Ebola virus cDNA and the NIH guidelines for research involving recombinant DNA molecules

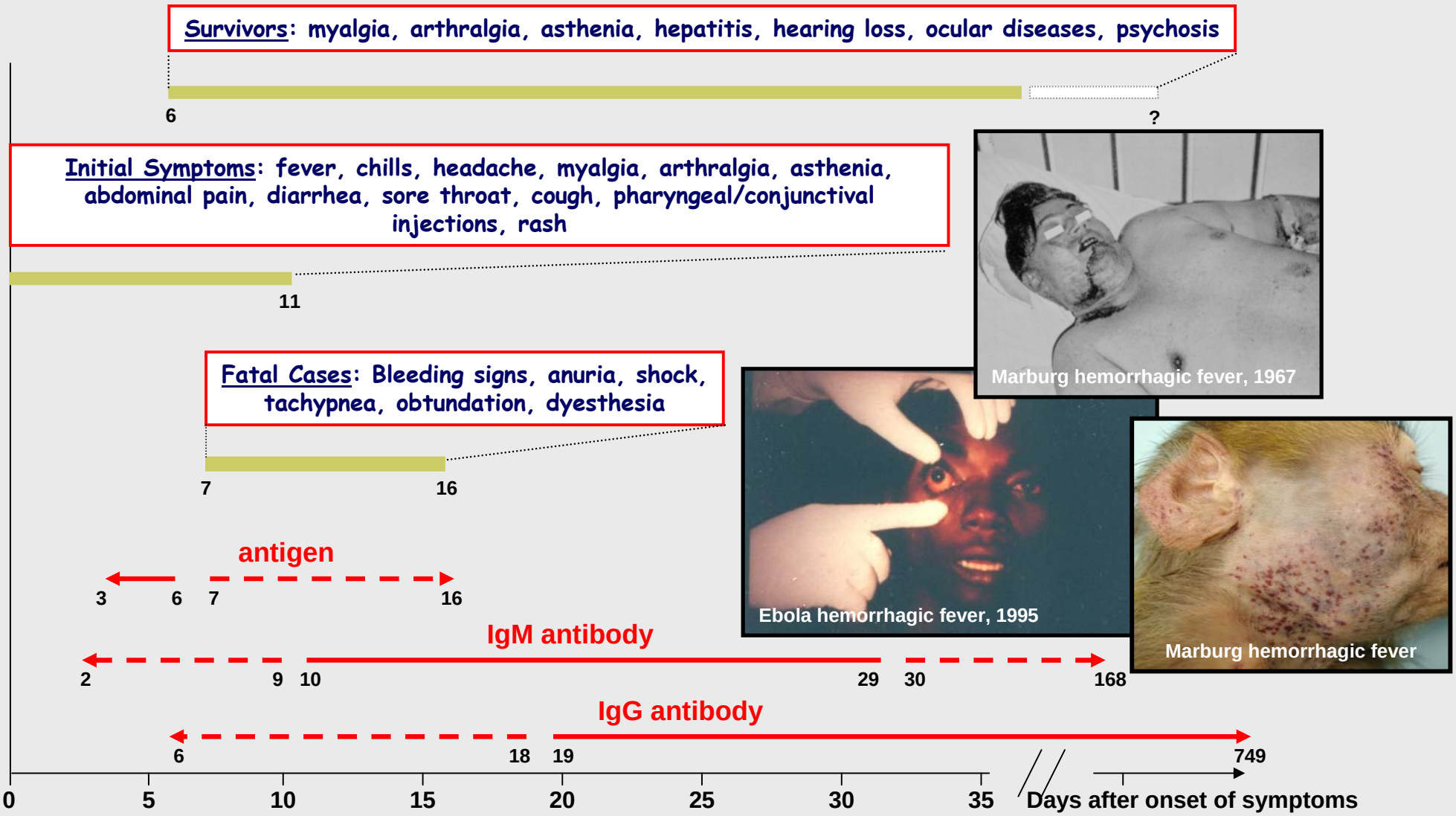
National Institutes of Health (NIH)
Recombinant DNA Advisory Committee (RAC)
116th Meeting
Tuesday, March 3-4, 2009

Heinz Feldmann, MD, PhD
Laboratory of Virology, DIR/ NIAID, NIH

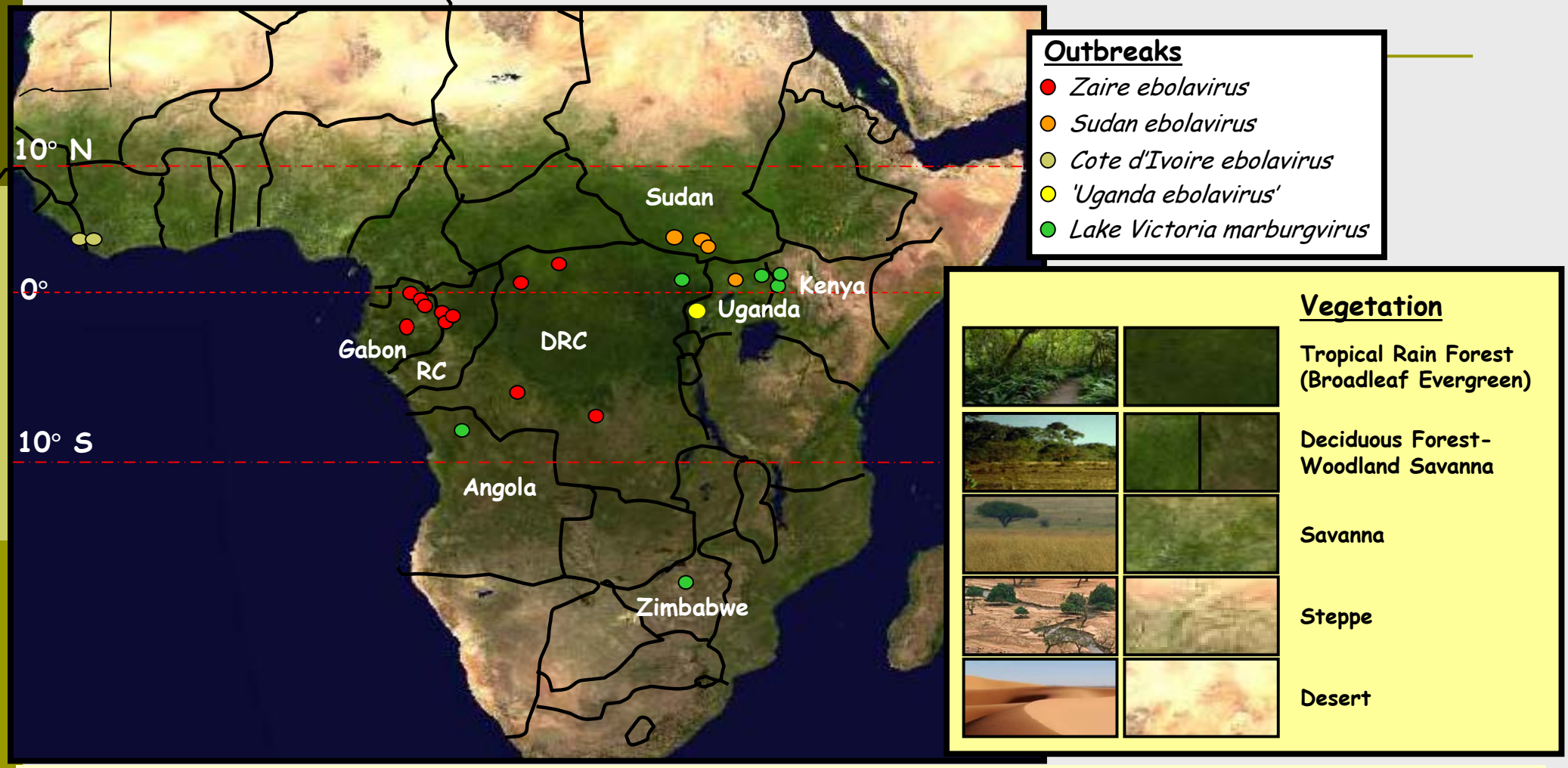
Ebola/Marbu

Treatment or prophylaxis:

