

Toward a Chimeric Live Attenuated Vaccine for Human Immunodeficiency Virus

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A New Live Attenuated Chimeric Vaccine Against HIV

Goal: To create a simple self-replicating entity that presents the major antigens of HIV *in vivo* until an appropriate immune response suppresses its growth, leaving the recipient immune to HIV.

- Combine the genome and replication machinery of a single strand RNA virus (Alphavirus) with the structural proteins of SHIV and SIV

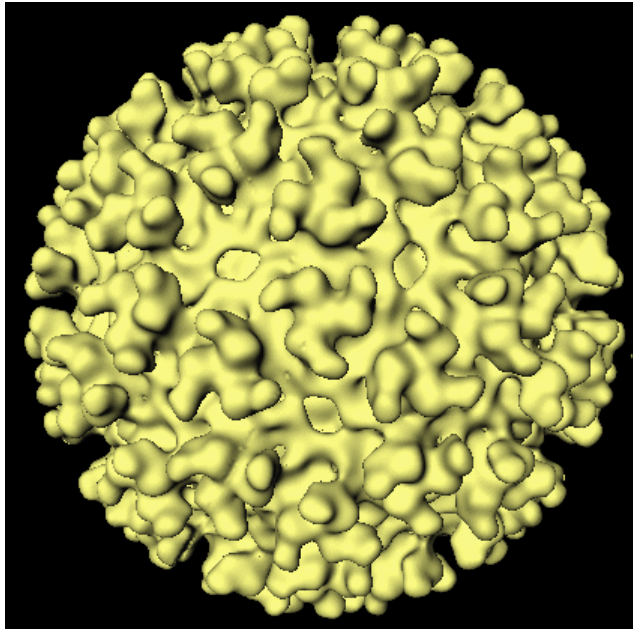
Obstacles for a Live-attenuated vaccine

- Recombination between vaccine virus and infecting virus
- Integration of retroviral genomes into host genome
- Persistence of vaccine virus
- Vaccine virus induced pathogenesis

Advantages of Alphavirus Chimeric System

- Exclusively cytoplasmic RNA virus with no DNA intermediate
- No persistent infection- genomic replication induces apoptosis
- Ease of genetic manipulation
- Highly sensitive to interferon, limited replicative capacity
- Capability of increasing interferon sensitivity (decreasing virulence) through targeted mutation
- Potential launch from a DNA vaccine

