

Cloning of full-length Ebolavirus and Marburgvirus cDNA clones in BSL-2 containment

RAC Meeting

March 11, 2010

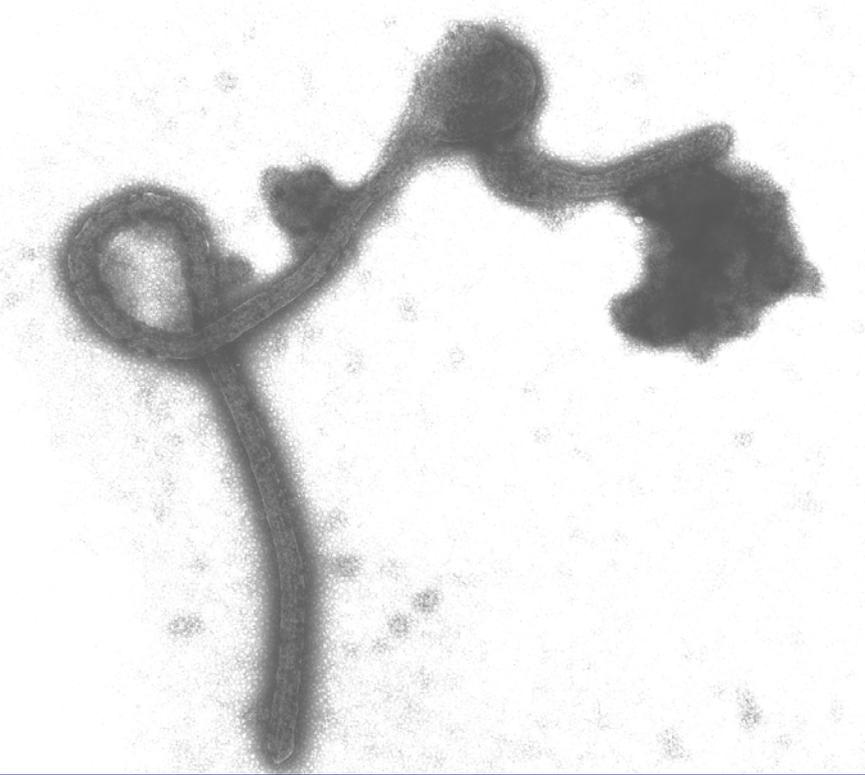
Principal Investigator

Yoshihiro Kawaoka, DVM, Ph.D.

University of Wisconsin-Madison

University of Wisconsin Team

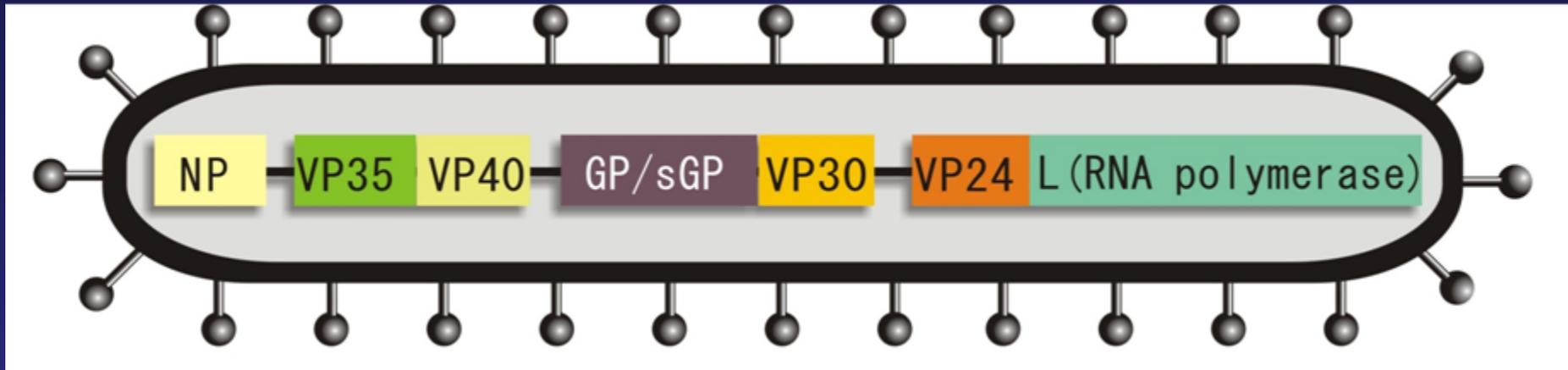
- **Gabriele (Gabi) Neumann, Ph.D.**
 - Res. Assoc. Prof., Kawaoka group
- **James Turk, M.S.**
 - Biological Safety Officer
 - Assistant Director, UW - Environment, Health, and Safety
- **Rebecca Moritz, M.S.**
 - Compliance Specialist
 - Alternate Responsible Official (ARO) for UW Select Agent Program



