# *In vitro* production of recombinant Nipah virus nucleoprotein for rapid test development

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## What is Nipah virus?

- Zoonotic paramyxovirus carried by bats endemic to South Asia and Australia.
- Single-stranded negative-sense RNA virus.
- Kills 40-75% of infected humans. Can spread from human-to-human.
- Prodrome manifests as non-specific flu-like symptoms progressing to encephalitis, coma, and death.
- No rapid diagnostics available.
- Some treatments available, critical to diagnose early.
- Viral Nucleoprotein was identified as an abundant and immunogenic antigen specific to Nipah virus.

## Overarching aim:

Develop a library of monoclonal antibodies for the generation of accessible immunodiagnostics to detect Nipah virus infection at the point of care.

## Problem:

Monoclonal antibodies are a critical component in immunodiagnostics. Mammalian expressed recombinant biomarkers (antigen) are expensive to purchase commercially but remain important for antibody screening and rapid test validation.

## NURA scope:

Produce recombinant Nipah virus (NiV) nucleoprotein (rNP; antigen) in-house via bacterial transformation to aid screening of antibodies for monoclonal antibody (mAb) libraries and immunodiagnostics.

## Hypothesis

By transforming *E.* coli with a plasmid containing the rNP gene, we can induce recombinant expression of antigen and purify it with an associated HIS-tag for much cheaper than purchase of mammalian expressed antigen.

## Background:

- Antibodies are soluble proteins generated by the adaptive immune system to identify and bind pathogens via associated biomarkers.
- Immunodiagnostics exploit antibody-antigen complexes for positive indication of infection.
- To develop diagnostics, mice are immunized against pathogen biomarkers (antigens), and their splenocytes (antibody-producing cells) are harvested for hybridoma fusion.
- For purification, a plasmid was constructed with genes encoding a HIS-tag modification to the rNP.
- HIS-tag purification, through nickel column chromatography, is an effective and inexpensive process to purify proteins by differentially immobilizing and eluting mixture components, resulting in isolation and purification.

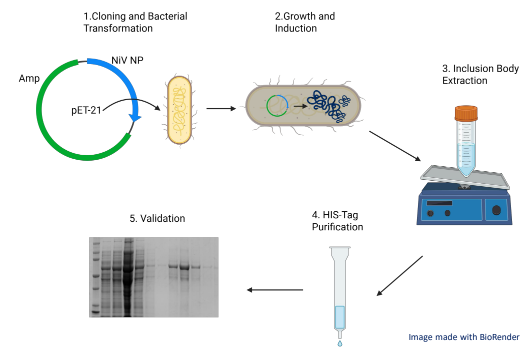


**Figure 1: Methodology for recombinant NP expression.** 1. Plasmid vectors containing the NiV NP gene are transformed into competent E. coli cells. 2. *E. coli* are grown in Nutrient broth containing ampicillin and induced using IPTG to make the rNP. 3. Proteins amass in inclusion bodies and are solubilized using Urea. 4. rNP is purified using a HIS tag purification column. 5. rNP is evaluated using an SDS gel with Coomassie stain to visualize protein.

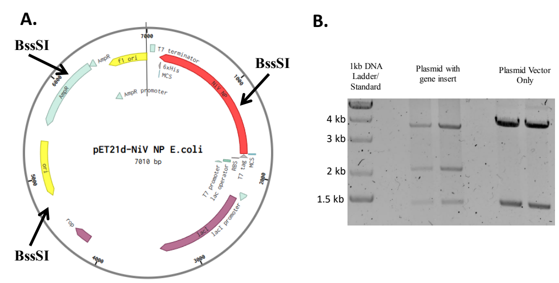


**Figure 2: Nucleoprotein plasmid diagram and DNA gel digest confirming nucleoprotein insert. A.** Nucleoprotein gene was inserted by collaborators into pET21d vector. Two cut sites for the restriction endonuclease BssSI are present in the vector, and one is located in the NiV NP gene, allowing for verification of insertion. **B.** In DNA digest gel electrophoresis stained with ethidium bromide, band sizes of 3593 bps, 2033 bps, and 1384 bps indicates proper gene insertion. For unmodified vector, two bands at 4000 bps and 1384 bps are seen as expected.


**Figure 3: Reactivity of mouse serum to mammalian and *E. coli* rNP.** The reactivity of the mouse serum to the rNPs was evaluated using a Western blot where both rNPs were run on a gel and transferred to a nitrocellulose membrane. Reactivity was confirmed by banding at the proper size of ~62 kDa. The serum from 5 mice immunized with mammalian rNP was then incubated on the membrane (lanes 2-6 and 11-15). A negative control serum from pre-immunization mice (lanes 8 and 17) and a positive control of α-HIS mAbs (lanes 9 and 18) were included.

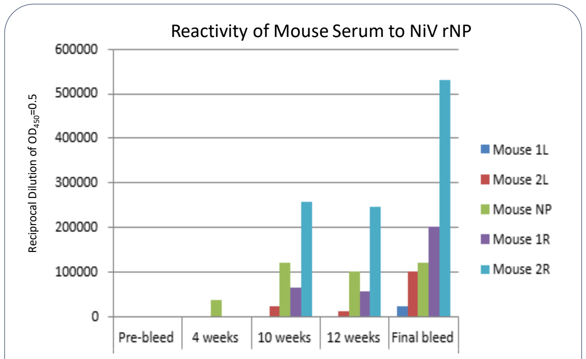


**Figure 4: Indirect ELISAs confirming mouse serum reactive with *E. coli* NiV rNP at different time points throughout immunization cycle.** Reciprocal murine titer dilution at $OD_{450}$=0.5 x 5 timepoints for immunizations of 5 respective mice (1L, 2L, NP, 1R, 2R).

## Results:

- Plasmid contains NiV NP gene (Figure 2.B).
- Transformation and protein expression successful, 1.2+ mg recombinant nucleoprotein produced, comparable to over $8,000 of commercial rNP.
- rNP has homology to mammalian expressed antigen used to immunize mice (Figure 3). Homology to native antigen was confirmed via western blot immunoassay.
- Polyclonal antibodies in mouse serum is reactive with rNP (Figures 3,4).

## Conclusions:

- Recombinant expression of viral antigens is a useful tool for antibody screening.
- In-house *in vitro* expression of rNP is a cost-effective, simple alternative to commercial product, and represents a safer study design than using live virus.
- pET21d expression of rNP in *E. coli* requires cell lysis and urea for protein extraction from inclusion bodies prior to nickel column chromatography.

## Future directions:

- Continue production and purification of rNP.
- Continue antibody screening with produced nucleoprotein to identify suitable pairs for a rapid diagnostic prototype (Figure 5).

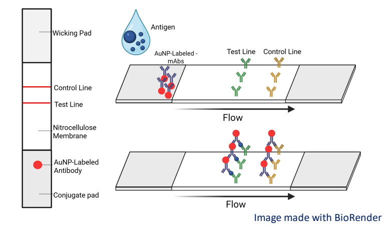


**Figure 5: Schematic of a rapid test.** A lateral flow immunoassay can utilize mAbs for a rapid and inexpensive diagnostic.

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