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IBE 2007
March30,2007
St. Louis, Missouri

Automated Antiviral Drug Screening Using Engineered Replication Systems

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Antiviral Market Opportunities

Big Markets (Chronic / high prevalence / high incidence)

- Human immunodeficiency virus (HIV)
- Hepatitis C Virus (HCV)
- Herpes viruses (HSV, VZV, EBV, CMV)

Modest Markets (acute / high incidence)

- Influenza (A & B)
- Respiratory syncytial virus (RSV)

Niche Markets (acute / low incidence)

- Respiratory viruses (SARS, PIV1-3, hMPV)
- Enteric viruses (rotavirus, enteroviruses, caliciviruses)
- Encephalitis viruses (VEE, JE, TBE)
- Hepatitis viruses (hepatitis A, hepatitis B, hepatitis E)
- Hemorrhagic fever viruses (Ebola, Marburg, Lassa fever, etc.)

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Serious Viral Threats

Avian Influenza/SARS

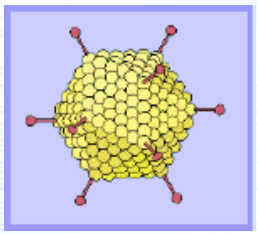
Bioterrorism Threats

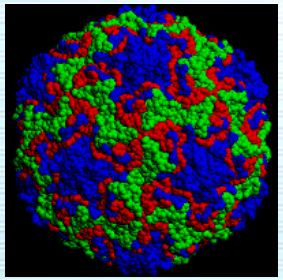
- »Hemorrhagic fever viruses
- »Venezuelan Equine Encephalitis

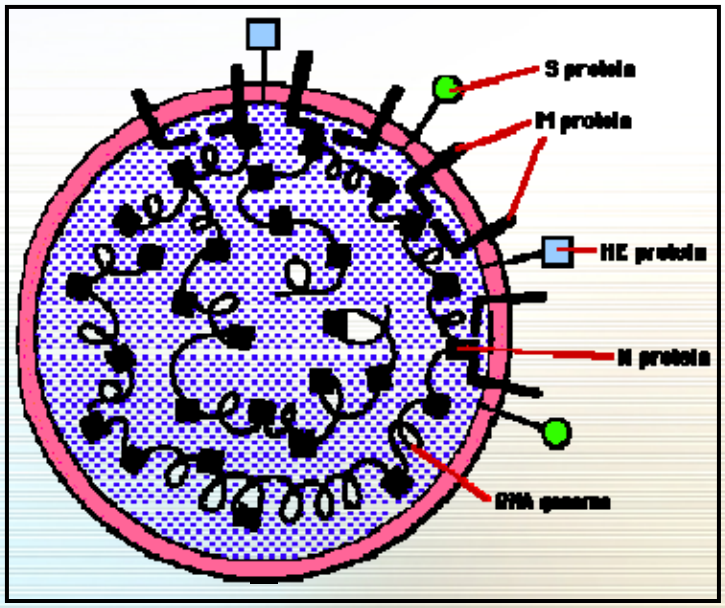
What is a Virus?

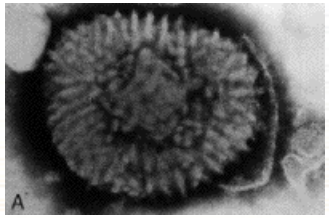
Virus: poison (Latin)

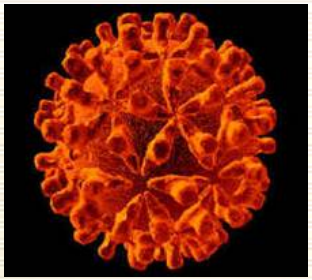
Submicroscopic entity that exists on the edge between biology and chemistry (between life forms and inanimate matter)