Venezuelan Equine Encephalitis Virus

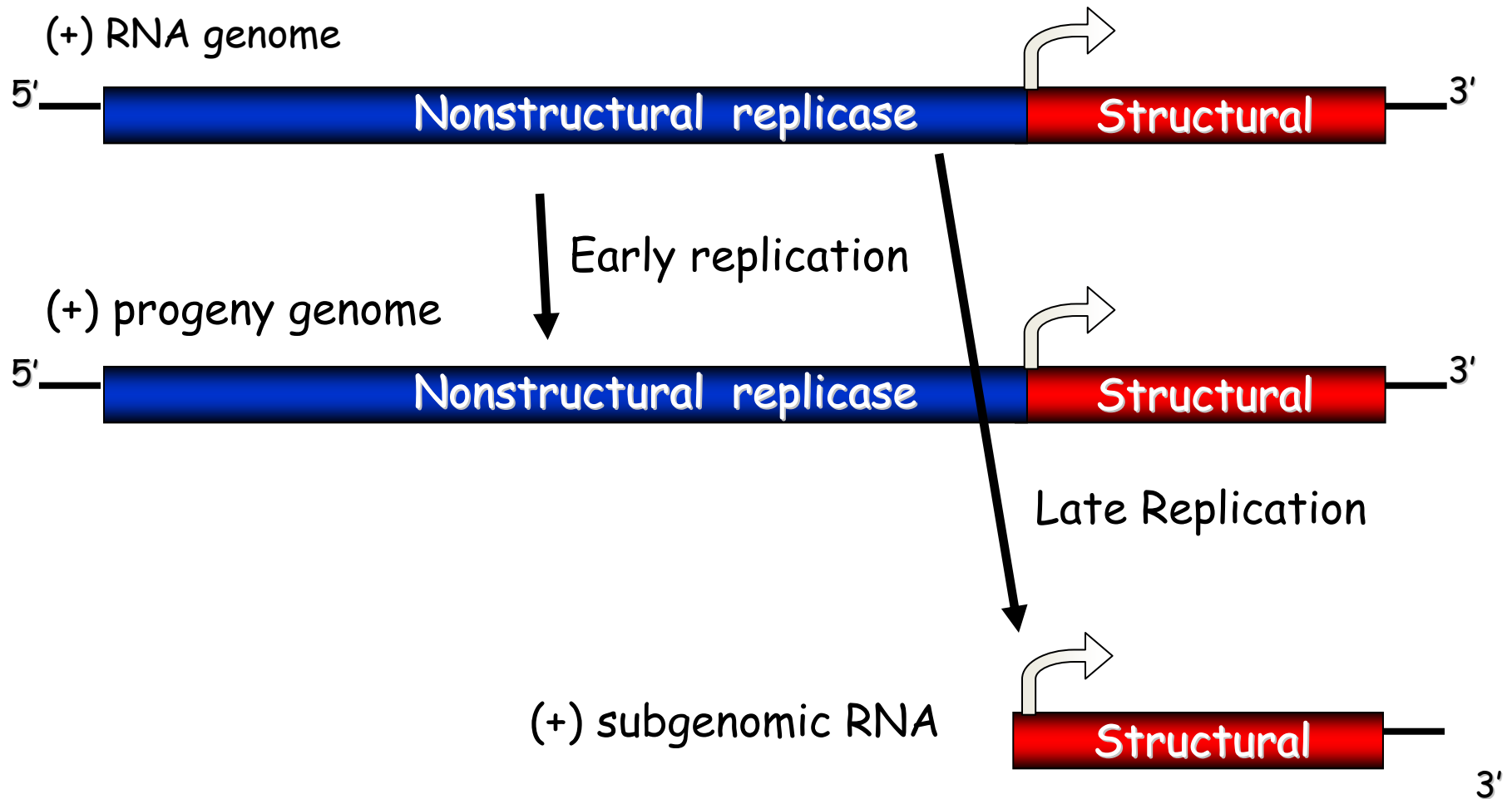


Paredes, et al.

