACCAAGATAAGGATATTTGTACCA  120
121 GCTACTAATAGTCCAGAGCTCAGATGGGAACTAACATTGTTTGCACTTGATGTGATTAGA  180
181 TCTCCGAGTGCTGCCGAGTCAATGAAAGTTGGAGCTGCTTTCACACTCATCTCTATGTAT  240
241 TCAGAGAGACCCGGGGCTCTCATTAGAAGTCTCCTCAATGACCCAGACATTGAAGCTGTA  300
301 ATAATAGATGTTGGATCAATGGTCAACGGAATACCAGTAATGGAGAGGAGAGGAGACAAG  360
361 GCTCAGGAGGAGATGGAAGGCTTGATGAGAATCCTCAAACTGCTCGAGACAGCAGCAAG  420
421 GGAAAAACACCTTTTGTGACAGCCGAGCTTACGGCCTACGGATAACAGACATGAGCACC  480
481 CTGGTCTCTGCAGTTATCACCATCGAGGCCAGATCTGGATACTGATCGCTAAAGCAGTT  540
541 ACAGCTCCCGACACTGCCGAGGAAAGTGAACTAGAAGATGGGCTAAATACGTCCAACAA  600
601 AAGAGGGTCAATCCGTTCTTTGCTCTAACTCAGCAATGGCTAACAGAAATGAGGAATCTG  660

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661 CTCTCCCAGAGTCTATCAGTAAGGAAGTTCATGGTTGAGATCCTCATAGAAGTCAAGAAA 720
 721 GGAGGATCTGCTAAAGGCAGAGCAGTAGAAATAATCTCAGACATCGGAACTATGTGAG 780
 781 GAAACTGGTATGGCAGGATTCTTCGCAACCATCAGATTCGGGTTGGAGACAAGGTATCCA 840
 841 GCACTTGCACTCAACGAATTCCAGAGTGACCTCAACACCATCAAAAGCTTGATGCTACTC 900
 5 901 TACAGAGAAATTGGCCCAAGAGCCCCTTATATGGTGCTTCTTGAAGAATCAATTCAGACT 960
 961 AAATTTGCCCCTGGAGGTTACCCATTATTGTGGAGCTTTGCCATGGGTGTGGCTACTACT 1020
 1021 ATTGACAGGTCTATGGGGGCATTGAATATCAATCGTGGTTATCTTGAGCCTATGTATTTT 1080
 1081 AGACTAGGCCAAAAATCAGCACGTCAACCATGCTGGAGGAATTGATCAGAACATGGCAAAT 1140
 1141 AGACTGGGACTAAGTTCAGATCAAGTTGCAGAACTCGCTGCTGCAGTTCAGGAAACATCA 1200
 10 1201 GCAGGAAGGCAAGAGAGTAATGTTTCAGGCTAGAGAGGCAAAATTTGCTGCAGGAGGTGTG 1260
 1261 CTCATTGGAGGCAGTGATCAAGATATCGATGAAGGGGAAGAACCTATAGAACAGAGTGGC 1320
 1321 AGACAGTCAGTTACCTTCAAAGGGGAGATGAGTATTTTCATCCCTTGCTAACAGTGTGCCG 1380
 1381 AGCAGTTCTGTGAGCACATCCGGTGGGACCAGATTGACTAATTCATTACTAAACCTCAGA 1440
 1441 TCAAGACTGGCTGCAAAGCAGCAAAAGAAGCCGCCTCATCCAATGCAACAGATGATCCA 1500
 15 1501 GCAATCAGCAACAGAACTCAAGGGGAATCAGAGAAGAAGAATAATCAAGACCTCAAACCT 1560
 1561 GCTCAAAATGACCTTGATTTTCGTCAGAGCTGATGTG 1596

It is another object of the present invention to provide recombinant plasmids harbouring part of the nucleocapsid gene.

It is yet another object of the present invention to provide a recombinant expression plasmid containing the truncated G gene of NiV having the following nucleotide sequence:

1 ATGGACAATCAGGCCGTGATCAAAGATGCGTTGCAGGGTATCCAACAGCAGATCAAAGGG 60
 61 CTTGCTGACAAAATCGGCACAGAGATAGGGCCCAAAGTATCACTGATTGACACATCCAGT 120
 121 ACCATTACTATCCCAGCTAACATTGGGCTGTTAGGTTCAAAGATCAGCCAGTCGACTGCA 180
 25 181 AGTATAAATGAGAATGTGAATGAAAATGCAAATTCACACTGCCTCCCTTGAAAATCCAC 240
 241 GAATGTAACATTTCTTGTCCTAACCCACTCCCTTTTAGAGAGTATAGGCCACAGACAGAA 300
 301 GGGGTGAGCAATCTAGTAGGATTACCTAATAATATTTGCCTGCAAAGACATCTAATCAG 360
 361 ATATTGAAGCCAAAGCTGATTTTCATACACTTTACCCGTAGTCGGTCAAAGTGGTACCTGT 420
 421 ATCAGACACCCATTGCTGGCTATGGACGAGGGCTATTTTGCATATAGCCACCTGGAAAGA 480
 30 481 ATCGGATCATGTTCAAGAGGGGTCTCCAAACAAAGAATAATAGGAGTTGGAGAGGTACTA 540
 541 GACAGAGGTGATGAAGTTCCTTCTTTATTTATGACCAATGTCTGGACCCACCAAATCCA 600
 601 AACACCGTTTACCACTGTAGTGCTGTATACAACAATGAATTCTATTATGTACTTTGTGCA 660
 661 GTGTCAACTGTTGGAGACCCTATTCTGAATAGCACCTACTGGTCCGGATCTCTAATGATG 720