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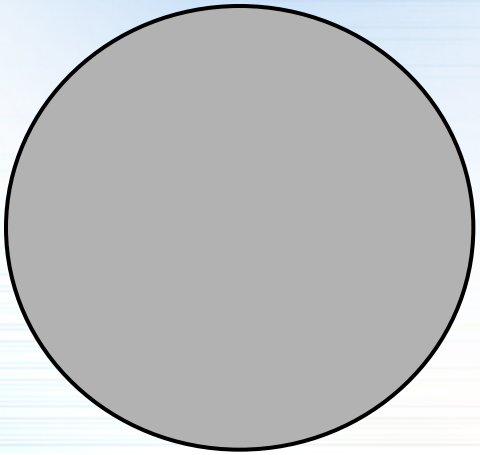
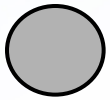
What is the size of a virus?



cell



bacterium

virus

1 micron (one millionth of a meter)

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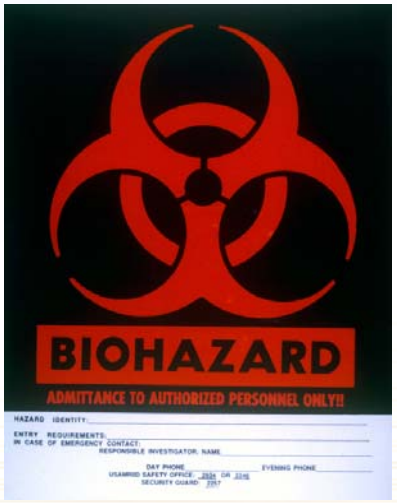
Antiviral Drug Discovery Challenges

- **Containment – Biosafety Level 4**



- **Lack of reliable animal models**
- **Very specific host cell types**
- **Measuring efficacy in vitro**

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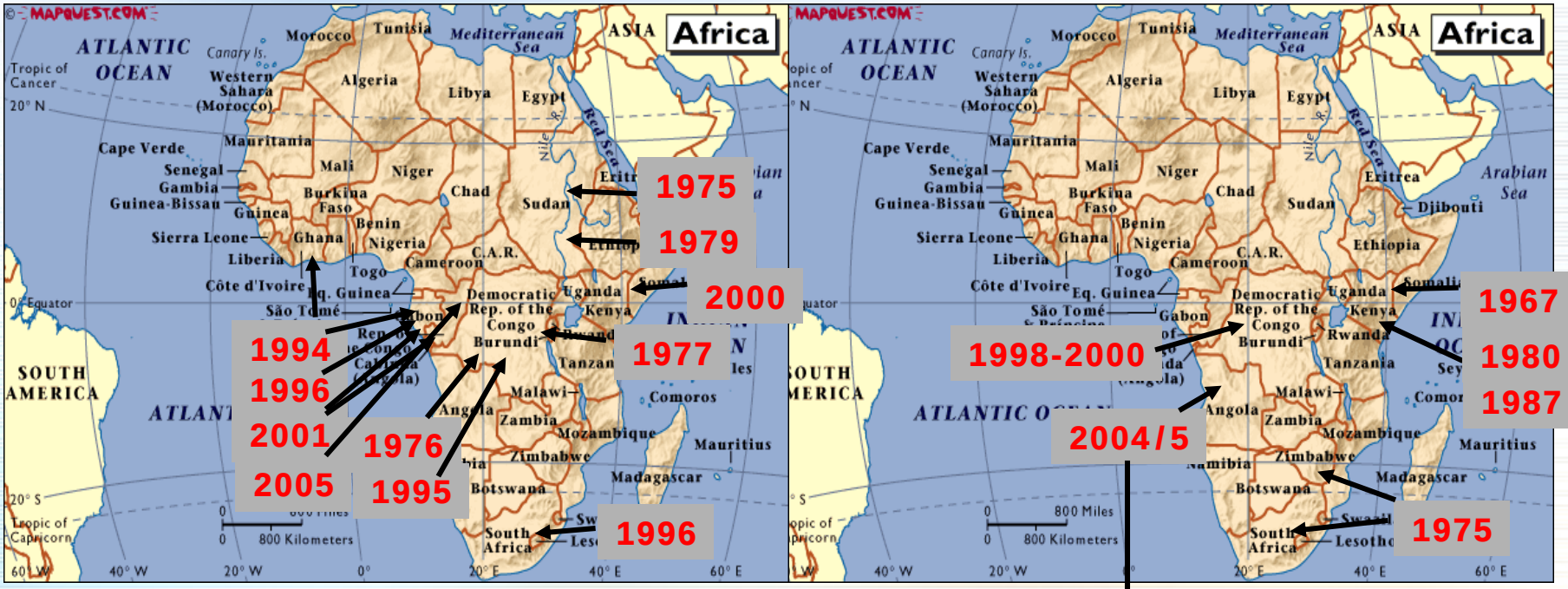
- Apath is an early stage drug discovery company focused on antiviral drug discovery
- Drug discovery platform is well suited to bioterrorism agents
- Screening platform based on subgenomic (replicons) and full length replication systems
  - 10 viruses (including 4 biodefense pathogens)