Filoviruses

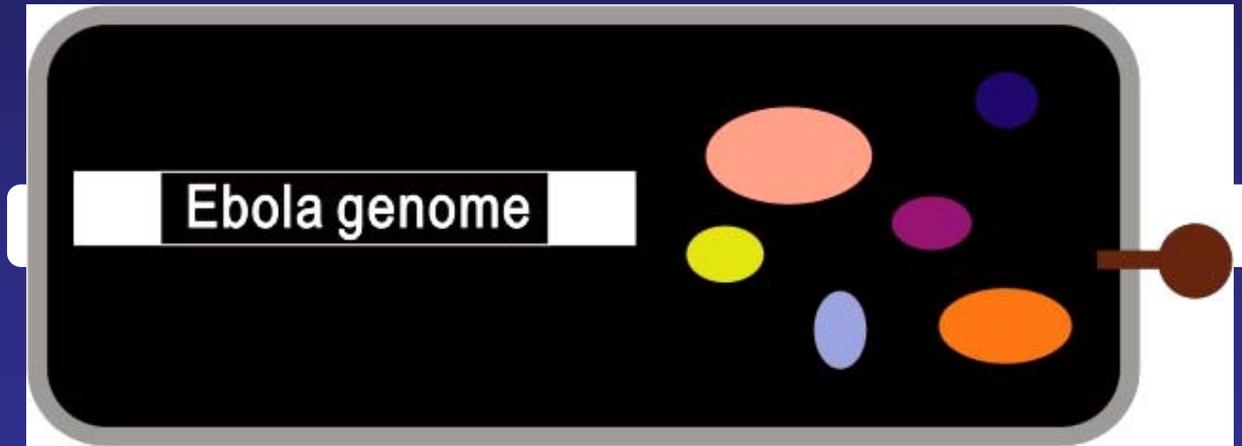
- Hemorrhagic fever
- Up to 90% mortality
- No vaccines or antiviral treatments
- BSL-4