721 ACCCGTCTAGCTGTGAAACCCAAGAGTAATGGTGGGGGTTACAATCAACATCAACTTGCC 780
 781 CTACGAAGTATCGAGAAAGGGAGGTATGATAAAGTTATGCCGTATGGACCTTCAGGCATC 840
 841 AAACAGGGTGACACCCTGTATTTTCCTGCTGTAGGATTTTGGTCAGGACAGAGTTTAAA 900
 901 TACAATGATTCAAATTGTCCCATCACGAAGTGTCAATACAGTAAACCTGAAAATTGCAGG 960
 5 961 CTATCTATGGGGATTAGACCAAACAGCCATTATATCCTTCGATCTGGACTATTAAAATA 1020
 1021 AATCTATCAGATGGGGAGAACCCCAAAGTTGTATTCATTGAAATATCTGATCAAAGATTA 1080
 1081 TCTATTGGATCTCCTAGCAAAATCTATGATTCTTTGGGTCAACCTGTTTTCTACCAAGCG 1140
 1141 TCATTTTCATGGGATACTATGATTAAATTTGGAGATGTTCTAACAGTCAACCCTCTGGTT 1200
 1201 GTCAATTGGCGTAATAACACGGTAATATCAAGACCCGGGCAATCACAATGCCCTAGATTC 1260
 10 1261 AATACATGTCCAGAGATCTGCTGGGAAGGAGTTTATAATGATGCATTCCTAATTGACAGA 1320
 1321 ATCAATTGGATAAGCGCGGGTGTATTCCTTGACAGCAATCAGACCGCAGAAAATCCTGTT 1380
 1381 TTTACTGTATTCAAAGATAATGAAATACTTTATAGGGCACAACTGGCTTCTGAGGACACC 1440
 1441 AATGCACAAAAACAATAACTAATTGTTTTCTCTTGAAGAATAAGATTGGTGCATATCA 1500
 1501 TTGGTTGAG 1509

15 It is yet another object of the present invention to provide transformed *E. coli* cells with the recombinant expression plasmids of the present invention.

It is a further object of the present invention to provide transformed *Spodoptera frugiperda* (Sf9) cells with the recombinant expression plasmids of the present invention.

20 It is yet another object of the present invention to provide a method for producing fused and truncated forms of nucleocapsid protein to high yield, which comprises culturing the transformed *E. coli* cells of the present invention in an appropriate medium and conditions to produce the said nucleocapsid proteins.

25 It is yet a further object of the present invention to provide a method for producing recombinant truncated G glycoprotein, which comprises culturing the transfected *Spodoptera frugiperda* (Sf9) cells of the present invention in an appropriate medium and conditions to produce the said glycoprotein.

It is yet a further object of the present invention to provide a full-length nucleocapsid protein of Nipah virus with the following amino acid sequence:

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It is another object of the present invention to provide a truncated G glycoprotein of Nipah virus with the following amino acid sequence:

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It is yet another object of the present invention to provide two methods for purifying recombinant nucleocapsid particles.

25 These and other objects of the present invention are accomplished by providing,

A recombinant expression plasmid containing Nipah virus nucleocapsid gene;

A recombinant plasmid containing the gene of truncated G glycoprotein of Nipah virus;

30 A recombinant nucleocapsid protein of Nipah virus that is capable of assembling into a particle form;

A method for producing recombinant nucleocapsid protein comprising:

- a) growing recombinant *E. coli* containing nucleocapsid protein genes of Nipah virus in a medium;
- b) allowing the recombinant protein to propagate in *E. coli*;
- 5 c) harvesting and lysing the *E. coli* cells; and
- d) purifying the lysate to obtain recombinant nucleocapsid protein.

A method for producing recombinant truncated nucleocapsid protein comprising:

- a) growing recombinant *E. coli* containing truncated nucleocapsid protein genes of Nipah virus in a medium;
- 10 b) allowing the recombinant protein to propagate in *E. coli*;
- c) harvesting and lysing the *E. coli* cells; and
- d) purifying the lysate to obtain recombinant truncated nucleocapsid protein.

A method for producing recombinant truncated G glycoprotein comprising:

- a) transfecting an insect cell culture with a baculovirus containing a recombinant plasmid carrying truncated G glycoprotein genes of Nipah virus;
- 15 b) allowing the recombinant protein to propagate in the insect cells;
- c) harvesting and lysing the insect cells; and
- d) purifying the lysate to obtain recombinant truncated G glycoprotein.

A method for purifying the recombinant nucleocapsid protein comprising:

- 20 a) precipitating the soluble recombinant protein with ammonium sulphate;
- b) dialysing the precipitated recombinant protein obtained in (a) with buffer;
- c) fractionating the recombinant protein using sucrose gradient centrifugation or nickel affinity column chromatography; and
- d) collecting and pooling the purified protein fractions for concentration of the
- 30 protein.

Brief Description of the Figures and Table

Table 1 is a summary of seven recombinant expression plasmids encoding the full-length, fused and truncated N proteins.

Figure 1 is a schematic diagram showing the full-length, fused and truncated nucleocapsid proteins of Nipah virus.

Figure 2 is a Western blot of N fusion protein of Nipah virus expressed by *E. coli* detected by swine anti-Nipah virus serum.

5 Figure 3 is a Western blot of fused and truncated nucleocapsid proteins of Nipah virus expressed by *E. coli* cells.

Figure 4 is a SDS-12% polyacrylamide gel of N fusion protein purified with an affinity chromatography column and sucrose gradient centrifugation.

Figure 5 shows the Western blot of purified recombinant nucleocapsid particles.

10 Figure 6 shows transmission electron microscopy (TEM) photographs of the purified recombinant nucleocapsid particles.

Figure 7 shows the Western blots of truncated G glycoprotein of Nipah virus expressed by *Spodoptera frugiperda* (Sf9) cells.

Detailed Description of the Figures and Table

15 Table 1 is a summary of seven recombinant expression plasmids encoding the full-length, fused and truncated N proteins. The nucleotide sequences of the primers used in reverse transcription-polymerase chain reaction (RT-PCR) are shown in the Detailed Description of the Invention. The table also shows the length of the coding regions and number of amino acids of the truncated mutants.

20 Figure 1 is a schematic diagram showing the full-length, fused and truncated nucleocapsid proteins of Nipah virus. (a) full-length N protein, (b) N_{fus} protein containing the *myc* and His-tag at the 3' end, (c) 25% deletion from the N-terminus of the N coding region; (d) 25% deletion from the C-terminus of the N coding region; (e) 50% deletion from the N-terminus of the N coding region; (f) 50% deletion from the

C-terminus of the N coding region; (g) 25% deletion from the N- and C-termini of the N coding region.