- no licensed drug or vaccine available
- experimental approaches are promising

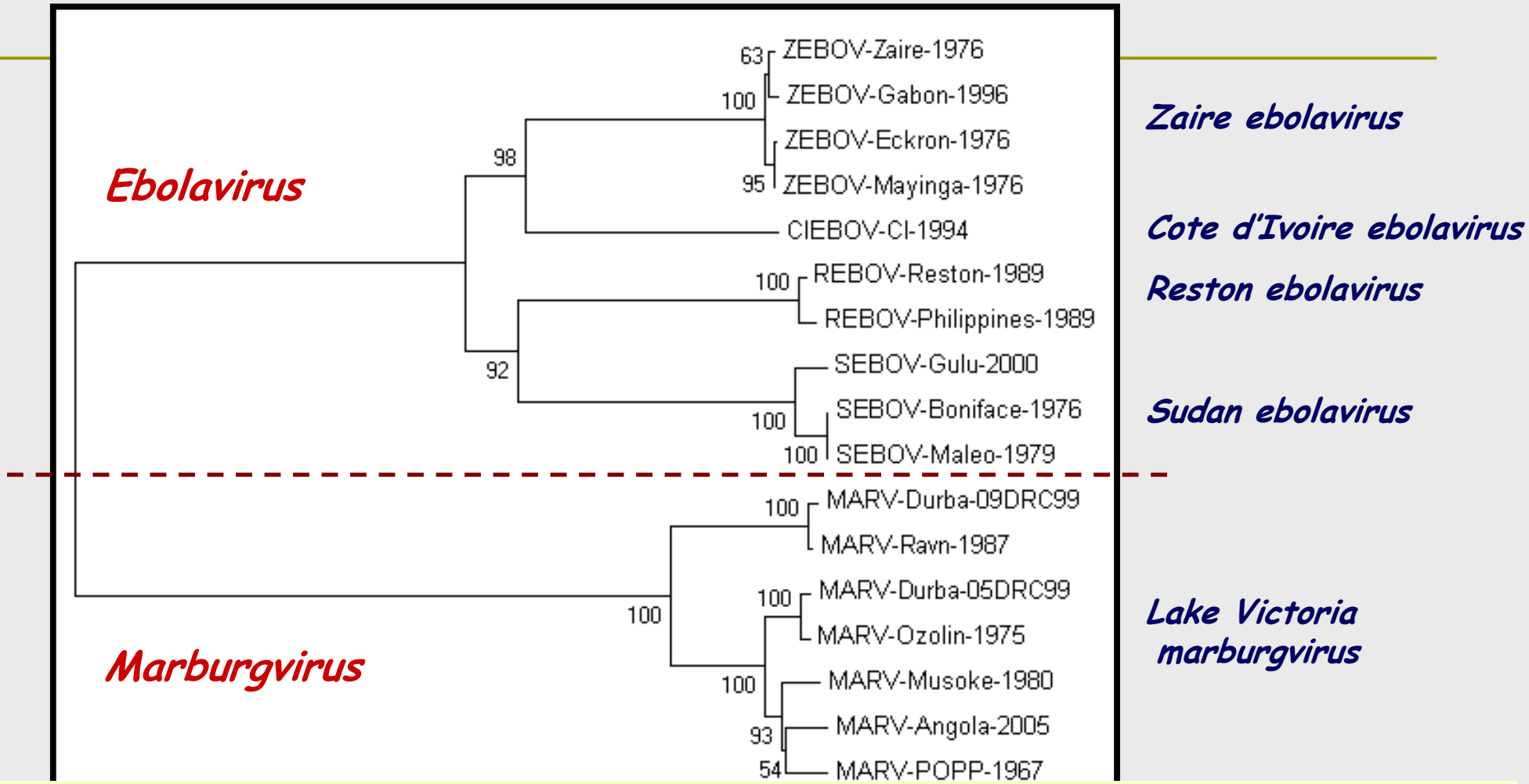


Filovirus hemorrhagic fever outbreaks



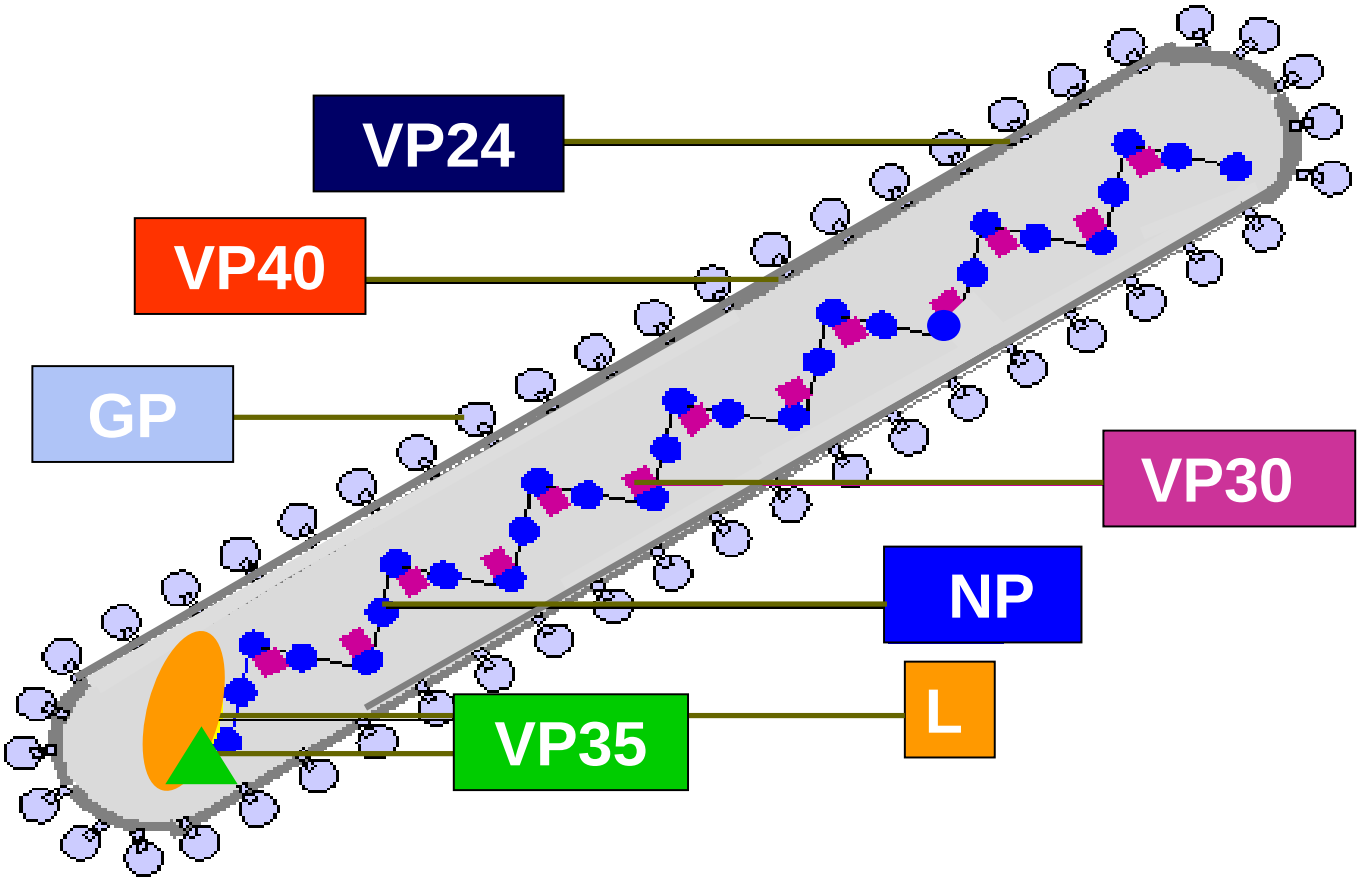
Ebola HF seems to be restricted to the humid rain forest of Central Africa, whereas Marburg HF is found in the drier areas of Central Africa