Ebolavirus genome organization

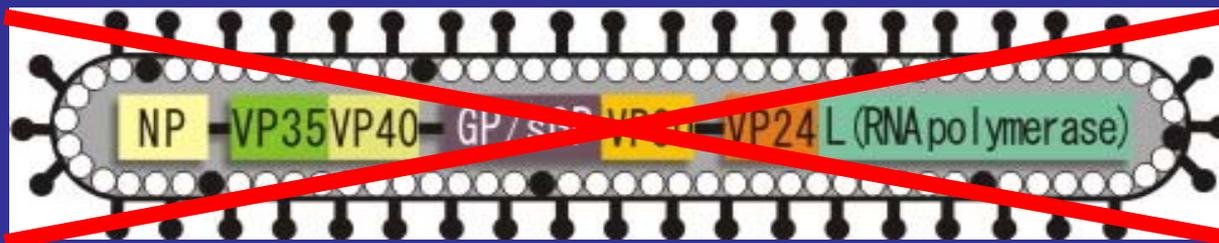
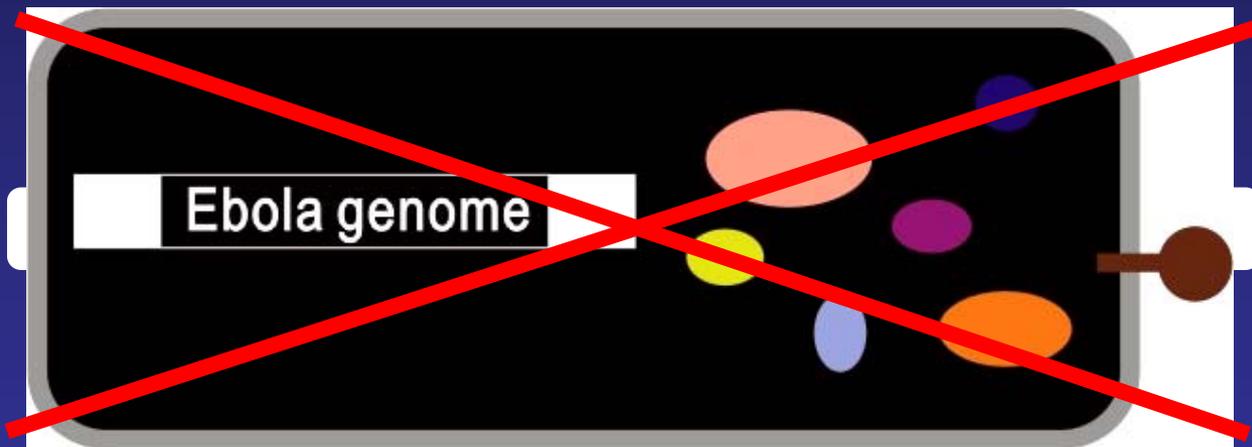


- **Genome:**
 - **Single-stranded RNA**
 - **Negative-polarity**
- **Viral RNA alone is NOT infectious**
- **Viral cDNA is NOT infectious**

Reverse genetics of Ebolavirus



Proposed work in 'restricted' BSL-2 lab



Work proposed in the 'restricted' BSL-2 lab

- Cloning of full-length Ebolavirus
and Marburgvirus cDNA clones**
- NO protein expression plasmids**
- NO cell culture work**

Rationale

- **Long-standing collaboration with**
 - **Heinz Feldmann, MD, PhD, Chief, Laboratory of Virology, NIH/NIAID Rocky Mountain Labs**
- **Several NIH grants/contracts with Dr. Feldmann**
 - **U54 AI081680, U54 AI057153, R01 AI077593**
- **Collaboration agreement in preparation**

Influenza Research Institute (IRI)



- Stand-alone facility
- 28,000 sq.-ft. facility, two stories
- Controlled access
- Kawaoka group only

Influenza Research Institute (IRI)



- Card readers
- Cameras with live feed to UW police
- Personnel who works at the IRI

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Security - Overview

'Restricted' BSL-2 lab:

WITHIN Select Agent (SA) perimeter

Security - Overview

'Restricted' BSL-2 lab: within Select Agent (SA) perimeter

Access to SA perimeter:

- ID card
- Biometrics
- SA approval
- SA training

Access to 'restricted' BSL-2 lab (within SA perimeter):

- High-security key
- Selected personnel (subgroup of SA-approved personnel)

Security - Details

Access to 'Restricted' BSL-2 Lab:

- Yoshihiro Kawaoka, DVM, Ph.D., PI
- Peter Halfmann, Ph.D., Assistant Scientist
- Martha McGregor, MS, Researcher
- Gabriele Neumann, Ph.D., Res. Assoc. Prof.