Ebola outbreaks

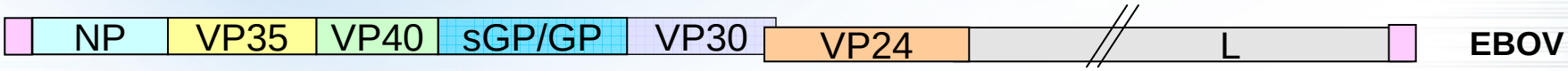
Marburg outbreaks



422 cases (356 deaths; >80% case-fatality)

Ebola virus

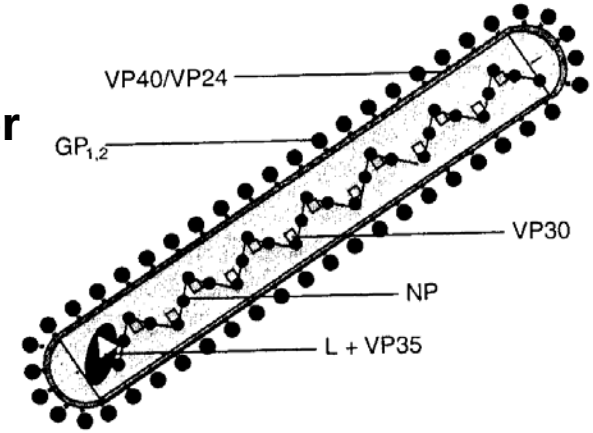
- **family: Filoviridae (filo (Latin): 'threadlike')**
- **enveloped**
- **genome: negative-sense, single-stranded RNA, 19 kb**

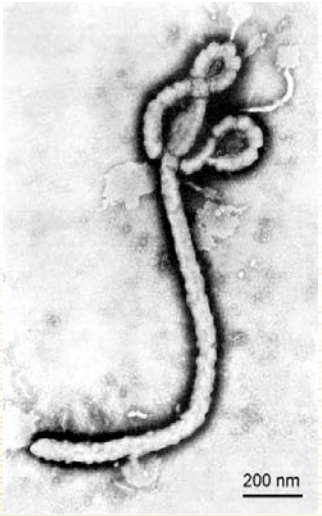


EBOV

Viral Proteins:

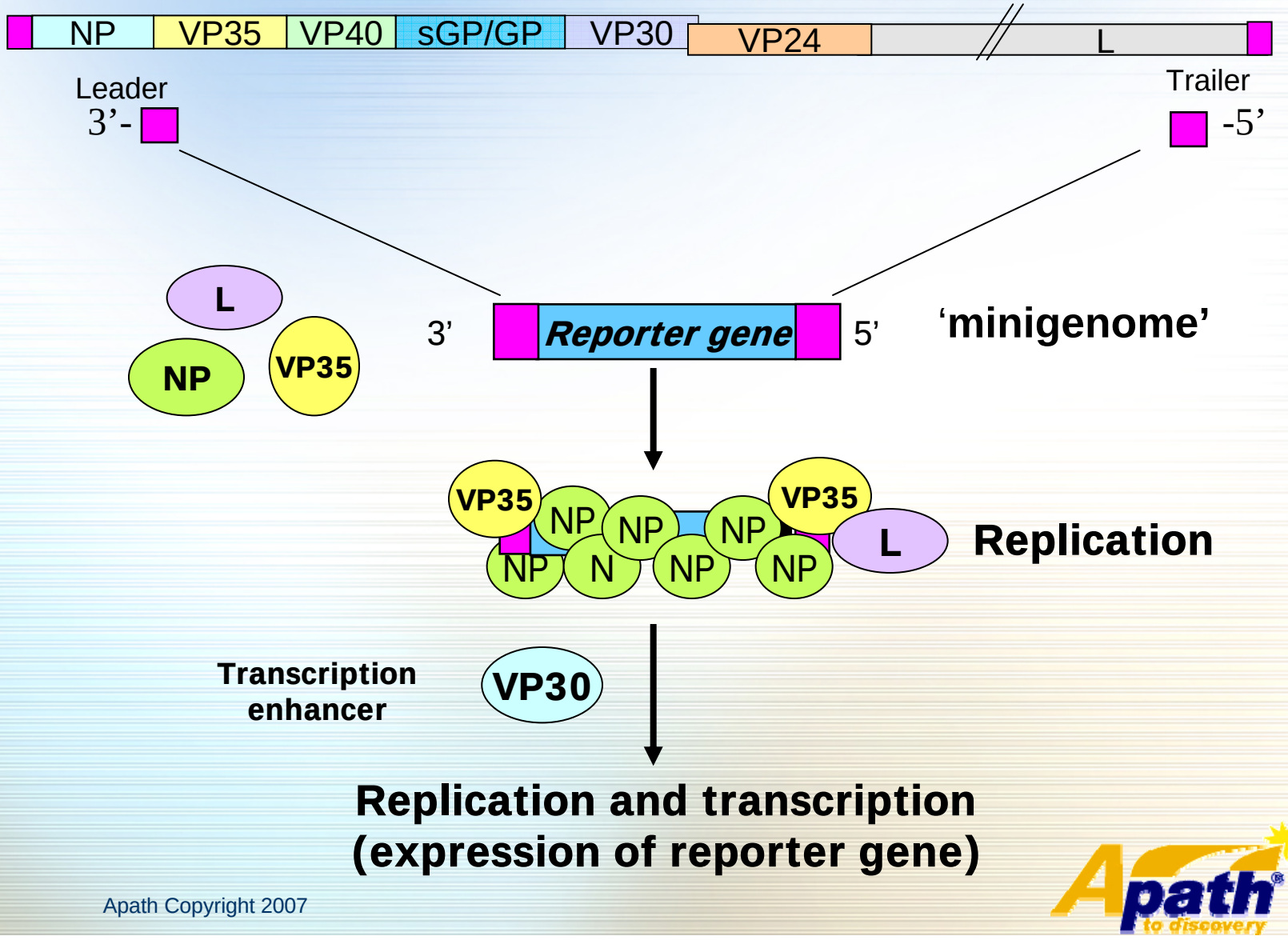
L = polymerase
VP35 = polymerase cofactor
NP = nucleoprotein
VP30 = transcription factor
GP = glycoprotein
VP40 = matrix protein
VP24 = matrix protein





EM image
Frederick A. Murphy, CDC

EBOV subgenomic replication

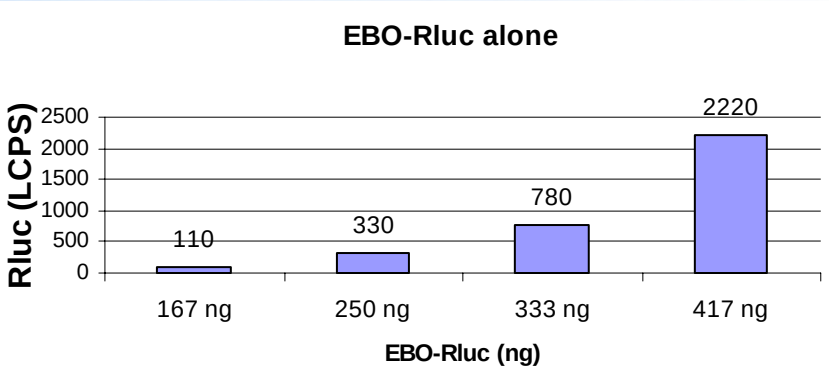


Rationale for replicon-based screen

- Focus on viral RNA replication, transcription and translation of viral proteins
- Cell-based assay that can be carried out at BSL-1/2
- Automated/High-throughput screening capability