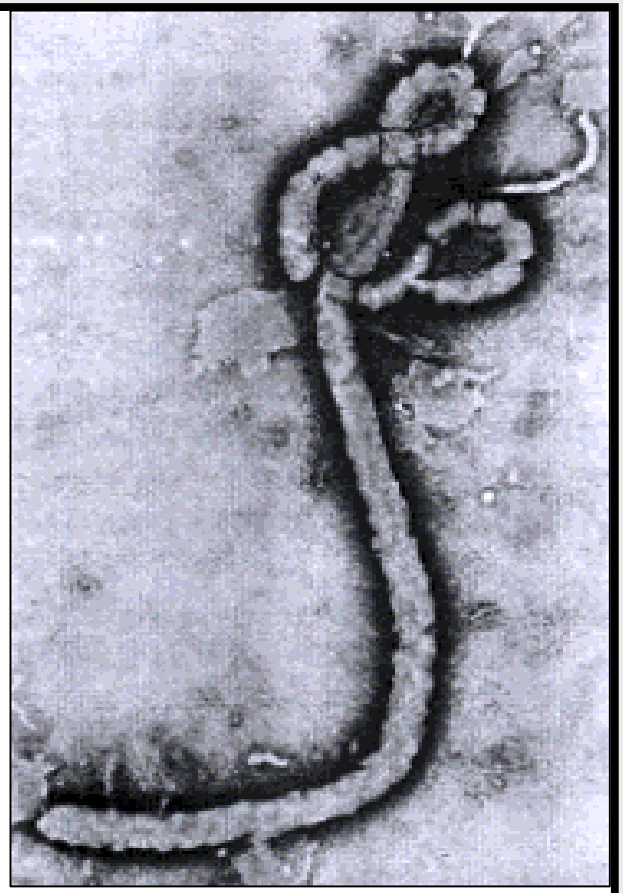
Filoviridae phylogeny (glycoprotein)

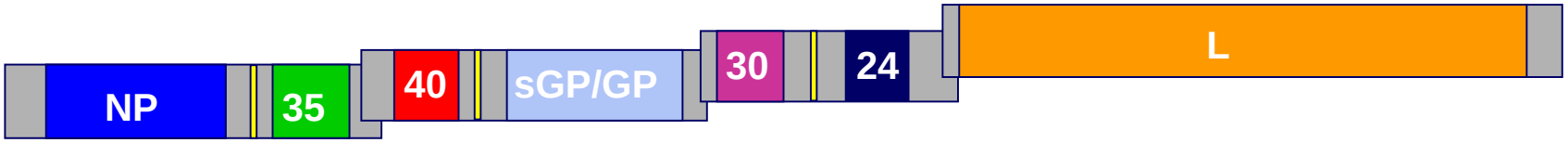


The causative agent of the recent Ebola HF outbreak in Uganda seems to represent a new species within the genus *Ebolavirus*

Ebola virus – morphology & genome

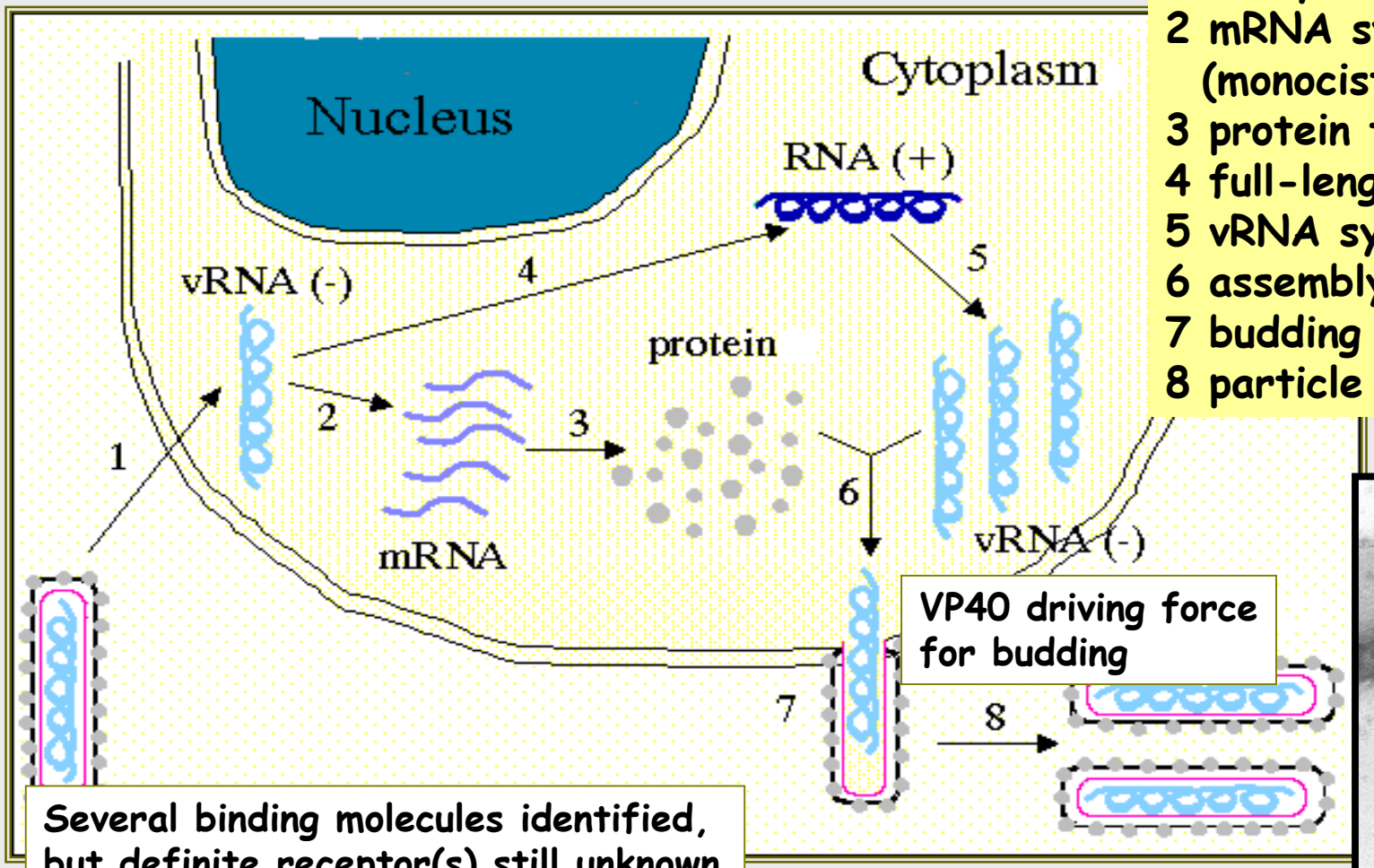






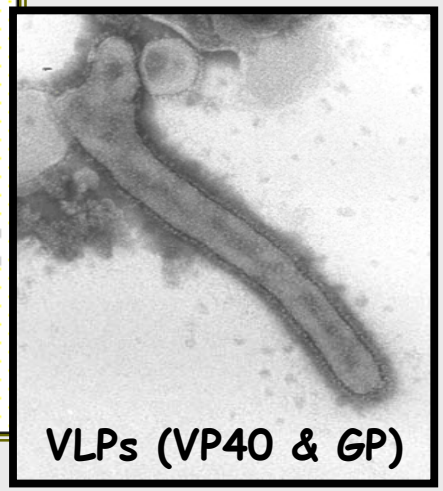
Virus replication

1 entry/fusion
2 mRNA synthesis (monocistronic)
3 protein translation
4 full-length cRNA synthesis
5 vRNA synthesis
6 assembly
7 budding
8 particle release



