



# RG4 Mononegavirales cDNA - Biosafety & Biosecurity Risks

Biosafety Working Group Assessment

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# Previous RAC Discussion: March 2009, Cloning Full-length Ebola cDNA 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*).”

# *NIH Guidelines*

## **Minor Actions: Section IV-C-1-b-(2).**

NIH/OBA shall carry out certain functions as delegated to it by the NIH Director. *Minor Actions* (as determined by NIH/OBA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to RAC and Institutional Biosafety Committee Chairs:

- ▶ **Section IV-C-1-b-(2)-(a).** Changing containment levels for experiments that are specified in Section III, *Experiments Covered by the NIH Guidelines* (except when a *Major Action* is involved);

Therefore, NIH OBA may lower containment for research falling under III-D, including research with RG4 agents that does not meet the conditions of Section III-D-2a.

# **RAC Discussion March 2009: Cloning Full-length Ebola cDNA into *E. coli* Proposal From Dr. Feldmann (RML)**

- **Prokaryotic plasmid carrying the full-length genome of Ebola will be handled in 'restricted' BSL2 with limited and controlled access**
  - ❖ the full-length plasmid will only be amplified in 'restricted' BSL2
  - ❖ no transcription and expression from plasmid in pro- or eukaryotes
  - ❖ bacteriophage T7 polymerase needed for transcription
  - ❖ filovirus helper plasmids needed for expression
  - ❖ plasmid is not a select agent

# Is Biosafety Level 2 Sufficient?

- **The complete cDNA from Ebola is not an irreversibly defective fraction of the genome; however,**
  - ▶ Neither the full-length RNA genome nor the cDNA of Ebola is capable of producing infectious virus in prokaryotic or lower eukaryotic cells
  - ▶ Infectious Ebola virus can be rescued from its cDNA under very deliberate conditions in mammalian cells but can NOT be rescued from prokaryotic or lower eukaryotic cells

# Research Involving cDNA from Ebola in *E. coli*

## From a biosecurity standpoint:

- Full-length nucleic acid of Ebola in the proper setting can generate infectious virus—a Select Agent
- However, full length Ebola RNA, by itself, is not currently considered to be a Select Agent nor is the cDNA

# Proposal from Dr. Feldmann, 'Restricted' BSL2:

- BSL2 negative to hallway and office area
- Limited and controlled access to 'restricted' BSL2
- Separate PPE in 'restricted' BSL2
- Specific SOP for entry/exit 'restricted' BSL2
- Material flow into 'restricted' BSL2; no return of material into regular BSL2
- Waste from 'restricted' BSL2 will be sealed and sterilized (no storage outside 'restricted' BSL2)

# Proposal From Dr. Feldmann for Ebola: Biosecurity Measures

- Routine screening of employees--a Public Trust level 5 background check is performed.
- System of inventory control to track who has access to the cDNA and what happens to the material
- Waste will be sealed prior to leaving the restricted laboratory area and subsequently be autoclaved.
- RML also has a comprehensive accident, injury and response plan.

# Previous RAC Discussion: March 2009, Cloning Full-length Ebola cDNA into *E. coli*

**BL2 containment with SOPs outlined by Dr. Feldmann appropriate, but prior to initiating the work RML should do the following:**

- Re-evaluate proposed security measures for this research using the BMBL “Principles of Biosecurity” and establish a security plan that includes the measures already proposed and any additional necessary elements including:
  - Appointment of an appropriate official to oversee the security plan on behalf of the Institution;
  - Establishment of a training program for the security aspects of the research in addition to biosafety, and
  - Periodic re-evaluation of the program and update as needed

# Dr. Feldman: Proposal to work with Risk Group 4 Mononegvirales - Marburg, Nipah and Hendra

- *Plasmids carrying the full-length genomes of non-segmented, negative-stranded RNA BSL4 Mononegavirales for amplification in prokaryotes such as Escherichia coli will be handled in 'restricted' BSL2 with limited and controlled access and under specific Standard Operating Procedures (SOPs)*

# Known Risk Group 4 (RG4) viral agents of the order Mononegavirales

## Fulminant viral hemorrhagic fever viruses

- **Filoviridae**

- ▶ **Ebola virus** – all species
- ▶ **Marburg** – all species

- **Paramyxoviridae - Henipaviruses**

- ▶ **Nipah virus** – all species
- ▶ **Hendra virus** – all species

# FILOVIRUSES (RG4)

<b>Virus</b>	<b>ss(-) RNA Genome</b>	<b>Natural Reservoir</b>	<b>Host Range</b>	<b>Transmissibility to humans</b>	<b>Case Fatality Rate (Humans)</b>	<b>Therapeutics</b>	<b>Number of Genes to rescue</b>
<b>Ebola</b>	<b>~19 KB</b>	<b>Bats (Pre-sumed)</b>	<b>forest antelope non-human primates</b>	<b>sporadic - localized large outbreaks</b>	<b>20% - 90% Endothelial Pathology</b>	<b>No licensed drug or vaccine</b>	<b>4 NP, VP35, VP30, L</b>
<b>Marburg</b>	<b>19.1 KB</b>	<b>Bats (Pre-sumed)</b>	<b>non-human primates</b>	<b>sporadic - localized large outbreaks</b>	<b>23% - 90% Endothelial Pathology</b>	<b>No licensed drug or vaccine</b>	<b>3 NP, VP35, L</b>