Figure 2 is a Western blot of N fusion protein of Nipah virus expressed by *E. coli* detected by swine anti-Nipah virus serum. Lane 1, protein markers in kDa; lane 2, negative control (pTrcHis2 in *E. coli* cells); lane 3, N_{fus} protein was fractionated on a SDS-12% polyacrylamide gel, electrotransferred to a nitrocellulose membrane and probed with the swine anti-NiV serum. The molecular mass of the N_{fus} protein is about 63 kDa.

Figure 3 is a Western blot of fused and truncated nucleocapsid proteins of Nipah virus expressed by *E. coli* cells. N_{fus} protein and its mutants were fractionated on a SDS-12% polyacrylamide gel, electrotransferred to a nitrocellulose membrane and probed with the anti-*myc* monoclonal antibody, which detected the *myc* epitope fused to the C-terminal end of N proteins. Lane 1, molecular markers in kDa; lane 2, negative control (pTrcHis2 in *E. coli* cells); lane 3, *E. coli* cells harbouring plasmid pTrcHis-N_{fus}; lane 4, *E. coli* cells harbouring plasmid pMN25_{fus}; lane 5, *E. coli* cells harbouring plasmid pMC25_{fus}; lane 6, *E. coli* cells harbouring plasmid pMN50_{fus}; lane 7, *E. coli* cells harbouring plasmid pMC50_{fus}; lane 8, *E. coli* cells harbouring plasmid pMNC25_{fus}.

Figure 4 is a SDS-12% polyacrylamide gel of N fusion protein purified with an affinity chromatography column and sucrose gradient centrifugation. Lane 1, molecular markers in kDa; lane 2, *E. coli* cells harbouring pTrcHis vector as negative control; lane 3, non-purified N_{fus} protein; lane 4, N fusion protein purified with an affinity chromatography column; lane 5, N fusion protein purified with sucrose gradient centrifugation.

Figure 5 shows the Western blot of purified recombinant nucleocapsid particles. Proteins were fractionated on a SDS-12% polyacrylamide gel, blotted onto a nitrocellulose membrane and probed with anti-NiV serum. Lane 1, molecular weight markers in kDa; lane 2, *E. coli* cells harbouring pTrcHis2 vector as negative control; lane 3, N_{fus} protein purified with sucrose gradient centrifugation.

Figure 6 shows transmission electron microscopy (TEM) photographs of the purified recombinant nucleocapsid particles. Arrows indicate nucleocapsid particles.

Figure 7 shows the Western blots of truncated G glycoprotein of Nipah virus expressed by *Spodoptera frugiperda* (Sf9) cells. Transfected insect cells were fractionated on SDS-PAGE. Their proteins were blotted onto nitrocellulose membranes and probed with (a) swine anti-NIV serum and (b) anti-G monoclonal antibody. Lane 1: non-transfected insect cells; lane 2: transfected insect cells and; lane M: molecular markers in kDa.

Detailed Description of the Invention

In order that the invention described herein will be more fully understood, the following detailed description is divided into two sections. The materials and methods employed in this invention are set forth in the examples. It should be understood that the examples are for illustration purposes only and are not to be construed as limiting this invention in any manner.

Construction of Recombinant Expression Vectors encoding Full-length, Fusion and Truncated N Proteins

The N coding region of the NiV swine isolate VRI 2794/99, which had been cloned into the expression vector, was prepared through reverse transcription-polymerase chain reaction (RT-PCR). Three primers were used for the amplification, which consisted of one forward and two reverse primers as listed below:

NF1 (24 nucleotides): 5' - atg agt gat atc ttt gaa gag gcg - 3' (a forward primer)

NR1 (27 nucleotides): 5' - tca cac atc agc tct gac gaa atc aag - 3' (a reverse primer)

NR2 (24 nucleotides): 5' - cac atc agc tct gac gaa atc aag - 3' (a reverse primer)

Incorporation of the primers designated as NF1 and NR1 (for the N gene) during Polymerase Chain Reaction (PCR) had amplified a DNA fragment containing a stop codon at the 3' end, while the presence of primers NF1 and NR2 gave rise to a DNA fragment without any stop codon. The PCR products were ligated to pTrcHis2 vector

(Invitrogen, USA) containing the coding region for the *myc* epitope and 6 His residues downstream of the multiple cloning site.

A total of five PCR products (Table 1) containing the coding region of the truncated mutants were produced using the following primers:

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| 5 | NF1 (24 nucleotides): | 5' - atg agt gat atc ttt gaa gag gcg - 3' (a forward primer) |
| | NF5 (24 nucleotides): | 5' - aaa act gct cga gac agc agc aag - 3' (a forward primer) |
| | NF6 (24 nucleotides): | 5' - ttc ttc gca acc atc aga ttc ggg - 3' (a forward primer) |
| | NR2 (24 nucleotides): | 5' - cac atc agc tct gac gaa atc aag - 3' (a reverse primer) |
| | NR5 (24 nucleotides): | 5' - tga tgt ttc ctg aac tgc agc agc - 3' (a reverse primer) |
| 10 | NR6 (24 nucleotides): | 5' - tcc tgc cat acc agt ttc ctc gag - 3' (a reverse primer) |

All the PCR products were ligated to the pTrcHis2 vector and introduced into *E. coli* strain BL 21 for expression.

- The nucleotide sequence of the N gene was determined by the ABI PRISM automated sequencer, model 377. The recombinant plasmid encoding the N fusion protein, pTrcHis-N_{fus}, was used as a template and the synthetic primers used in the sequencing reactions of the N gene are as follows:

For the sequencing of the N gene coding region

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| | pTrcHis2F (21 nucleotides): | 5' - gag gta tat att aat gta tcg - 3' |
| | sNF1 (24 nucleotides): | 5' - atg agt gat atc ttt gaa gag gcg - 3' |
| 20 | sNF2 (24 nucleotides): | 5' - aaa act gct cga gac agc agc aag - 3' |
| | sNF3 (24 nucleotides): | 5' - ttc ttc gca acc atc aga ttc ggg - 3' |
| | sNF4 (24 nucleotides): | 5' - cga gac agc agc aag gga aaa aca - 3' |
| | pTrcHis2R (18 nucleotides): | 5' - gat tta atc tgt atc agg - 3' |
| | sNR1 (27 nucleotides): | 5' - tca cac atc agc tct gac gaa atc aag - 3' |
| 25 | sNR2 (24 nucleotides): | 5' - cac atc agc tct gac gaa atc aag - 3' |
| | sNR3 (24 nucleotides): | 5' - tcc tgc cat acc agt ttc ctc gag - 3' |
| | sNR4 (24 nucleotides): | 5' - tga tgt ttc ctg aac tgc agc agc - 3' |
| | sNR5 (24 nucleotides): | 5' - gag gat tct cat caa gcc ttc cat - 3' |

The N coding region was sequenced from 5' to 3' direction and reversely from 3' to 5' direction.