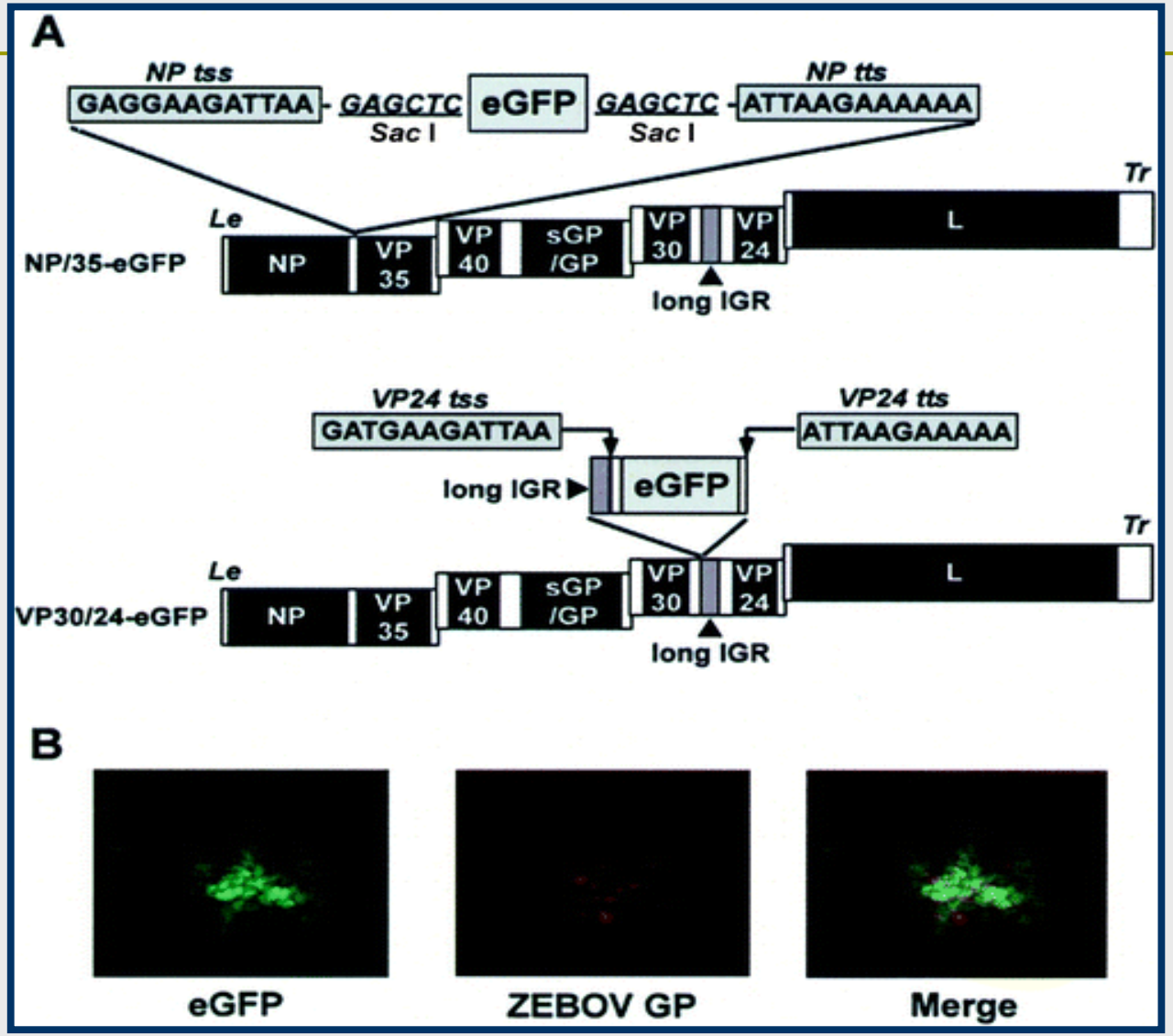
VP40 driving force for budding

Several binding molecules identified, but definite receptor(s) still unknown

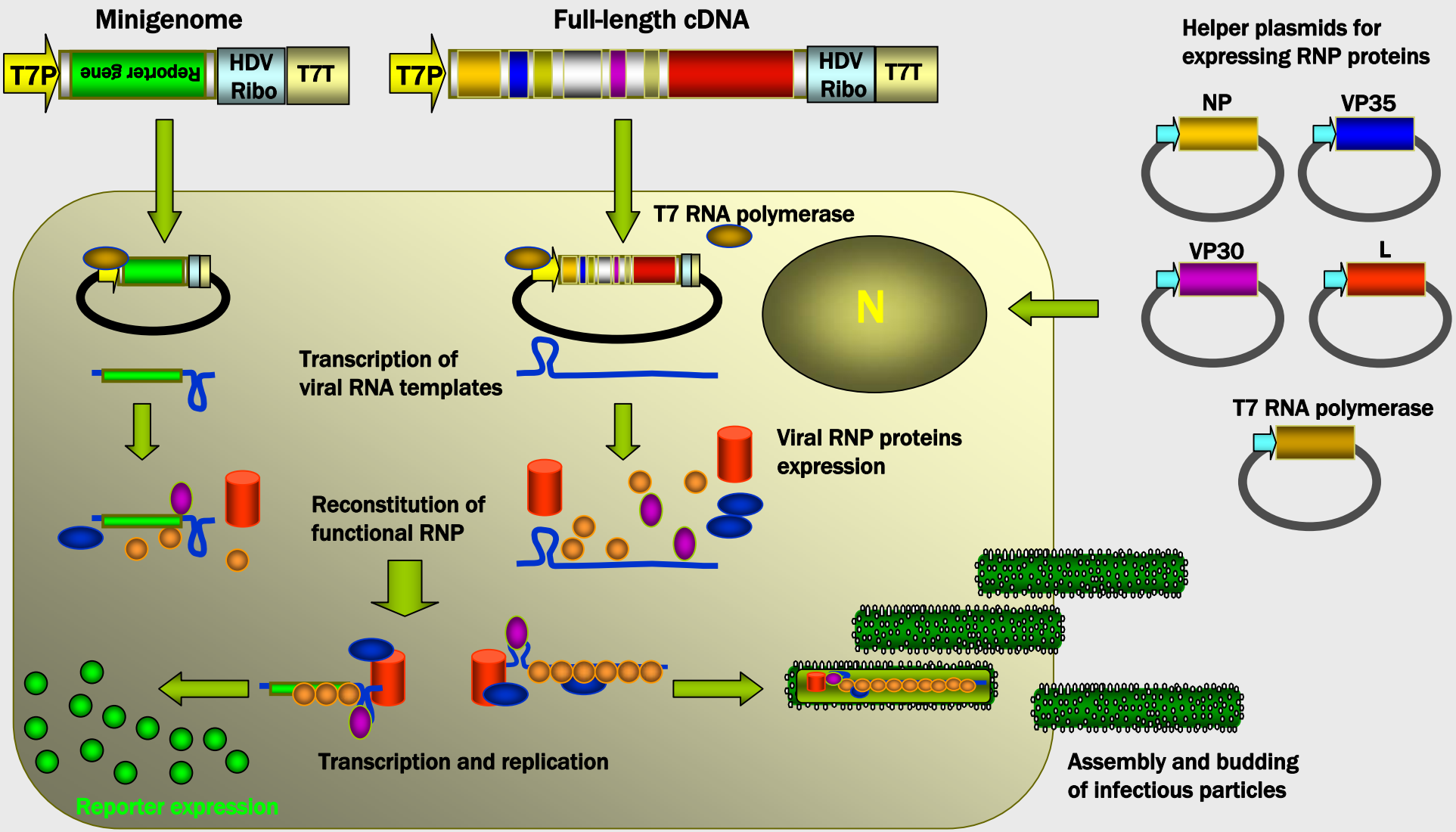


VLPs (VP40 & GP)