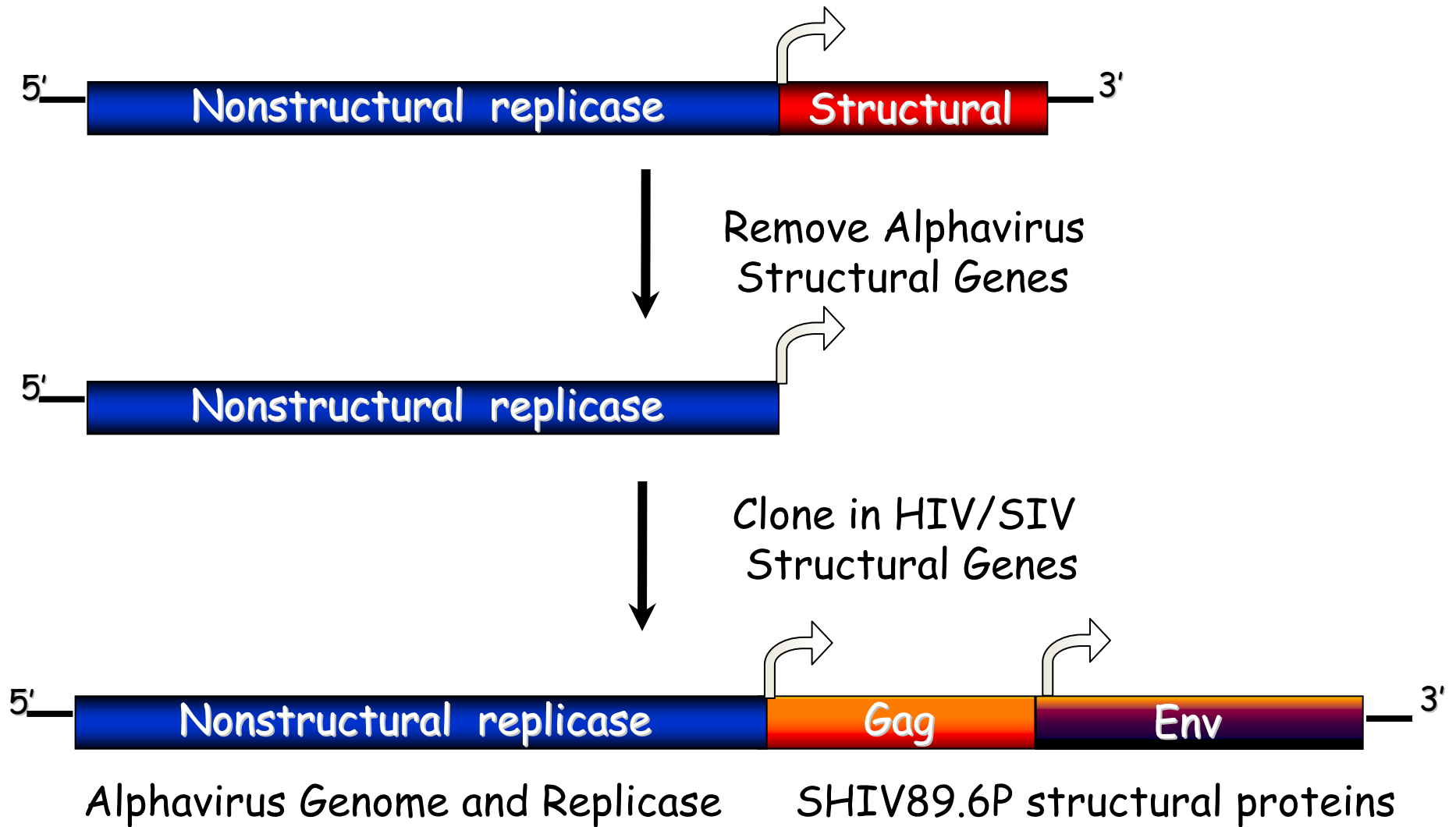
- Family: *Togaviridae*
- Genus: *Alphavirus*
- + ssRNA genome
- Enveloped
- Non-structural "replicase"
- Structural: C, EI, E2