Expression and Western Blotting of Full-length, Fusion and Truncated N Proteins

5 Transformants carrying the recombinant expression plasmids were grown in Luria broth (LB), and protein expression was induced by adding isopropyl- β -D-thiogalactopyranoside (IPTG). Figure 1 depicts the primary structure of the full-length, fusion and truncated N proteins. The number of amino acids of each protein is given in Table 1.

10 Cells extracts were analysed with SDS-PAGE under denaturing conditions and the proteins were transferred to nitrocellulose membranes. The N_{fus} protein was detected with swine anti-NiV serum and the result is shown in Figure 2. A protein band with apparent molecular weight of about 63 kDa was detected. The N_{fus} and truncated N proteins were detected by the anti-*myc* monoclonal antibody (Figure 3).

15 Purification and Electron Microscopic Analysis of N_{fus} Protein

Affinity Chromatography and sucrose gradients centrifugation were employed to purify recombinant nucleocapsid particles. Overnight culture was first spun down to collect *E. coli* cells. The cells were lysed by sonication and soluble proteins were precipitated with ammonium sulfate and it was found that most of the recombinant
20 N_{fus} protein was precipitated at 10 and 20% of ammonium sulfate saturation. Precipitated proteins were dialyzed and split into two, one was purified with affinity chromatography and the other was on sucrose gradient centrifugation. Nearly all the host proteins were removed by these purification methods (Figure 4). However, two major bands were observed in which one of them corresponds to the expected size and
25 the other one with a smaller size. Both of the purified bands were detected by the antiserum from an infected swine (Figure 5). This suggests that the protein with a smaller size is a degraded product of the N_{fus} protein. The N_{fus} protein either purified with affinity chromatography or sucrose gradient centrifugation self-assembled into ring-like particles (Figure 6).

Cloning and Expression of Truncated G Gene in Baculovirus

The partial G gene (nucleotides 231 to 1737) of NiV was amplified with a forward primer (5'-gggggatccatggacaatcaggccgtgatc-3') and a reverse primer (5'-ggggggaagcttctcaaccaatgatatgcacca-3'). The PCR product was cloned into pFASTBAC vector (Gibco-BRL, USA). The translated product lacks 77 amino acids from the N-terminal end of the G glycoprotein. In order to generate recombinant bacmid, the recombinant pFAST vector (Gibco-BRL, USA) was transformed into DH10BAC competent cells for subjecting to site-specific transposition. To confirm transposition, PCR of the transposition region was performed using pUC/M13 amplification primers located on either site of the region. Recombinant bacmid DNA was isolated from overnight cultures and transfected into insect cells, *Spodoptera frugiperda* (Sf9).

For the expression of recombinant protein by infection, insect cells were infected with recombinant baculoviruses collected from transfection experiment. Figure 7 shows the results of Western blot analysis of 96 h samples using anti-G monoclonal antibodies (MAbs) and swine anti-NiV serum. In both cases, two bands at position about 52 and 65 kDa were observed. The lower protein band corresponds to the expected size of the truncated G protein. The larger molecular weight band is most probably due to post-translational modification of the truncated G protein.

EXAMPLES

Example 1: Viral RNA extraction

Infected Vero cell culture (250 μ l) was mixed with TRIZOL LS reagent (750 μ l; Gibco BRL, USA) and incubated for 5 min at room temperature. After incubation, chloroform (250 μ l) was added and the mixture was mixed vigorously for about 15 s and incubated at room temperature for 10 min. The mixture was centrifuged at 13,000 xg for 15 min at 4°C. The viral RNA (vRNA) was then precipitated by adding isopropanol (500 μ l) to the aqueous phase and left at room temperature for 10 min. The precipitated vRNA was centrifuged at 13,000 xg for 10 min and the pellet obtained was washed once with 75% (v/v) diethyl pyrocarbonate (DEPC) (Sigma, USA) treated ethanol (Hamburg). The pellet was dissolved in DEPC treated distilled water (20 μ l) and the dissolved vRNA was stored at -70°C.

Example 2: Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

For the amplification of DNA fragments encoding the full-length N protein, synthesis of the first strand cDNA was carried out in a reaction mixture (50 μ l) containing the primers (NF1 and NR1 or NR2; 1 μ M), deoxynucleoside triphosphate (0.2 mM; Promega, USA), AMV reverse transcriptase (5 U), Tfl DNA polymerase (5 U; Promega, USA), MgSO₄ (1 mM) and AMV/Tfl reaction buffer (1 x; Promega, USA). The mixture was initially incubated at 48°C for 45 min to synthesise the first strand of cDNA and then heated at 94°C for 5 min to inactivate the reverse transcriptase and also for cDNA denaturation. This was immediately subjected to a second strand cDNA synthesis and PCR amplification for 40 cycles with the following profile: denaturation at 94°C for 30 s, annealing at 55°C for 1 min and extension at 68°C for 2 min, followed by a 7 min extension at 68°C to ensure a complete synthesis of the PCR product.