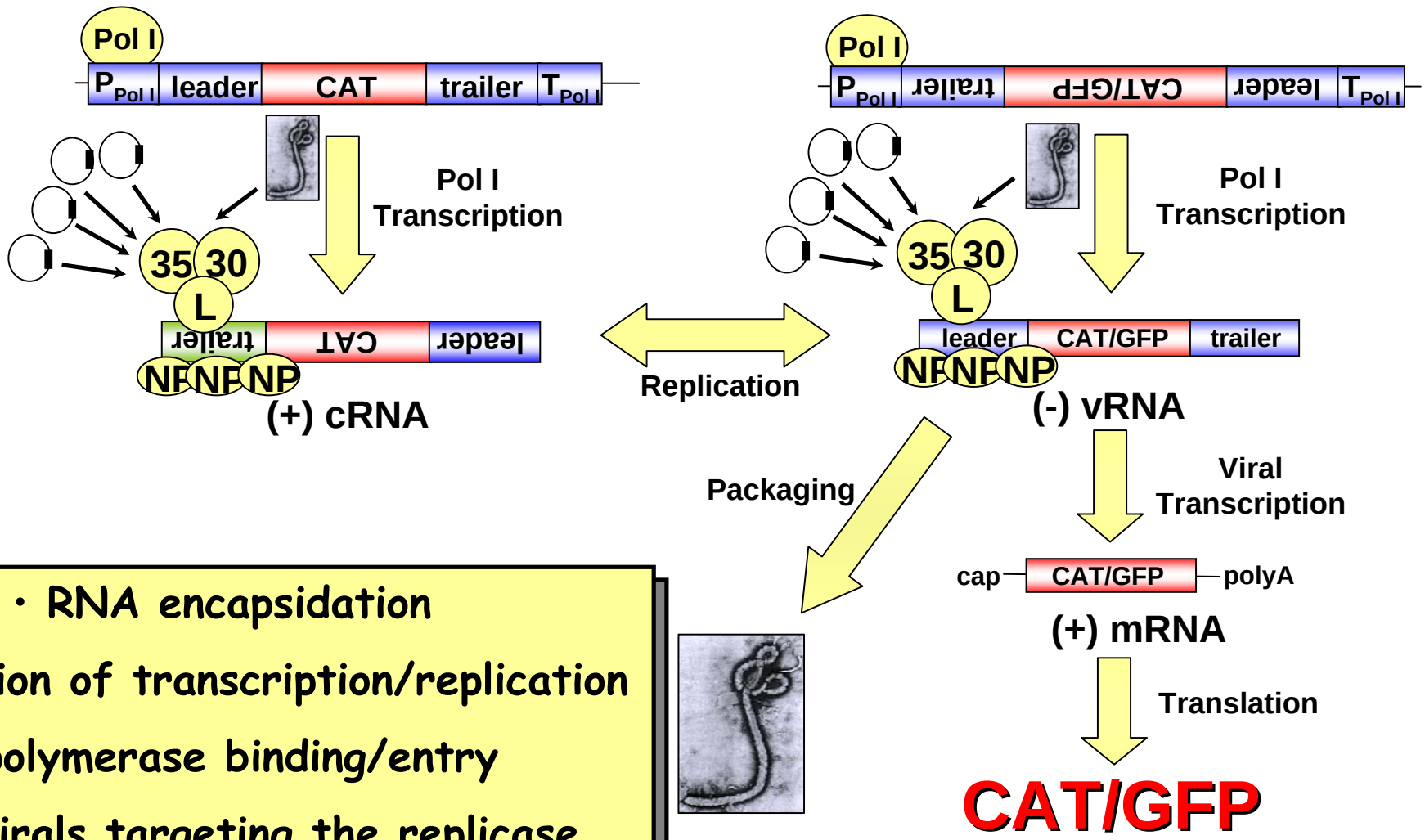
Transcriptional units



Reverse genetics systems

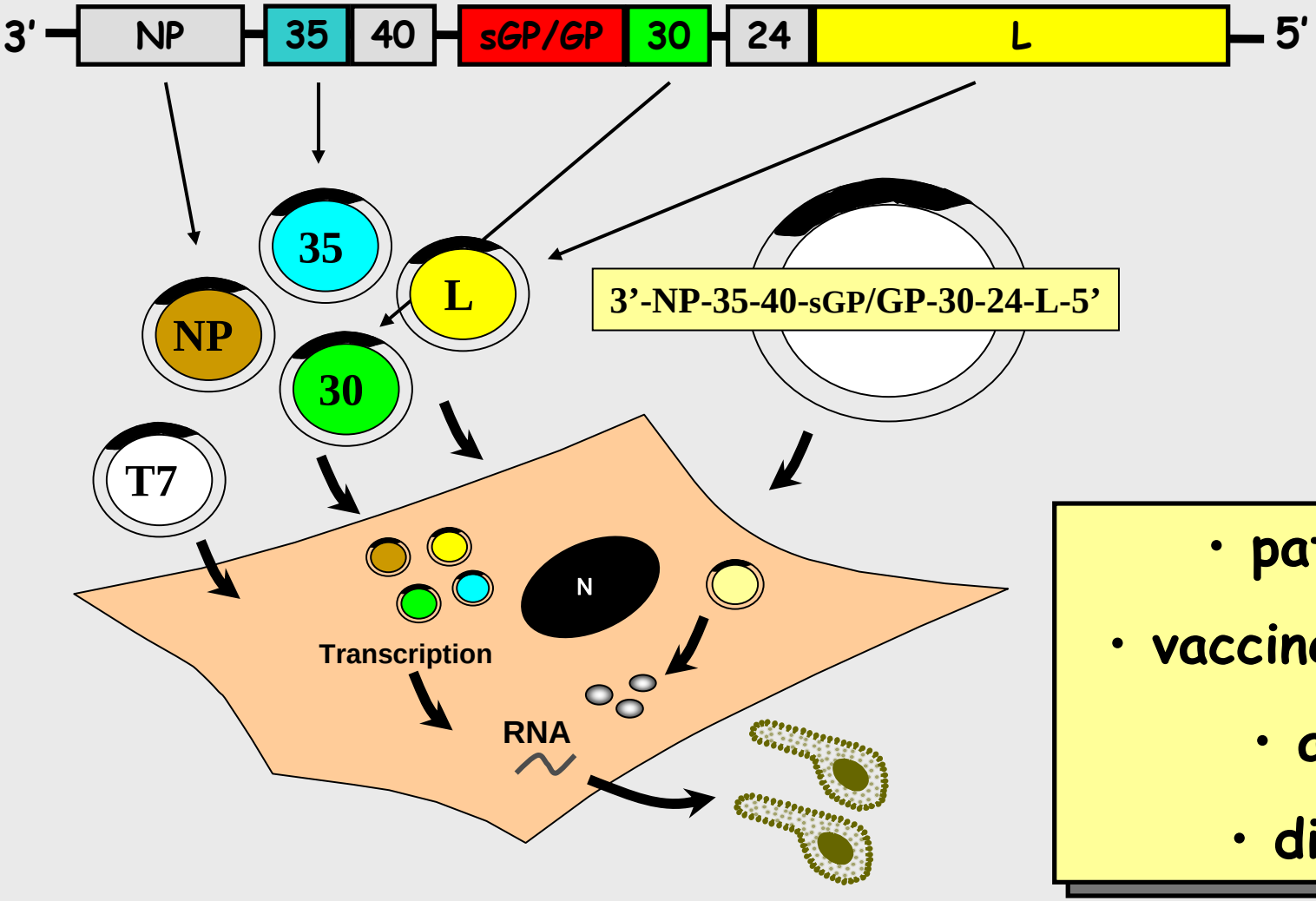


Minigenome system



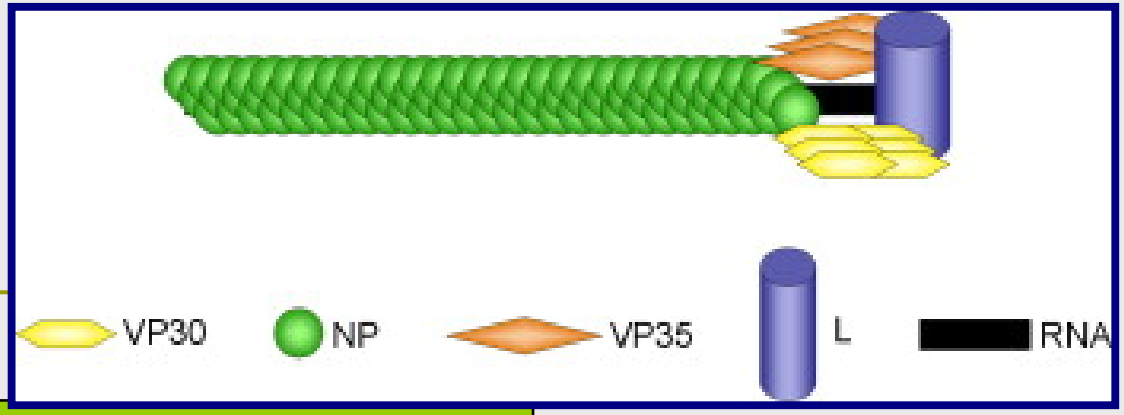
- RNA encapsidation
- regulation of transcription/replication
- polymerase binding/entry
- antivirals targeting the replicase