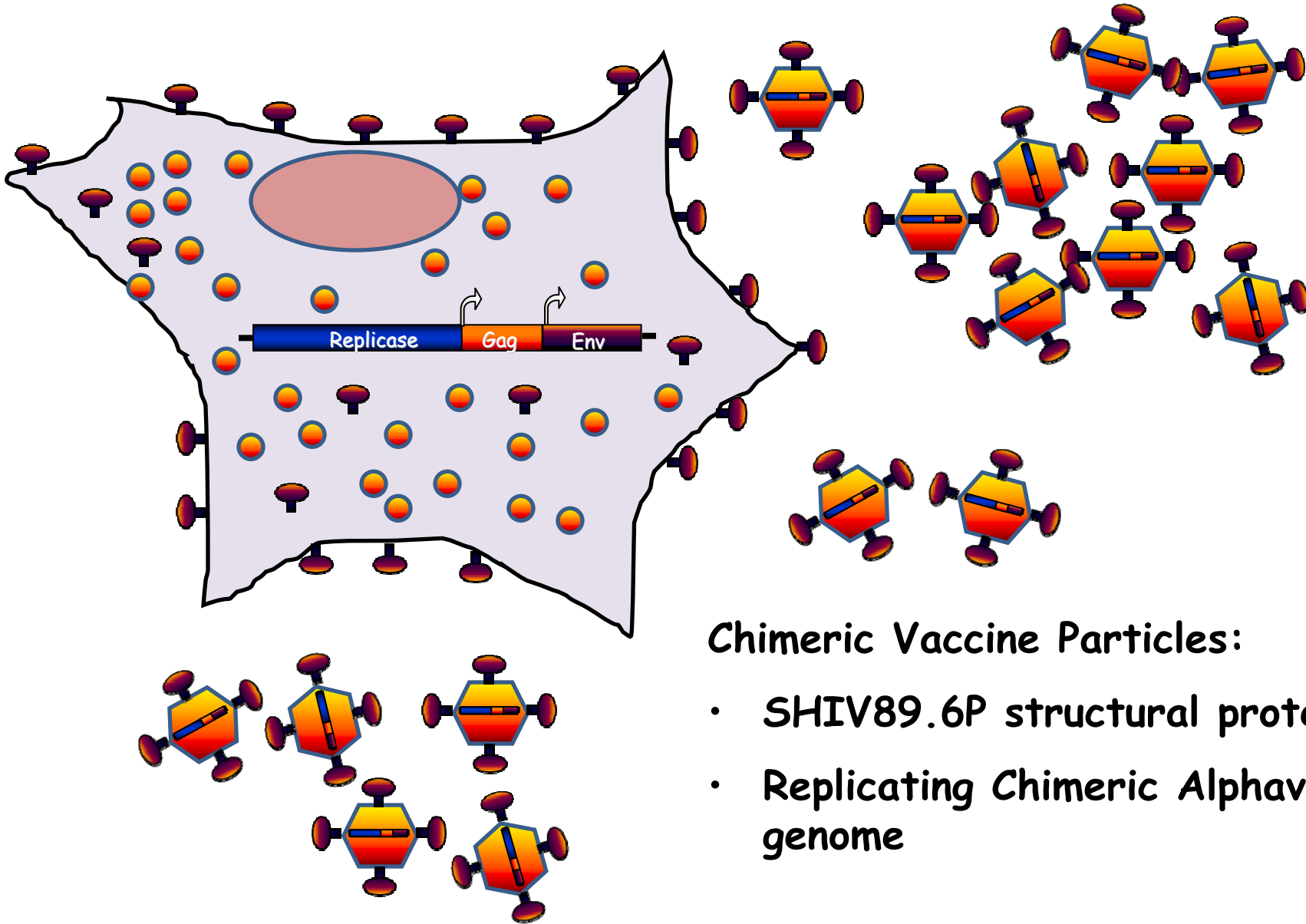
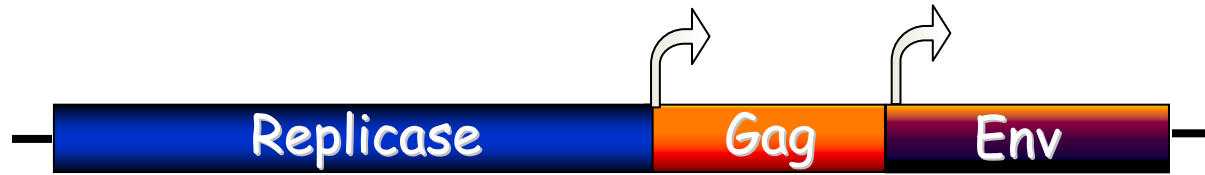
Alphavirus RNA Replication