For the amplification of DNA fragments encoding truncated N proteins, PCR, were carried out in a reaction mixture (50 μ l) containing the primers (1 μ M of forward and reverse primers; Table 1), deoxynucleoside triphosphates (0.2 mM; Promega, USA), *Taq* DNA polymerase (Finzyme, Finland; 1 U) and MgCl₂ (1 mM). The mixture was initially denatured at 94°C for 2 min, followed by 30 cycles with the following profile: denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a 7 min extension at 72°C to ensure a complete synthesis of the PCR products. At the completion of the reaction, the PCR products were analysed on 1.2% (w/v) agarose gels in Tris-Acetate-EDTA buffer (TAE; 40 mM Tris-acetate, 1 mM EDTA). The agarose gel was stained with ethidium bromide (0.5 μ g/ml) and observed over ultraviolet light.

For the amplification of DNA fragment encoding truncated G protein, PCR was carried out in a reaction mixture (50 μ l) containing the primers (1 μ M of forward and reverse primers), deoxynucleoside triphosphates (0.2 mM; Promega, USA), *Taq* DNA polymerase (Promega, 1 U), MgCl₂ (1 mM) and a recombinant pCITE vector (20 ng) harbouring the G gene of Nipah virus. The mixture was initially denatured at 94°C for 2 min, followed by 30 cycles with the following profile: denaturation at 94°C for 1

min, annealing at 55°C for 45 seconds and extension at 72°C for 2 min, followed by a 7 min extension at 72°C to ensure a complete synthesis of the PCR products. At the completion of the reaction, the PCR product was analysed on 1.2% (w/v) agarose gels in Tris-Acetate-EDTA buffer (TAE; 40 mM Tris-acetate, 1 mM EDTA). The agarose gel was stained with ethidium bromide (0.5 µg/ml) and observed over ultraviolet light.

Example 3: Purification of the Amplified PCR Products

All the PCR products were analysed on 1.2% (w/v) agarose gels by using Tris-Acetate-EDTA (TAE; 40 mM Tris-acetate, 1 mM EDTA). After the staining with ethidium bromide (0.5 µg/ml), the DNA bands with the correct size were excised from the gels and purified with the QIAquick® Gel Extraction Kit (QiaGen, Germany) according to the manufacturer's procedure. After purification, the PCR products (5 µl) were again analysed with agarose gel electrophoresis to determine the recovery of the PCR products.

Example 4: Ligation

The purified PCR products (100 ng) encoding the full-length and truncated N proteins were ligated with the pTrcHis2 TOPO expression vector (Invitrogen, USA; 10 ng), at 25°C for 5 min. The PCR product containing nucleotides 231 to 1737 of G gene was ligated with the pFASTBACH1 (Gibco- BRL, USA), that had been digested with *Bam*HI and *Hind*III .

Example 5: Transformation and Transfection

For the transformation of recombinant plasmids encoding the full-length, fused and truncated N proteins. The ligation mixtures were mixed with *E. coli* TOP 10 competent cells (50 µl; Invitrogen, USA) and incubated on ice for 30 min. Cells were then heated for 30 s at 42°C, solution SOC (2% trypton, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose; 250 µl) was then added, and incubated at 37°C for 1 h with shaking at 250 rpm. Cells (30 µl and 50 µl) were then plated on a Luria Bertani (LB) agar plates supplemented with ampicillin (50 µg/ml) and the plates were then incubated overnight at 37°C.

- Competent *E. coli* DH10BAC cells, containing Bacmid (baculovirus shuttle vector plasmid) and helper plasmid, were used to generate recombinant Bacmids according to manufacturer's (BAC-TO-BAC Baculovirus Expression System, Gibco-BRL) instructions. Briefly, the recombinant pFASTBACH1 plasmids were transformed into
- 5 MAX EFFICIENCY DH10BAC Competent Cells and plated on Miller's Luria Agar (LA) plates containing kanamycin (50 µg/ml), gentamicin (7 µg/ml), tetracycline (10 µg/ml), Bluo-gal (100–300 µg/ml), and IPTG (40 µg/ml). After 24 to 48-h incubation at 37°C, large, white colonies were selected for overnight culture. High-molecular weight DNA was isolated from the overnight cultures as described in the manual.
- 10 The extracted recombinant bacmid DNA was transfected into insect cells using CELLFECTIN Reagent. *Spodoptera frugiperda* (Sf9) cells were cultured at 27°C in Sf-900 II SFM. For each transfection, 9×10^5 cells were seeded in 35 mm wells of a 6-well plate and allowed to attach for around 1 h. Lipid reagent and DNA were diluted separately with Sf-900 II SFM (100 µl), then combined to form lipid-DNA
- 15 complexes. The lipid-DNA complexes were diluted to 1 ml with SFM and added to cells. Cells were then incubated at 27°C, for 5 h.

Example 6: Screening for Positive Clones

- In order to screen for transformants carrying the full-length, fused and truncated N genes. A dozen single colonies were randomly chosen and cultured overnight in 5 ml
- 20 of LB medium supplemented with ampicillin (50 µg/ml). Recombinant plasmids DNA were isolated by using the alkaline lysis method and the orientation of the insert in the putative clones was confirmed by PCR using the primer pair of pTrcHis2F (5'-gag gta tat att aat gta tcg -3') and NR2 (5'-cac atc agc tct gac gaa atc aag-3').

- To confirm transposition in DH10BAC Cells, PCR of the transposition region was
- 25 adopted by pUC/M13 amplification primers located on either site of the region.

Example 7: Protein Expression

For the expression of full length, fused and truncated N proteins, the identified positive clones were grown overnight in LB medium (3 ml) supplemented with ampicillin (50 µg/ml) at 37°C. The next day, LB medium (10 ml) supplemented with

ampicillin (50 µg/ml) was inoculated with the overnight culture (0.2 ml) and incubated at 37°C with shaking at 250 rpm. When the cultures reached OD₆₀₀ about 0.6 to 0.8, IPTG (isopropyl-1-thio-β-D-thiogalactopyranoside; 1 mM) was added to induce protein expression and continued shaking for 3 to 5 h. The cells (1 ml culture) were harvested by centrifugation at 12,000 xg for 30 s at room temperature, and then subjected to polyacrylamide gel electrophoresis (SDS-PAGE). For the expression of truncated proteins, the transformations were grown at 25°C.