- Approved for work with Select Agents

Work Flow

- **Mutate subgenomic cDNA fragments in 'regular' BSL-2 lab**
- **Take (mutated) subgenomic cDNA fragment to 'restricted' BSL-2 lab**
(see next slides for more details)
- **Clone (mutated) subgenomic cDNA fragments into full-length cDNAs clones**
(see next slides for more details)
- **Ship (mutated) cDNA clones to Dr. Feldmann**
(see next slides for more details)

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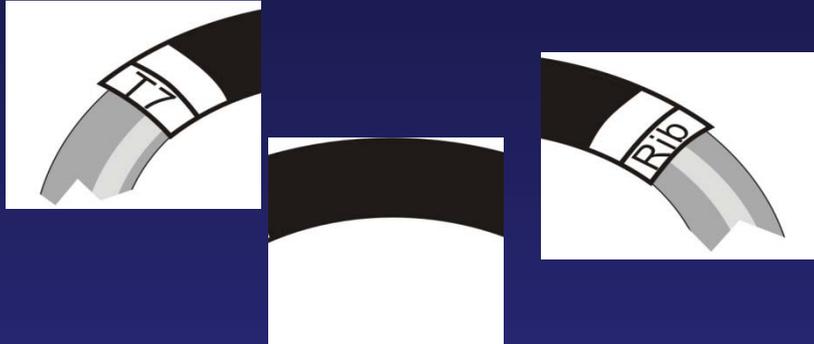
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Work Flow



- 'Regular' BSL-2:**
- Subgenomic fragments (pieces of viral cDNA)

'Regular' BSL-2

'Restricted' BSL-2

- Combine subgenomic fragments into full-length viral cDNA



Flow of Material - IN

- **(Mutated) subgenomic cDNA fragments will be brought from 'regular' BSL-2 lab to 'restricted' BSL-2 lab**
- **Full-length cDNAs will be obtained from Dr. H. Feldmann**
 - **cDNA clones will be shipped as Dangerous Goods**
 - **Sealed transport container will be brought directly to 'restricted' BSL-2 lab**
 - **Sealed transport container will be opened in 'restricted' BSL-2 lab**

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Flow of Material - OUT

- **cDNA clones will be shipped as plasmids in liquid form**
- **Place screw cap tube in secondary transport container with absorbant material**
- **Spray outside of transport container with 70% EtOH**
- **Remove from 'restricted' BSL-2 lab**
- **Ship as Dangerous Goods**

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Equipment in 'restricted' BSL-2 lab

- **Thermocycler (PCR)**
- **Eppendorf centrifuge**
- **Waterbath**
- **Test tube shaker**
- **Refrigerator/freezer**
- **Ultracold freezer (-80C)**

Pressure in 'restricted' BSL-2

- Negative pressure relative to adjacent room (clean cage wash area)

Entry protocol

- **Enter BSL-3/SA locker room**
- **Change into scrubs, inner shoe covers, hall shoes, outer shoe covers**
- **Enter BSL-3/SA 'secure' hallway**
- **Enter BSL-3/SA clean cage wash area**
- **Enter 'restricted' BSL-2 lab**
- **Put on lab coat, gloves, and eye protection**

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Exit Protocol

- **Spray gloves with 70% EtOH**
- Remove eye protection, outer shoe covers, gloves, lab coat
- Exit 'restricted' BSL-2 lab to clean cage wash area
- Put on new outer shoe covers
- Walk over to sink in clean wash area, wash hands
- Proceed to locker room
- Remove outer shoe covers, shoes, inner shoe covers
- Remove scrubs
- Change into street clothes
- Exit BSL-3/SA perimeter

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Liquid Waste

- **Disinfect with 10% bleach**
- **Let sit over night**
- **Pour down sink in clean cage wash area
(within BSL-3/SA perimeter)**

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Solid Trash

- **Double-bag and seal**
- **Spray outside of trash bag with 70% EtOH**
- **Let sit for >20'**
- **Take to autoclave (secure hallway within BSL-3/SA perimeter)**
- **Autoclave immediately**
- **(if autoclave is not available, bag will be kept in trash container in 'restricted' BSL-2 lab)**