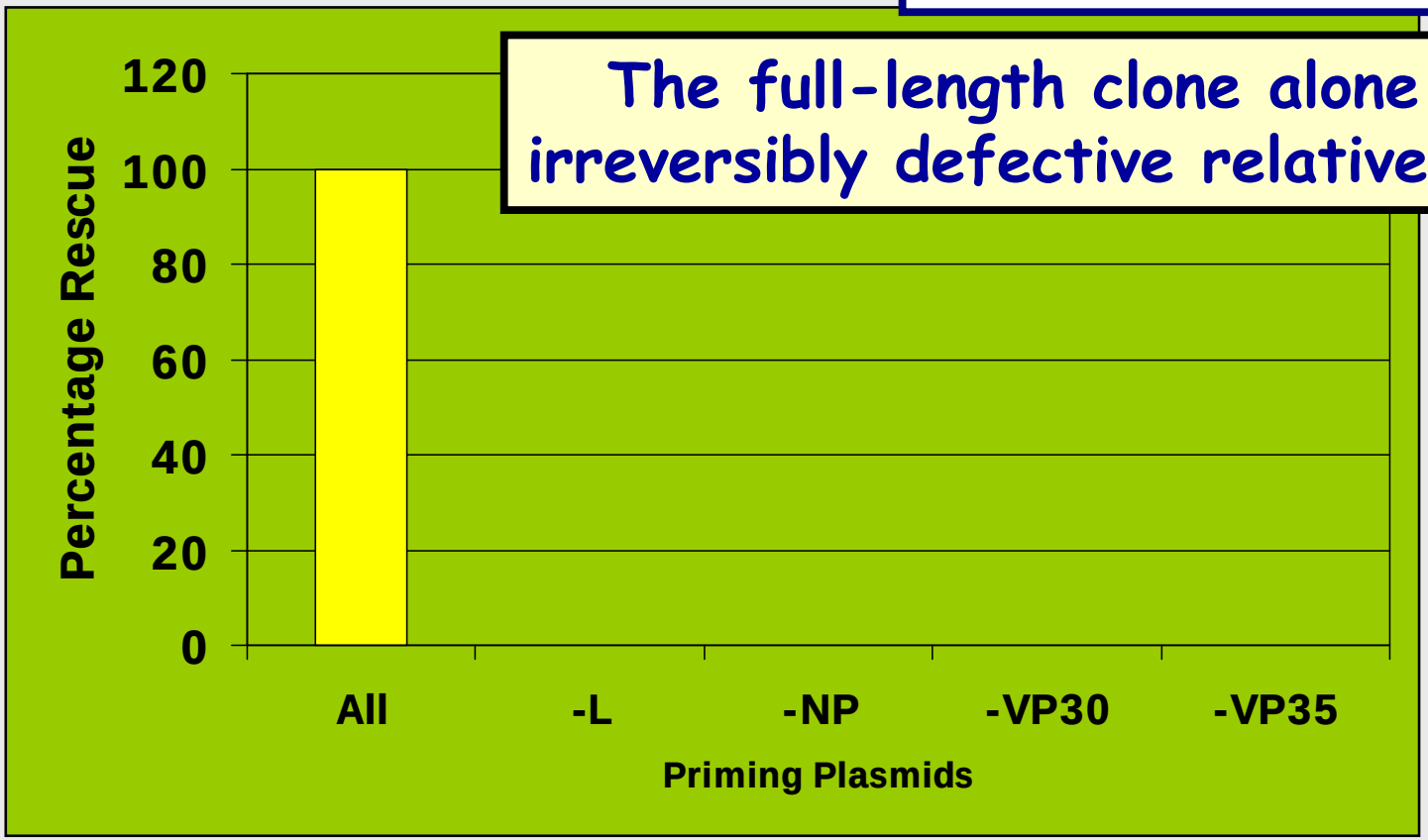
Full-length clone system



- pathogenesis
- vaccine development
- antivirals
- diagnostics

Rescue efficacy





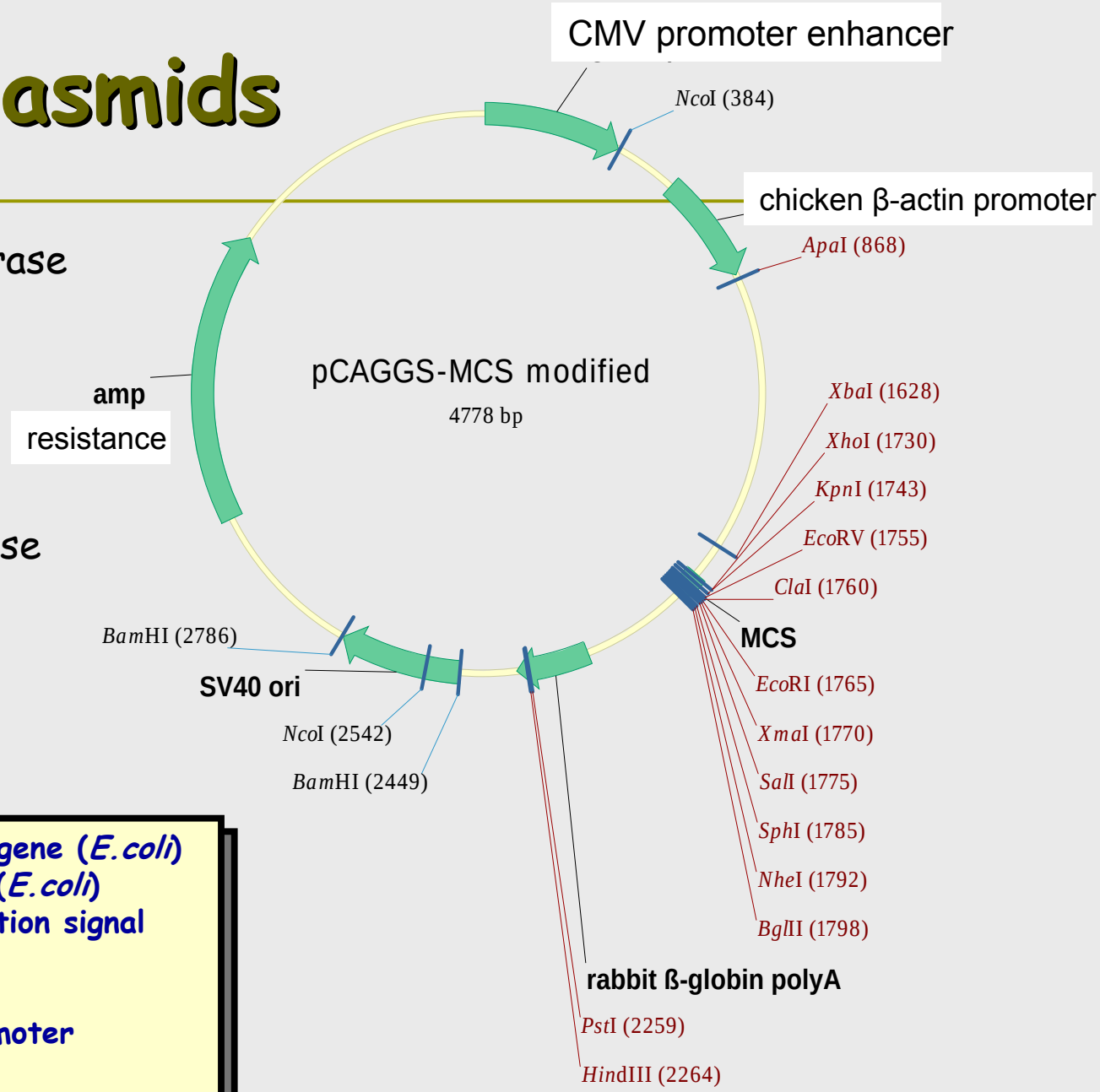
The full-length clone alone is totally and irreversibly defective relative to its infectivity