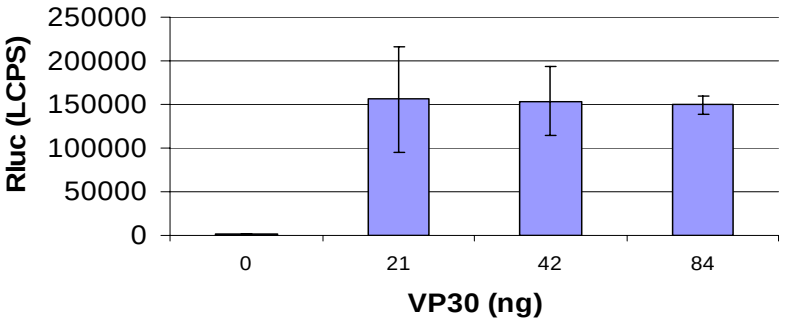
Reporter gene expression is dependent on viral proteins

Minigenome with reporter gene



T7 pol expression vectors for:

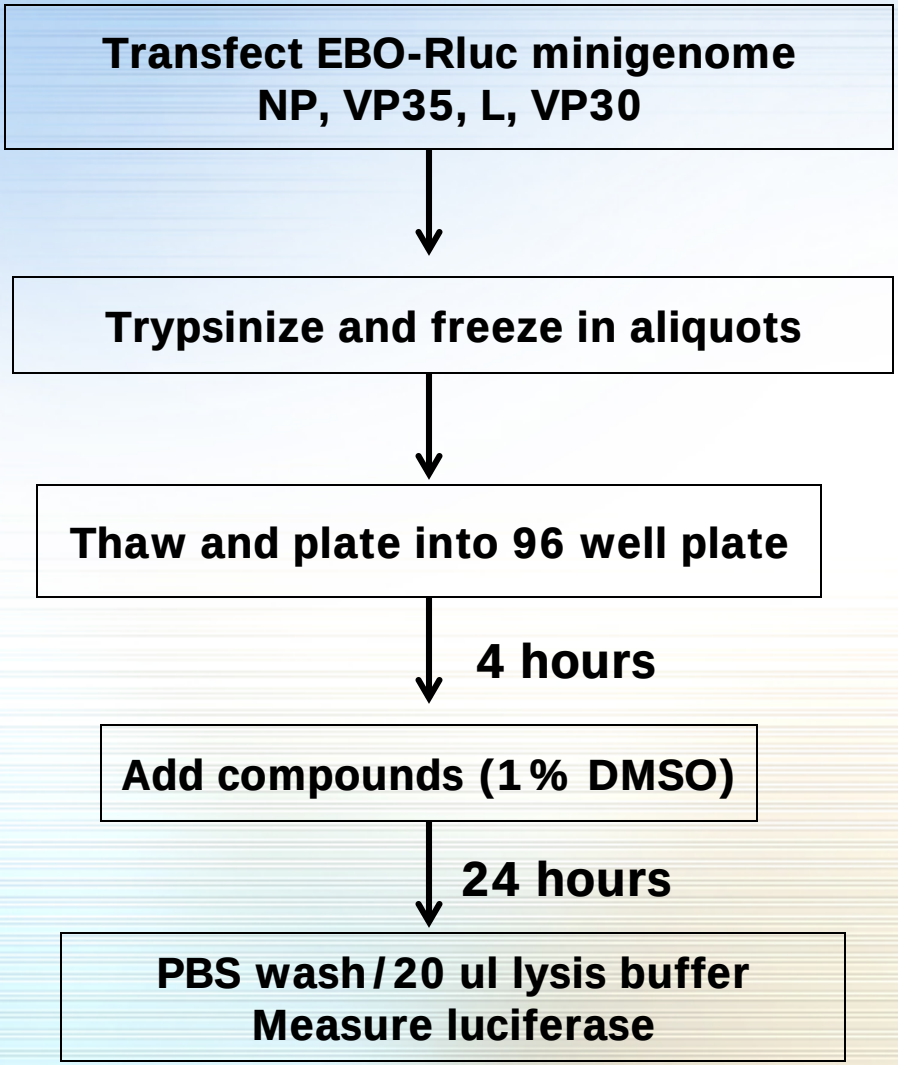
- NP
- VP35
- L
- VP30



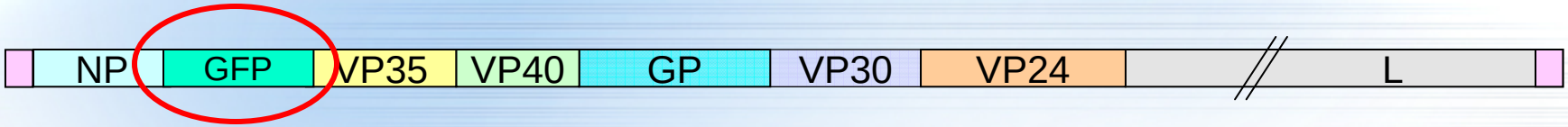
Signal: ca 150000 LCPS
Noise: ca 100 LCPS
S/N: 1500

EBO-Rluc:	**167 ng**
NP:	**208 ng**
VP35:	**208 ng**
L:	**208 ng**

EBO-Rluc screening setup



Ebola-GFP recombinant virus



- Zaire strain of Ebola virus
- High level of GFP expression
- Not cytolytic

Ebola-GFP infection assay:

- Vero E6 cells in 96 plates
- Infection (MOI = 0.1)
- IFN a control (IC90)
- 48h incubation
- Formalin fixation

} BCL-4

- Wash out formalin with PBS and soak in PBS (1h)

- GFP detection: Spectrofluorometer (bottom read)
- Signal to noise: S/N = $\geq$12
- Cytotoxicity: crystal violet staining (CC50 @ Apath by ATP-content)