# PARAMYXOVIRUSES (RG4)

<b>Virus</b>	<b>ss(-) RNA Genome</b>	<b>Natural Reservoir</b>	<b>Host Range</b>	<b>Transmissibility to humans</b>	<b>Case Fatality Rate (Humans)</b>	<b>Therapeutics</b>	<b>Number of Genes to rescue</b>
<b>Nipah (NiV)</b>	<b>~18 KB</b>	<b>Bats</b>	<b>All mammals esp. swine, cows, dogs, cats, humans</b>	<b>sporadic large outbreaks</b>	<b>38 – 92% Endothelial &amp; neuronal pathology</b>	<b>No licensed drug or vaccine  Ribavirin (off-label use)</b>	<b>3  N, P, L</b>
<b>Hendra (HeV)</b>	<b>~18KB</b>	<b>Bats</b>	<b>(?) – horses, cats, humans</b>	<b>7 documented outbreaks</b>	<b>high fatality rate (?) (% Unk) Respiratory &amp; neuronal pathology</b>	<b>No licensed drug or vaccine</b>	<b>3  N, P, L</b>

# Key Biological Properties of RG4 Mononegavirales

- Single-stranded, negative-sense RNA viruses
- 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 its derived full-length cDNA are considered to be Select Agents

# cDNA Manipulations of RG4 Mononegavirales

- The full-length RNA genome can produce infectious virus only with the addition of 3 or 4 essential viral proteins.
- The full-length cDNA of any of these viruses can produce infectious virus only if the full-length RNA genome is produced and 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 shared biological properties of these RG4 agents, the biosafety level recommended for cloning the full-length cDNA of Ebola into non-pathogenic *E. coli* should also apply to Marburg, Nipah and Hendra virus cDNA work.
- **Biosecurity**: All research with these Risk 4 agents raise similar biosecurity concerns and the RAC's assessment and recommendation for Ebola virus biosecurity shall apply to all other RG4 viruses of the order Mononegavirales.

# General Requirements for cDNA cloning of RG4 Mononegavirales full-length genomes

## ■ Biosafety and Containment

- Cloning of full length RG4 Mononegavirales cDNA in any non-pathogenic prokaryote (e.g. *E. coli* K12) can safely be performed at BL2
  - ▶ Physical and procedural measures will be undertaken to limit access to the laboratory area where
    - Work will be performed
    - Reagents will be stored
    - Waste will be decontaminated

# Biosecurity for cDNA cloning of RG4 Mononegavirales in *E. coli*

## ■ Biosecurity

- In keeping with the approach outlined in the 5<sup>th</sup> edition of the NIH/CDC *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), the PI and Institution are responsible for developing and implementing an appropriate biosecurity plan that addresses the principles of biosecurity outlined in the BMBL and is commensurate with the risk.

# Biosecurity for cDNA cloning of RG4 Mononegavirales in *E. coli*

This biosecurity plan, at a minimum, shall include the following:

- Appointment of a responsible institutional official to oversee the Biosecurity Plan
- Appropriate security screening for personnel who will have access to the complete cDNA
- Establishment of an inventory system that tracks:
  - ▶ The quantity of the cDNA and the rescue plasmids
  - ▶ Personnel access to these constructs
- Policies that prevent the complete cDNA and helper plasmids being worked with simultaneously in the same lab
- A mechanism for periodic review and update as necessary of the security plan

# Future Requests - General

- OBA will review future requests to lower containment for research with the full-length cDNA of RG4 viral agents in the order Mononegavirales in non-pathogenic *E. coli*
- Approval to conduct such research at BL2 will be specific to a PI at a specific institution

# Condition to Lower Containment

- Lowering of containment will only be available to PIs who plan to use the cDNA to rescue virus at BL-4 or those with research agreements with another PI to rescue virus at BL-4
  - ▶ The biosecurity risk of conducting this research at BL2 is undertaken to facilitate the ultimate goal of this research: to study the impact of manipulations to the cDNA (genome) on the full virus

# Future Requests - Required Information

- A written description of the biosafety risk assessment undertaken in accordance with the *NIH Guidelines* and the *BMBL* to include:
  - ▶ Physical and procedural measures to limit access to the laboratory or area where
    - Work will be performed
    - Reagents will be stored
    - Waste will be decontaminated

# Future Requests - Required Information

## ■ Biosecurity

- ▶ Establish a written biosecurity plan that is consistent with the “Principles of Biosecurity” in the 5<sup>th</sup> edition of the BMBL and includes:
  - Appointment of an appropriate official to oversee the security plan on behalf of the Institution
  - Establishment of a training program for the security aspects of the research in addition to biosafety
  - Periodic re-evaluation of the program and provisions to update it as needed

# Future Requests - Required Information

## ■ Biosecurity

- ▶ Describe the policies and procedures in place to ensure:
  - Appropriate security screening for personnel who will have access to the complete cDNA
  - Control and accountability for all research materials
  - Access control to the laboratory and materials
  - Material transfer procedures
  - Training, emergency planning and program management