For the expression of truncated G glycoprotein, *Spodoptera frugiperda* (Sf9) cells were cultured at 27°C in Sf-900 II SFM. For each transfection, 9×10^5 cells were seeded in 35 mm wells of a 6-well plate and allowed to attach for around 1 h. Lipid reagent and DNA were diluted separately with Sf-900 II SFM (100 µl), then combined to form lipid-DNA complexes. The lipid-DNA complexes were diluted to 1 ml with SFM and added to cells. Cells were incubated for 5 h at 27°C. The transfection medium was removed and replaced with a fresh medium. Cells were analysed for protein expression at 24 to 72 h post transfection. Viral supernatant was collected at 72 h post-transfection.

Example 8: SDS-PAGE and Western Blotting

For the Western blotting of full-length, fused and truncated N protein, *E. coli* cell pellets (from 1 ml culture) were resuspended in 2x SDS-PAGE sample buffer [100 mM Tris-Cl (pH 8.8), 200 mM β-mercaptoethanol, 4% SDS, 0.2% bromophenol blue, 20% glycerol; 50 µl] and boiled for 10 min. For the detection of truncated G protein, cells were analyzed for protein expression at 24 to 72 h post transfection. Viral supernatant was collected at 72 h post-transfection. Control or infected cells were washed with PBS. To extract total soluble protein, cells were lysed directly in Tris-HCl (62.5 mM, pH 6.8) containing SDS (2%) and boiled for 10 min. The treated samples (5 µl) were then analysed on SDS-12% polyacrylamide gel and electrophoresed at 32 mA. The proteins on the gel were then transferred onto a nitrocellulose membrane and blocked with skim milk (KPL, USA; 1:10 dilution) for 1 h. The swine serum (anti-NiV, 1: 500) or anti-*myc* or anti-His monoclonal antibody was added to the membrane and shaken for 1 h. The membrane was then washed thoroughly with TTBS (100 mM Tris-HCl, 0.9% NaCl, 0.01% Tween-20; pH 7.5).

Secondary antibody conjugated to alkaline phosphatase was then added and incubated for another 1 h. After washing, colour development was carried out using the BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) substrate (Promega, USA).

5 **Example 9: Purification of Recombinant N_{fus} protein**

The recombinant *E. coli* strain BL21 was cultured in LB broth (400 ml) at 25°C overnight. The cells were harvested by centrifugation at 8000 xg for 15 min. The pellet was suspended in 25 mM HEPES (n-2-Hydroxyethyl-piperazine-n-2-ethanesulfonic acid; pH 7.6, 16 ml) and subjected to sonication. The lysates were
10 centrifuged at 18000 xg to separate soluble fraction from inclusion body. The soluble form of recombinant protein was precipitated with a range of 10% to 60% ammonium sulfate before dialyzing against 6 times changing of Tris-Glycine Buffer (0.05M Tris; 0.07M Glycine; pH 8.0). Half of the dialyzed sample was applied to a 10% to 60% sucrose gradient and centrifuged at 36000 xg, for 4 h. Fractions were collected and
15 analyzed by SDS-PAGE. The rest of the dialyzed sample was then subjected to a column packed with nickel resin (Invitrogen, USA). The fractions with the purified protein were pooled and concentrated using Centricon (Amicon, USA) with 10 kDa cut off.

20 **Example 10: Electron Microscopy**

N_{fus} protein (20 µl) purified with Nickel column and sucrose gradient was absorbed to carbon-coated grids and stained with 2% uranyl acetate. The grids were viewed under an electron microscope.

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It should be understood that the preceding is merely a detailed description of certain preferred embodiments. It therefore should be apparent to those skilled in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. It is intended to encompass all such modifications within the scope of the appended claims.

15

All references, patents and patent publications that are recited in this application are incorporated in their entirety by reference.

Table 1

N mutants	Number of amino acids	Primers used		Length of PCR product (bp)	% of amino acids deleted from the N- or C-terminus of N protein
		Forward	Reverse		
N full-length	533	NF1	NR1	1599	-
N _{fus}	532	NF1	NR2	1596	-
MN25 _{fus}	399	NF5	NR2	1197	25 % of N-terminus
MC25 _{fus}	399	NF1	NR5	1197	25 % of C-terminus
MN50 _{fus}	266	NF6	NR2	798	50 % of N-terminus
MC50 _{fus}	266	NF1	NR6	798	50 % of C-terminus
MNC25 _{fus}	266	NF5	NR5	798	25 % of NC-terminus

Claims:

1. A recombinant expression plasmid containing Nipah virus nucleocapsid gene.
2. The Nipah virus nucleocapsid gene of claim 1 having the following nucleotide sequence:

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5      1  ATGAGTGATATCTTTGAAGAGGCGGCTAGTTTTAGGAGTTATCAATCTAAGTTAGGGAGC  60
      61  GATGGGAGGGCTAGTGCAGCAACTGCTACTTTGACAACCAAGATAAGGATATTTGTACCA  120
      121 GCTACTAATAGTCCAGAGCTCAGATGGGAACTAACATTGTTTGCACTTGATGTGATTAGA  180
      181 TCTCCGAGTGCTGCCGAGTCAATGAAAGTTGGAGCTGCTTTCACACTCATCTCTATGTAT  240
      241 TCAGAGAGACCCGGGGCTCTCATTAGAAGTCTCCTCAATGACCCAGACATTGAAGCTGTA  300
10     301 ATAATAGATGTTGGATCAATGGTCAACGGAATACCAGTAATGGAGAGGAGAGGAGACAAG  360
      361 GCTCAGGAGGAGATGGAAGGCTTGATGAGAATCCTCAAACTGCTCGAGACAGCAGCAAG  420
      421 GGAAAAACACCTTTTGTGACAGCCGAGCTTACGGCCTACGGATAACAGACATGAGCACC  480
      481 CTGGTCTCTGCAGTTATCACCATCGAGGCCAGATCTGGATACTGATCGCTAAAGCAGTT  540
      541 ACAGCTCCCGACACTGCCGAGGAAAGTGAACTAGAAGATGGGCTAAATACGTCCAACAA  600
15     601 AAGAGGGTCAATCCGTTCTTTGCTCTAACTCAGCAATGGCTAACAGAAATGAGGAATCTG  660
      661 CTCTCCAGAGTCTATCAGTAAGGAAGTTCATGGTTGAGATCCTCATAGAAGTCAAGAAA  720
      721 GGAGGATCTGCTAAAGGCAGAGCAGTAGAAATAATCTCAGACATCGGAACTATGTGAG  780
      781 GAAACTGGTATGGCAGGATTCTTCGCAACCATCAGATTCGGGTTGGAGACAAGGTATCCA  840
      841 GCACTTGCACTCAACGAATTCAGAGTGACCTCAACACCATCAAAAGCTTGATGCTACTC  900
20     901 TACAGAGAAATTGGCCCAAGAGCCCCTTATATGGTGCTTCTTGAAGAATCAATTCAGACT  960
      961 AAATTTGCCCTGGAGGTTACCCATTATTGTGGAGCTTTGCCATGGGTGTGGCTACTACT  1020
      1021 ATTGACAGGTCTATGGGGGCATTGAATATCAATCGTGGTTATCTTGAGCCTATGTATTT  1080
      1081 AGACTAGGCCAAAATCAGCACGTCACCATGCTGGAGGAATTGATCAGAACATGGCAAAT  1140
      1141 AGACTGGGACTAAGTTCAGATCAAGTTGCAGAACTCGCTGCTGCAGTTCAGGAAACATCA  1200
25     1201 GCAGGAAGGCAAGAGAGTAATGTTTCAGGCTAGAGAGGCCAAAATTTGCTGCAGGAGGTGTG  1260
      1261 CTCATTGGAGGCAGTGATCAAGATATCGATGAAGGGGAAGAACCTATAGAACAGAGTGGC  1320
      1321 AGACAGTCAGTTACCTTCAAAGGGGAGATGAGTATTTTCATCCCTTGCTAACAGTGTGCCG  1380
      1381 AGCAGTTCGTGTGAGCACATCCGGTGGGACCAGATTGACTAATTCATTACTAAACCTCAGA  1440
      1441 TCAAGACTGGCTGCAAAGCAGCAAAGAAGCCGCCTCATCCAATGCAACAGATGATCCA  1500
30     1501 GCAATCAGCAACAGAACTCAAGGGGAATCAGAGAAGAAGAATAATCAAGACCTCAAACCT  1560
      1561 GCTCAAATGACCTTGATTTTCGTCAGAGCTGATGTG  1596

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3. A recombinant plasmid containing a part of the nucleocapsid gene as claimed in claim 1 or 2.
4. A recombinant plasmid containing the gene of truncated G glycoprotein of Nipah virus.
5. The gene of truncated G glycoprotein of claim 4 having the following nucleotide sequence:

1 ATGGACAATCAGGCCGTGATCAAAGATGCGTTGCAGGGTATCCAACAGCAGATCAAAGGG 60

61 CTTGCTGACAAAATCGGCACAGAGATAGGGCCCCAAAGTATCACTGATTGACACATCCAGT 120

121 ACCATTACTATCCCAGCTAACATTGGGCTGTTAGGTTCAAAGATCAGCCAGTCGACTGCA 180

10 181 AGTATAAATGAGAATGTGAATGAAAAATGCAAATTCACACTGCCTCCCTTGAAAATCCAC 240

241 GAATGTAACATTTCTTGTCTTAACCCACTCCCTTTTAGAGAGTATAGGCCACAGACAGAA 300

301 GGGGTGAGCAATCTAGTAGGATTACCTAATAATATTTGCCTGCAAAAGACATCTAATCAG 360

361 ATATTGAAGCCAAAGCTGATTTTATACACTTTACCCGTAGTCGGTCAAAGTGGTACCTGT 420

421 ATCACAGACCCATTGCTGGCTATGGACGAGGGCTATTTTGCATATAGCCACCTGGAAAGA 480

15 481 ATCGGATCATGTTCAAGAGGGGTCTCCAAACAAAGAATAATAGGAGTTGGAGAGGTACTA 540

541 GACAGAGGTGATGAAGTTCCTTCTTTATTTATGACCAATGTCTGGACCCACCAAATCCA 600

601 AACACCGTTTACCACTGTAGTGCTGTATACAACAATGAATTCTATTATGTACTTTGTGCA 660

661 GTGTCAACTGTTGGAGACCCTATTCTGAATAGCACCTACTGGTCCGGATCTCTAATGATG 720

721 ACCCGTCTAGCTGTGAAACCCAAGAGTAATGGTGGGGGTACAATCAACATCAACTTGCC 780

20 781 CTACGAAGTATCGAGAAAGGGAGGTATGATAAAGTTATGCCGTATGGACCTTCAGGCATC 840

841 AAACAGGGTGACACCCTGTATTTTCTGCTGTAGGATTTTGGTCAGGACAGAGTTTAAA 900

901 TACAATGATTCAAATTGTCCCATCACGAAGTGTCAATACAGTAAACCTGAAAATTGCAGG 960

961 CTATCTATGGGGATTAGACCAAACAGCCATTATATCCTTCGATCTGGACTATTAAAATAC 1020

1021 AATCTATCAGATGGGGAGAACCCCAAAGTTGTATTCATTGAAATATCTGATCAAAGATTA 1080

25 1081 TCTATTGGATCTCCTAGCAAAATCTATGATTCTTTGGGTCAACCTGTTTTCTACCAAGCG 1140

1141 TCATTTTCATGGGATACTATGATTAAATTTGGAGATGTTCTAACAGTCAACCCTCTGGTT 1200

1201 GTCAATTGGCGTAATAACACGGTAATATCAAGACCCGGGCAATCACAATGCCCTAGATTC 1260

1261 AATACATGTCCAGAGATCTGCTGGGAAGGAGTTTATAATGATGCATTCTAATTGACAGA 1320

1321 ATCAATTGGATAAGCGCGGGTGTATTCCTTGACAGCAATCAGACCGCAGAAAATCCTGTT 1380

1381 TTTACTGTATTCAAAGATAATGAAATACTTTATAGGGCACAACCTGGCTTCTGAGGACACC 1440
 1441 AATGCACAAAAACAATAACTAATTGTTTTCTCTTGAAGAATAAGATTTGGTGCATATCA 1500
 1501 TTGGTTGAG 1509

- 5 6. The nucleocapsid protein of claim 1 or 2, having the following amino acid sequence:

1 MSDIFEEAAS FRSYQSKLGS DGRASAATAT LTTKIRIFVP ATNSPELRWE LTLFALDVIR 60
 61 SPSAAESMKV GAAFTLISMY SERPGALIRS LLNDPDIEAV IIDVGSMVNG IPVMERRGDK 120
 121 AQEEMEGLMR ILKTARDSSK GKTPFVDSRA YGLRITDMST LVSAVITIEA QIWILIAKAV 180
 181 TAPDTAESE TRRWAKYVQQ KRVNPFALT QQWLTEMRL LSQSLSVRKF MVEILIEVKK 240
 10 241 GGSAGRAVE IISDIGNIVE ETGMAGFFAT IRFGLETRYP ALALNEFQSD LNTIKSLMLL 300
 301 YREJGPRAPY MVLLEESIQT KFAPGGYPLL WSFAMGVATT IDRSMGALNI NRGYLEPMYF 360
 361 RLGQKSARHH AGGIDQNMN RLGLSSDQVA ELAAVQETS AGRQESNVQA REAKFAAGGV 420
 421 LIGGSQDID EGEEPIEQSG RQSVTFKREM SISSLANSVP SSSVSTSGGT RLTNSSLNLR 480
 481 SRLAAKAAKE AASSNATDDP AISNRTQGES EKKNQDLKP AQNDLDFVRA DV 532

- 15 7. The truncated nucleocapsid protein of claim 3 having a part of the amino acid sequence as claimed in claim 6.

8. The truncated G glycoprotein of claim 4 or 5 having the following amino acid sequence:

1 MDNQAVIKDA LQGIQQQIKG LADKIGTEIG PKVSLIDTSS TITIPANIGL LGSKISQSTA 60
 20 61 SINENVNEKC KFTLPPLKIH ECNISCPNPL PFREYRPQTE GVSNLVGLPN NICLQKTSNQ 120
 121 ILKPKLISYT LPVVGQSGTC ITDPLLAMDE GYFAYSHLER IGSCSRGVSK QRIIGVGEVL 180
 181 DRGDEVPSLF MTNVWTPPNP NTVYHCSAVY NNEFYVVLCA VSTVGDPIIN STYWSGSLMM 240
 241 TRLAVKPKSN GGGYNQHQLA LRSIEKGRYD KVMPYGPSGI KQGDITLYFPA VGFLVRTEFK 300
 301 YNDSNCPITK CQYSKPENCR LSMGIRPNH YILRSGLLY NLSDGENPKV VFIEISDQRL 360
 25 361 SIGSPSKIYD SLGQPVFYQA SFSWDTMIKF GDVLTVNPLV VNWRNNTVIS RPGQSQCPRF 420
 421 NTCPEICWEG VYNDAFLIDR INWISAGVFL DSNQTAENPV FTVFKDNEIL YRAQLASEDT 480
 481 NAQKTITNCF LLKNKIWCIS LVE 503

9. A transformed *Escherichia coli* having the recombinant expression plasmid as claimed in claim 1 or 3.
- 30 10. A transformed *E. coli* having the recombinant plasmid according to claim 4.

11. A fused form of NiV nucleocapsid protein having the amino acid sequence as claimed in claim 6.
12. A fused form of NiV truncated nucleocapsid protein having a part of the amino acid sequence as claimed in claim 7.
- 5 13. The fused form of NiV nucleocapsid protein of claim 11, wherein said protein consists of at least one immunogenic component.
14. The fused form of NiV truncated nucleocapsid protein of claim 12, wherein said protein consists of at least one immunogenic component.
- 10 15. The immunogenic component of claim 13 or 14, wherein said immunogenic component is linked to the carboxy-terminus of NiV nucleocapsid protein.
16. A recombinant nucleocapsid protein of Nipah virus that is capable of assembling into a particle form.
17. A pharmaceutical composition comprising a therapeutically effective amount of the recombinant nucleocapsid protein as claimed in claim 6 or 7.
- 15 18. A pharmaceutical composition comprising a therapeutically effective amount of the recombinant G glycoprotein as claimed in claim 8.
19. The pharmaceutical composition according to claim 17 or 18, wherein said composition is for therapeutic, prophylactic and prevention purposes.
- 20 20. A diagnostic reagent comprising an effective amount of the recombinant nucleocapsid protein as claimed in claim 6 or 7.
21. A diagnostic reagent comprising an effective amount of the recombinant G glycoprotein as claimed in claim 8.

22. A method for producing recombinant nucleocapsid protein of claim 6 comprising:
- a) growing recombinant *Escherichia coli* containing nucleocapsid protein genes of Nipah virus in a medium;
 - 5 b) allowing the recombinant protein to propagate in *Escherichia coli*;
 - c) harvesting and lysing the *Escherichia coli* cells; and
 - d) purifying the lysate to obtain recombinant nucleocapsid protein.
23. A method for producing recombinant truncated nucleocapsid protein of claim 7 comprising:
- 10 a) growing recombinant *Escherichia coli* containing truncated nucleocapsid protein genes of Nipah virus in a medium;
 - b) allowing the recombinant protein to propagate in *Escherichia coli*;
 - c) harvesting and lysing the *Escherichia coli* cells; and
 - d) purifying the lysate to obtain recombinant truncated nucleocapsid protein.
- 15 24. A method for producing recombinant truncated G glycoprotein of claim 8 comprising:
- a) transfecting an insect cell culture with a baculovirus containing a recombinant plasmid carrying truncated G glycoprotein genes of Nipah virus;
 - 20 b) allowing the recombinant protein to propagate in the insect cells;
 - c) harvesting and lysing the insect cells; and
 - d) purifying the lysate to obtain recombinant truncated G glycoprotein.
25. A method for purifying recombinant nucleocapsid protein as claimed in claim 6 comprising:
- 25 a) precipitating the soluble recombinant protein with ammonium sulphate;
 - b) dialysing the precipitated recombinant protein obtained in (a) with buffer;
 - c) fractionating the recombinant protein using sucrose gradient centrifugation or nickel affinity column chromatography; and
 - 30 d) collecting and pooling the purified protein fractions for concentration of the protein.