Towner et al. *Virology:* 332(1):20-7; Feb. 5, 2005

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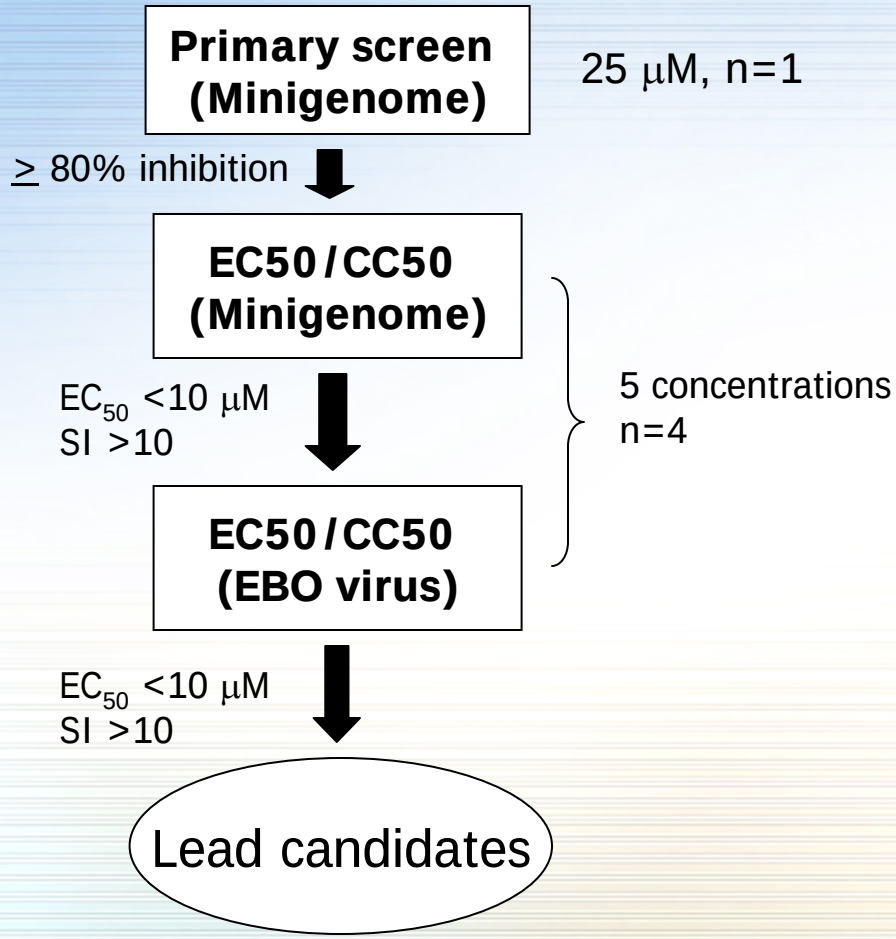
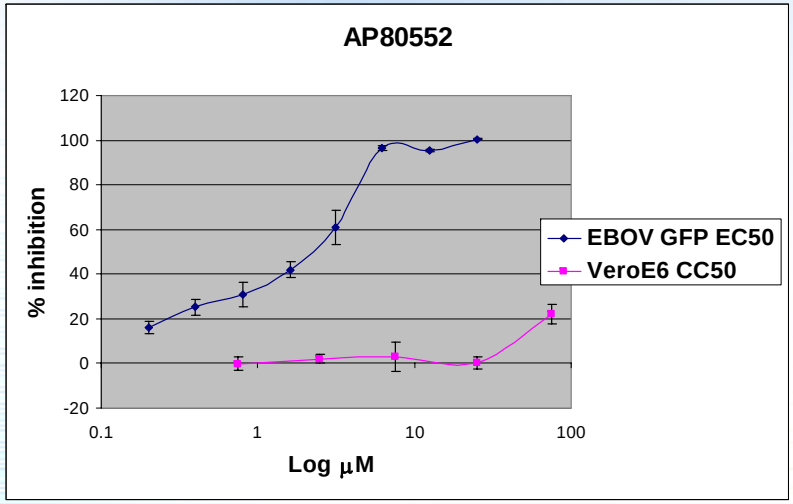
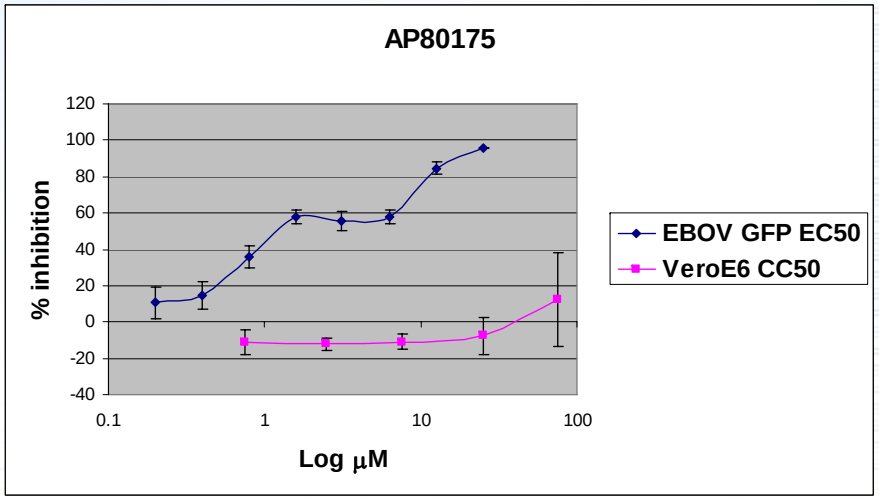
Screening protocol



Novel 2^0 Sulfonamide lead candidates





EC50 3.4
CC50: >75

EC50 1.8
CC50: >75

Summary

- Subgenomic replication represents a useful cell-based screening tool for identifying inhibitors of viruses (particularly BSL3 and 4 viruses).
- A number of lead candidates have been identified.
- Novel sulfonamide lead compounds have been identified
- Mouse efficacy studies have been initiated at USAMRIID under Project Bioshield

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