- No reports of successful rescue from transfection with only the full-length genomic plasmid (>100 times).
- No reports of successful rescue with one ribonucleoprotein component missing (see graph above) (>150 times)

Expression plasmids

L = RNA-dependent RNA polymerase
NP = nulceocapsid protein
VP35 = virion protein 35
VP30 = virion protein 30

T7 = bacteriophage T7
DNA-dependent RNA polymerase

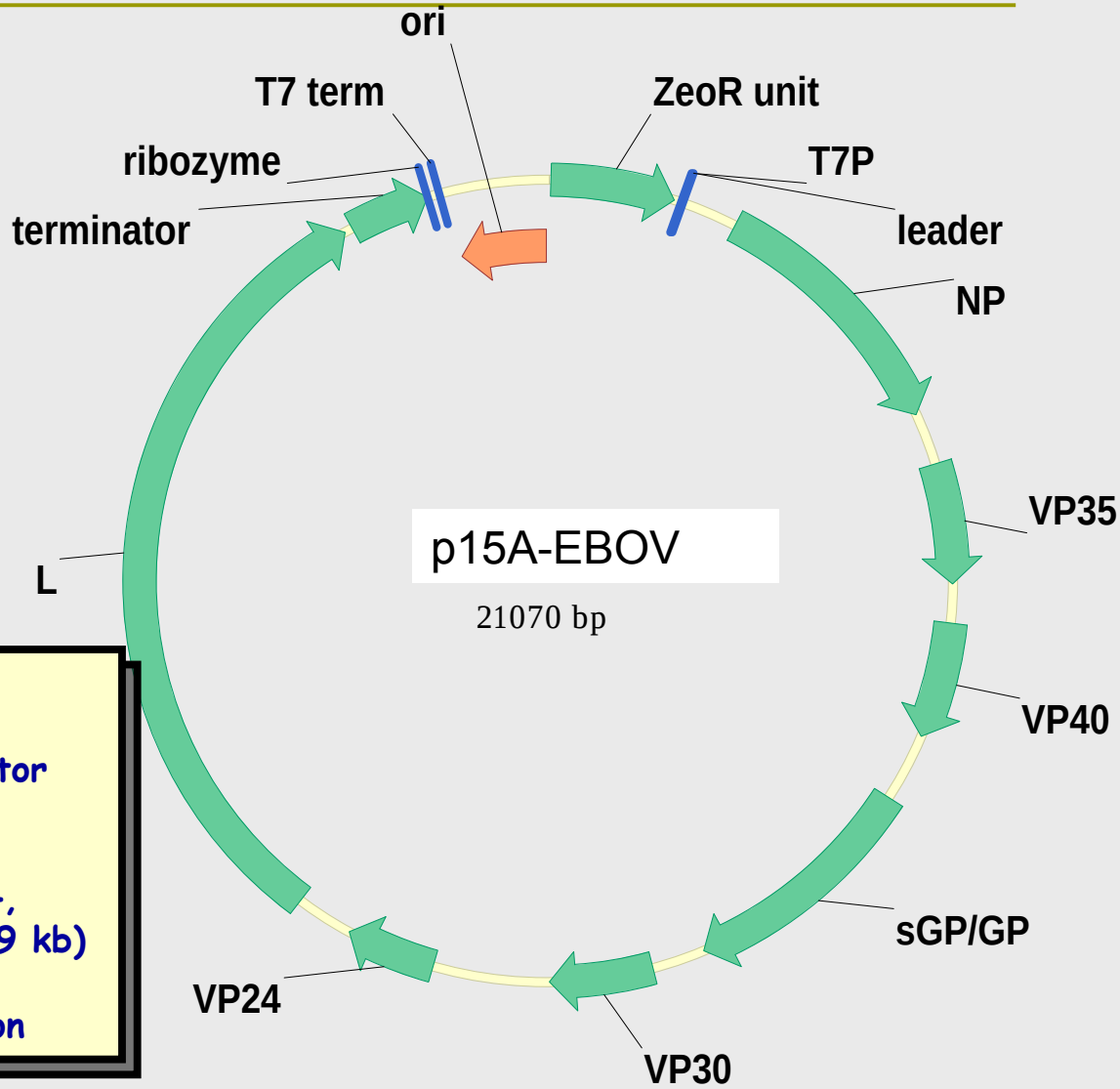


amp resistance: ampicillin resistance gene (*E.coli*)
SV40 ori: SV40 origin of replication (*E.coli*)
rabbit β-globulin polyA: poly-adenylation signal
MCS: multiple cloning site

CMV enhancer & chicken β-actin promoter for expression in mammalian cells

Full-length genome plasmid

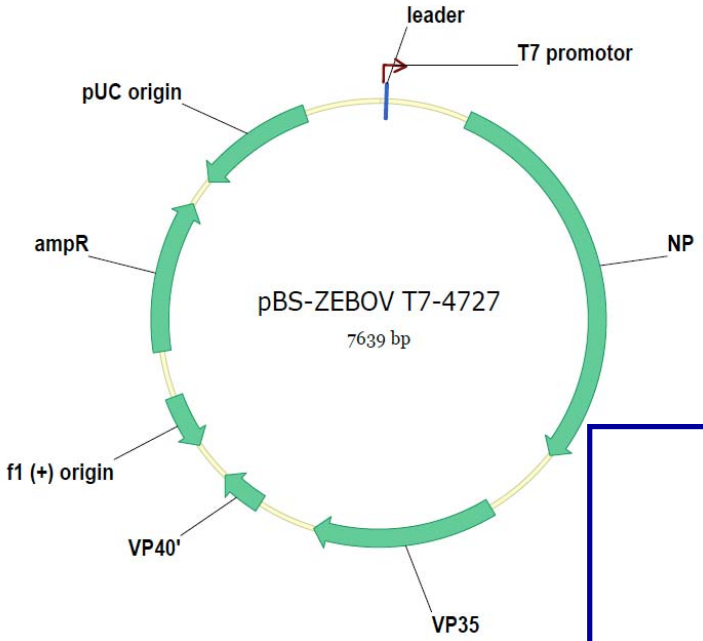
- no expression plasmid
- low copy plasmid (~10 copies)



