

Cloning cDNA from Risk Group 4 Flaviviruses: Biosafety & Biosecurity

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March 12, 2013



National Institutes
of Health

Overview

- Biosafety conditions for the cloning of Risk Group (RG) 4 viruses in non-pathogenic bacteria under the *NIH Guidelines*
- Office of Biotechnology Activities (OBA) Guidance for cloning of cDNA from RG 4 negative stranded viruses (e.g. Ebola, Marburg, Hendra) in *E. coli*
- Application of Guidance to RG4 positive-stranded RNA Flaviviruses

Risk Group (RG) 4 Flaviviruses

Non-segmented, positive sense,
single-stranded RNA viruses



- **Tick-borne Hemorrhagic Fever Group:**
(aka Tick-borne Encephalitis Group)

Kyasanur, Alkhurma, Omsk
all are Select Agents

Cloning Full-length cDNA of RG 4 RNA Viruses – *NIH Guidelines*

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 Full-length cDNA of negative sense RG 4 RNA Viruses

NIH Guidelines

In September 2009, after consultation with the RAC, OBA issued a set of recommendations to allow for the cloning of full-length cDNA genomes of Mononegavirales under BL2 containment:

Points to Consider When Applying to the NIH Office of Biotechnology Activities (NIH OBA) for a Reduction in Biosafety Level (BL) Containment for Experiments Involving the Cloning of Full-length cDNA Constructs Derived from Risk Group (RG) 4 Viruses of the Order *Mononegavirales*

http://oba.od.nih.gov/oba/rac/PTC_RG4_MNGV.pdf

Request for Lowering of Containment

- In light of this Guidance that allows the cloning of full-length cDNAs of RG4 Mononegavirales (e.g. Ebola, Marburg, Nipah and Hendra) in *E. coli* at Biosafety Level 2 (BL2) containment if certain biosafety and biosecurity practices are in place, OBA was asked to consider whether the same could apply to the RG4 Flaviviruses.

Cloning cDNAs of Positive Sense RG4 RNA viruses in *E. coli*

- Unlike the negative sense RG4 viruses, positive sense RG4 RNA genomes alone can produce infectious virus by relying on cellular machinery to initiate replication.
- Introduction of the RNA genome alone into tissue culture cells or directly into animals can result in viral replication.
- Full-length cDNA constructs containing eukaryotic promoters can be fully infectious in mammalian cells and *in vivo*.

Key Characteristics	Mononegavirales (e. g Ebola, Marburg, Hendra)	Flaviviruses
Genome	Negative-stranded RNA genome is not infectious (in absence of other viral proteins)	Positive-stranded RNA genome is infectious
cDNA In mammalian cells	Rescue of infectious virus in mammalian cells requires RNA and addition of supporting viral proteins in stoichiometric quantities	cDNA alone can yield infectious virus in mammalian cells if promoters or cryptic transcription start sites are present
cDNA In prokaryotic cells	Virus cannot be rescued from cDNA	Virus cannot be rescued from cDNA
Select Agent Status	Yes	Yes

Cloning cDNAs of Positive Sense RG4 RNA viruses in *E. coli*

Does the “Points to Consider” guidance document adequately address the biosafety concerns related to the cloning of full-length cDNA of RG4 Flaviviruses?

- Based on recommendations of the Biosafety Working Group (BSWG)
 - The same security concerns apply
 - However, the biosafety concerns differ because of the infectious nature of the RNA, thus a potential risk of infection should the cDNA be transcribed by known or unintentional/unknown cellular processes.

“Points to Consider” Guidance as applied to the Flaviviruses cDNA

- Research is 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

“Points to Consider” Guidance as applied to the Flaviviruses

- 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

“Points to Consider” Guidance: Recommendations for Flaviviruses

Enhanced BL2 Practices specific for Flaviviruses:

- Work is performed in a dedicated strict prokaryotic cloning laboratory
 - No mammalian cell tissue culture work
 - No *in vitro* transcription work
- Full-length cDNA clones are appropriately secured
- Supporting expression plasmids are not stored in the dedicated cloning laboratory

“Points to Consider” Guidance: Recommendations for Flaviviruses

Enhanced BL2 Practices, cont...:

- The use of sharps is to be avoided
- Use of glassware is to be avoided
 - Plastic alternatives are recommended
- Personal Protective Equipment (PPE) must include adequate mucosal membrane protection

“Points to Consider” Guidance: Recommendations for Flaviviruses

Enhanced BL2 Practices:

PPE – continued...

- Suite-dedicated or disposable lab coats
- Gloves (latex, nitrile, vinyl etc.) are chosen to resist those chemicals used in the cloning procedures

Questions?