

Cloning cDNA from Risk Group 4 Viruses Ebola, Marburg, Nipah, Hendra: Biosafety & Biosecurity

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Risk Group (RG) 4 *Mononegavirales*

- Non-segmented, negative sense, single-stranded RNA viruses
 - Ebola and Marburg (Family: *Filoviridae*)
 - Nipah and Hendra (Family: *Paramyxoviridae*)

Request for Lowering of Containment

- Institutional Biosafety Committee (IBC), Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, NIH contacted OBA regarding whether containment for cloning of the cDNA from Ebola, Marburg, Nipah and Hendra into *E. coli* could be done at Biosafety Level (BL) 2.

Biological Properties of RG4 viruses of the order *Mononegavirales*

- No DNA is produced during viral replication.
- Viral RNA genome and its cDNA copy are not inherently infectious in either mammalian or in prokaryotic cells; additional functional viral proteins are required for replication or rescue in mammalian cells and rescue *is not possible in prokaryotes*.
- Neither the RNA genome nor the full-length cDNA are considered to be Select Agents.

Cloning Full-length cDNA of RG 4 RNA Viruses: Nipah, Hendra, Ebola or Marburg into *E. coli*

Cloning nucleic acid from a risk group 4 (RG4) agent into non-pathogenic bacteria or a lower eukaryote falls under Section III-D-2-a of the *NIH Guidelines*, which states in part:

“.... Experiments in which DNA from Risk Group 4 agents is transferred to non-pathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a **totally and irreversible defective fraction** of the agent’s genome is present in a given recombinant. **In the absence of such a demonstration, BL4 containment shall be used.** The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III-F, *Exempt Experiments*).”

Cloning cDNA of Ebola, Marburg, Nipah and Hendra in *E. coli*

- The full-length cDNA of any of these viruses can produce infectious virus in mammalian cells only if the full-length RNA genome is produced and then only with the addition of 3 or 4 essential viral proteins.

Research Involving DNA from RG4 Mononegavirales Agents in Non-Pathogenic *E. coli*

- **Biosafety**: Given the biological properties of these RG4 agents, BL2 is appropriate for cloning of the full-length cDNA of Ebola, Marburg, Hendra, or Nipah into non-pathogenic prokaryotes such as *E. coli*.
- **Biosecurity**: Research with these RG4 agents at BL2 raises biosecurity concerns and therefore additional biosecurity provisions must be in place for such work.

OBA Recommendation

- Based on RAC's recommendation, OBA advised the Rocky Mountain Lab's IBC and the PI, Dr. Heinz Feldmann, that lowering of containment to BL2 for work with the cDNA of Ebola, Marburg, Nipah, and Hendra in non-pathogenic *E. coli* could be considered provided the following biosafety and biosecurity conditions were met:

Biosafety and Biosecurity for RML Research

- Research done in a dedicated BL2 laboratory with physical and procedural measures to:
 - Limit access
 - Control Inventory, material flow and waste
 - Maintain separation of full length cDNA and rescue plasmids
 - Ensure all personnel have adequate training

Biosafety and Biosecurity for RML Research, cont . . .

- Written Biosecurity plan:
 - Developed using approach outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th ed.*
 - All personnel with access to the cDNA have at least a Public Trust Level 5 security clearance
 - Appointment official at Institution for oversight of the research
 - Periodic re-evaluation
 - Annual Report to IBC and copy to OBA

Future Requests to OBA

- OBA will review future requests to lower containment for research with the full-length cDNA of Ebola, Marburg, Nipah and Hendra in non-pathogenic *E. coli* in consultation with the RAC as needed.
- OBA approval to allow the IBC to lower containment for such research to BL2 will be specific to a PI at a specific institution.
- The IBC is not required to lower containment based on OBA's assessment; an IBC must perform its own risk assessment.

Future Requests to OBA Required Information

■ General

- Description of the biosafety risk assessment undertaken in accordance with the *NIH Guidelines*.
- Description of the biosecurity assessment and a plan that is consistent with the “Principles of Laboratory Biosecurity” outlined in Section VI of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (5th edition) (*BMBL*).
 - Investigators or institutions that are approved to work with Select Agents are to provide non-sensitive information in the application document in accordance with applicable regulations.

Future Requests to OBA Required Information

■ Section 1:

Statement of Work and Justification

- Objectives and rationale for proposed work including information to justify the need for research with recombinant *E. coli* containing the full-length cDNA of these RG4 viruses.

Section 2:

Description of the Biological System(s)

- Identify all biological reagents needed to rescue infectious recombinant virus and proposed containment for all experiments using any of these reagents.
- Types of recombinant manipulations and the biosafety containment for each of these operations.

Section 3:

Facilities and Operating Procedures

- Indicate the containment level for all areas contained within the laboratory space.
- Identify dedicated areas for experiments with the full-length cDNA clones.
- Describe the physical or procedural measures to ensure that adequate controls exist to restrict access to full length cDNA clones.

Facilities and Operating Procedures, cont...

A Description of:

- Methods for the decontamination or destruction of discarded and waste reagents.
- System of inventory control.
- Procedures used to ensure appropriate control over full-length cDNA reagents should these be moved to other laboratory areas that are not adjacent to the dedicated experimental area.

Section 4: Personnel

- Describe the level of expertise, experience and training of personnel who will have access to full-length cDNA reagents (e.g. cDNA, plasmid constructs, transformed *E. coli*).
- Describe the screening policies and procedures used to evaluate individuals who have access to full-length cDNA reagents.

Section 5:

Incident Response, Incident Reporting and Training

- **Describe the institutional policies and procedures for managing:**
 - Biosafety incidents (exposures or laboratory acquired infections);
 - Biosecurity incidents (thefts or threats);
 - Hazardous materials incidents (spills or releases);
 - Policies and procedures for coordination with outside health authorities, and
 - Biosafety and Biosecurity training.

Section 6:

Compliance Assurance and Documentation Requirements

- Appoint an institutional official who will:
 - Be responsible for periodic re-evaluation of the biosafety and biosecurity plan.
 - Be responsible for filing an annual report with the IBC; the report will include:
 - Any incidents of containment breaches or laboratory exposures;
 - Changes in biosafety or biosecurity procedures, and
 - Documentation of on-going periodic training of personnel in all applicable procedures.

Questions?