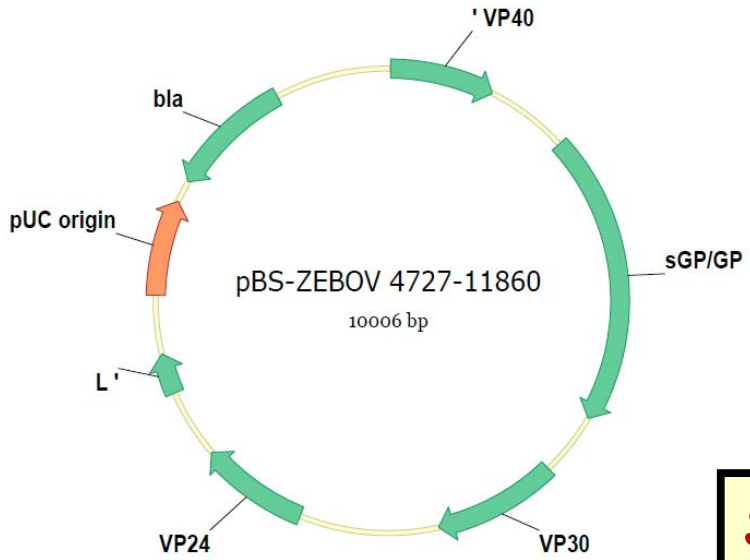
ZeoR unit: zeocin-resistance gene (*E.coli*)
Ori: origin of replication (E. coli)
T7 term: bacteriophageT7 polymerase terminator
T7P: T7 promotor

Leader, NP, VP35, VP40, sGP/GP, VP30, VP24,
L & terminator represent the viral genome (~19 kb)

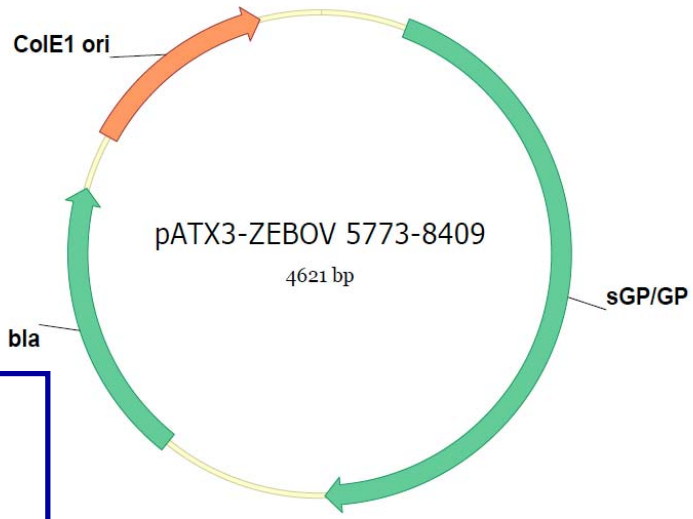
Transcription into (+)-sense RNA; no expression



pUC origin	origin of replication
ampR	ampicillin resistance gene
T7 promotor	T7 promotor for bacteriophage T7 polymerase
leader	EBOV leader sequence
NP	EBOV gene for nucleoprotein
VP35	EBOV VP35 gene
VP40'	3 prime end of EBOV VP40 gene



pUC ori	origin of replication
bla	ampicillin resistance gene
'VP40	3 prime end of ZEBOV 76 VP40
sGP/GP	ZEBOV 76 gene for soluble GP and GP
VP30	ZEBOV 76 VP30 gene
VP24	ZEBOV 76 VP24 gene
L'	5 prime end of ZEBOV 76 polymerase gene



ColE1 ori	origin of replicatopm
bla	ampicillin resistance gene
sGP/GP	ZEBOV 76 gene encoding soluble GP and GP

Subgenomic plasmids

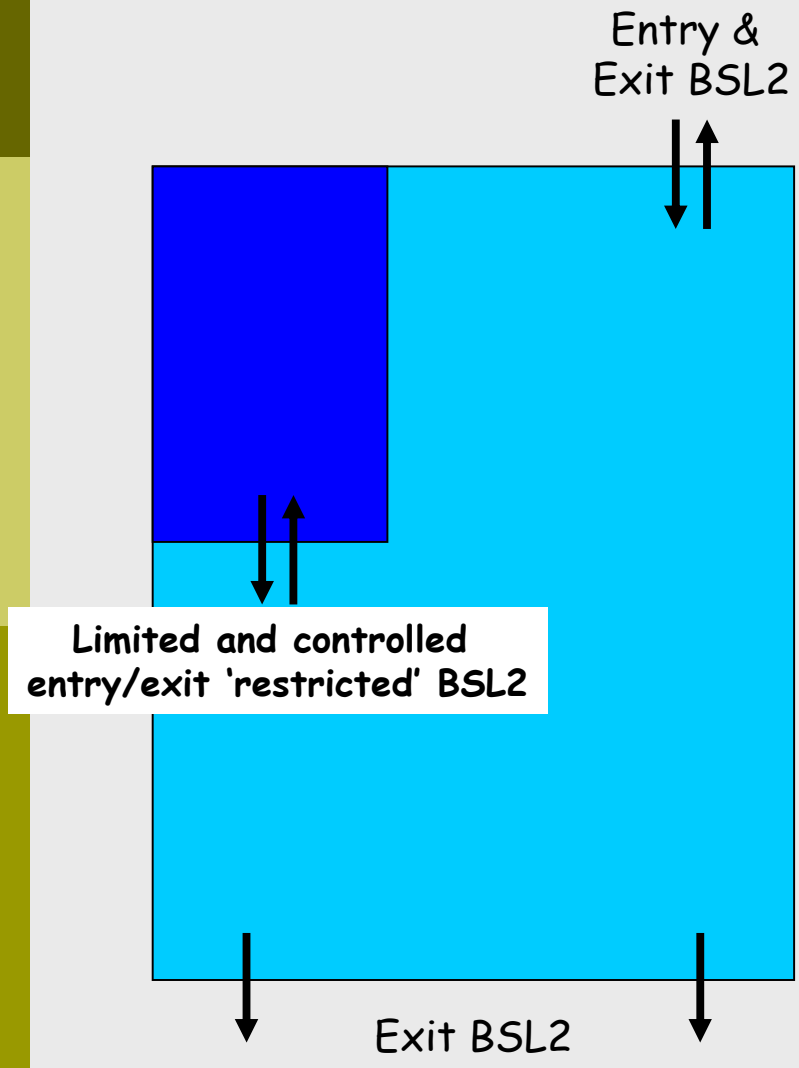
- < 2/3 of the genome
- mutagenesis plasmids

Proposal

"The plasmid carrying the full-length genome of Ebola or any filovirus should be handled in 'restricted' BSL2 with limited and controlled access"

- plasmid DNA is non-infectious
- 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 has been safely used since 2001 (Winnipeg) and longer (lyon) in several laboratories under BSL2 or 'restricted' BSL2 conditions
- plasmid is not a select agent

Proposed physical requirements



- 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)

Proposed 'entry/exit' procedures

- Personnel needs institutional security clearance
- Access is 'keypad-controlled'
- Prior to entry into 'restricted' BSL2 remove BSL2 lab coat
- Entry procedure: put on gloves (2 pairs), disposable lab coat and shoe covers
- Exit procedure: remove outer pair of gloves, then lab coat, then shoe covers, then inner pair of gloves, wash hands
- Plasmid will be transported in sealed container to BSL4 for transfection/rescue
- Waste will be sealed prior to removal; waste will be immediately sterilized (no storage outside 'restricted' BSL2)

Proposed use of 'restricted' BSL2

- Room dedicated for handling of full-length genomic plasmids
- Work performed:
  - (1) re-ligation of mutated subgenomic fragments into full-length clone
  - (2) transformation of appropriate *E.coli* in the presence of zeocin
  - (3) selection and characterization of positive clones
  - (4) amplification of full-length clones in *E.coli*
  - (5) storage of *E.coli* carrying full-length genomic plasmids (glycerol stocks)
  - (6) storage of full-length plasmid DNA
- All procedures will be documented in a room-specific laboratory journal
- All stored *E.coli* stocks and plasmid DNA preparations will be recorded
- Staff has to be security cleared and trained on all procedures

Note **- all transfections for rescue experiments will be performed in BSL4**

CAUTION: Increased exposure risk for lab staff !!!

Thank You for Your Attention!