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Solid Trash

Autoclave:

- **Parameters will be verified before trash is removed**
- **Logs of all runs are kept**
- **Validated monthly with biological indicators**

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Large Spill (> 10 ml)

- **Alert co-workers, leave room immediately**
- **Close door, post “DO NOT ENTER” sign**
- **Remove contaminated PPE**
- **Wash hands, face, and exposed skin**
- **Replace PPE, wait 30’ before re-entry**
- **Carefully pour disinfectant (10% bleach) around spill, cover spill with disinfectant-soaked paper towels**
- **Allow to sit for 20’**
- **Transfer contaminated material into autoclave bag**
- **Wash or mop entire area with disinfectant, discard gloves**
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- **Report to supervisor (see slide on ‘Incident Reporting’)**
- **Submit ‘First Report’ form (see slide on ‘Incident Reporting’)**

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- Submit 'First Report' form (see slide on 'Incident Reporting')

Small Spill (< 10 ml)

- Carefully pour disinfectant (10% bleach) around spill
- Cover spill with disinfectant-soaked paper towels
- Allow to sit for 20'
- Transfer contaminated material into autoclave bag
- Discard gloves
- **Autoclave all contaminated materials**
- Report to supervisor (see slide on 'Incident Reporting')
- Submit 'First Report' form (see slide on 'Incident Reporting')

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- Autoclave all contaminated materials
- Report to supervisor (see slide on 'Incident Reporting')
- **Submit 'First Report' form (see slide on 'Incident Reporting')**

Communication

- **Phone in clean cage wash area**
- **Portable radio in 'restricted' BSL-2 lab**

Visitors / Maintenance Staff

- **Log-in**
- **Escorted/supervised at all times**

Inventory

- **CDC and APHIS-approved inventory plans are in place**
- **Will use same plan for full-length filovirus cDNAs**
- **Will log the creation, usage, and destruction of**
 - **each plasmid**
 - **each glycerin stock**
- **Monthly inventory by two people**

Training

Training requirements for work with Select Agents in BSL-3:

- Three online training modules (Biosafety, Sharps, NIH Guidelines)
- Annual mandatory refresher training (Biosafety Dept.)
- Annual mandatory refresher training (Kawaoka group)
- Additional training as needed (for example, for revised SOPs)
- All training is documented
- Must demonstrate proficiency

Incident Reporting and Response

- **Personnel will immediately inform PI or designated representative**
- **PI will inform Responsible Official for Select Agent Program (Bill Mellon) or alternate (Jeff Zebrowski, Darren Berger, Rebecca Moritz)**
- **Lab will submit UW 'First Report' form
(to be submitted within 24 hours of incident)**
- **Reportable incidents:**
 - **Biosafety incidents (exposures)**
 - **Hazardous materials incidents (spills or releases)**
 - **Biosecurity incidents (thefts or threats)**

Comparison – Safety/Security

Feldmann (Approved)

Kawaoka (Pending)

Access to facility

Controlled

Controlled

Access to 'restricted'
BSL-2 lab

Controlled

Controlled

Work in 'restricted'
BSL-2 lab

Institutional
security clearance

Select Agent
Approval

Negative Pressure

Yes

Yes

Entry Protocol

Yes

Yes

Exit Protocol

Yes

Yes

Separate PPE

Yes

Yes

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Yes

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Yes

Exit Protocol

Yes

Yes

Separate PPE

Yes

Yes

Comparison – Proposed Work

Feldmann (Approved)

Kawaoka (Pending)

Full-length cDNA clones

Yes

Yes

Protein expression
plasmids

No

No

Cell culture

No

No

Virus rescue

No

No

Record keeping

Yes

Yes

Comparison – Proposed Work

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Kawaoka (Pending)

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Yes

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Protein expression
plasmids

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Cell culture

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Virus rescue

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Record keeping

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**Thank you for your
attention!**