Alphavirus/SHIV Chimeric virus concept



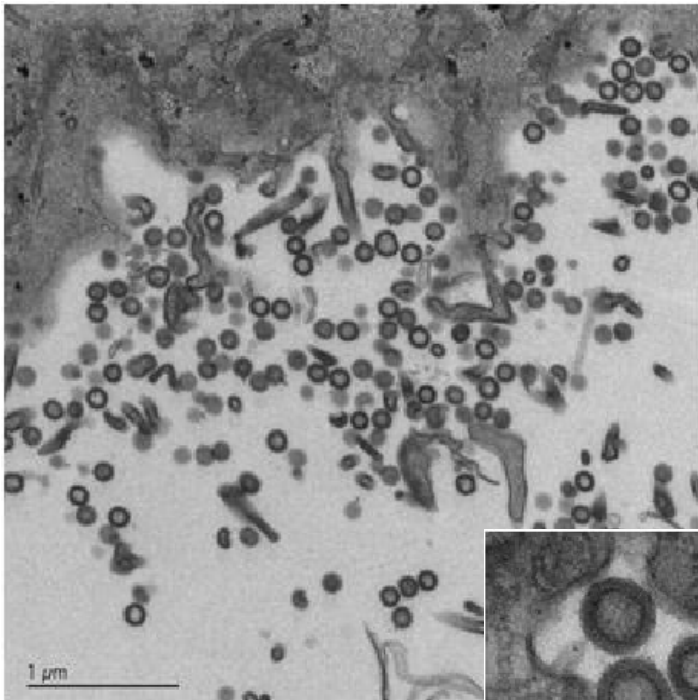
Chimeric Virus Genome



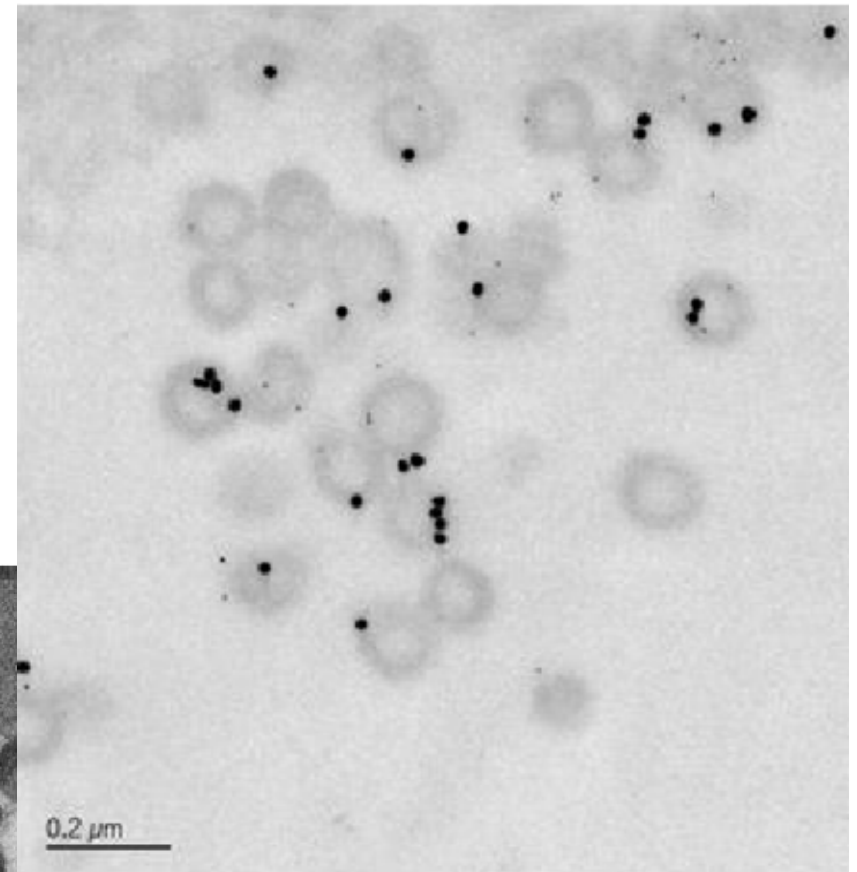
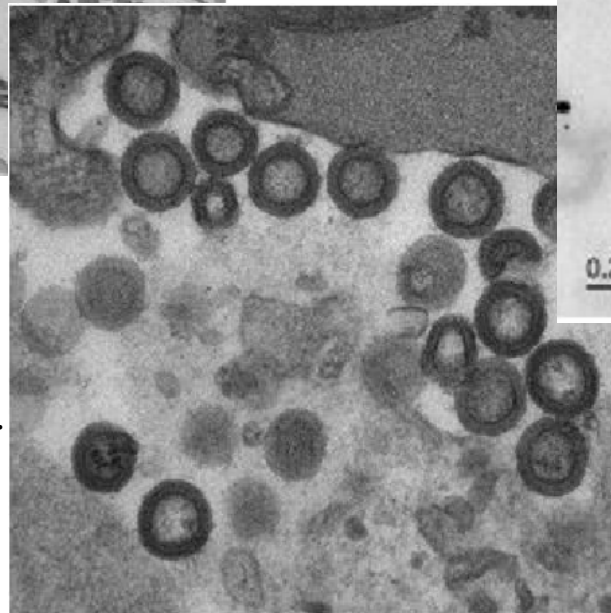
Chimeric Vaccine Particles:

- SHIV89.6P structural proteins
- Replicating Chimeric Alphavirus genome

Particle formation from chimeric genomes

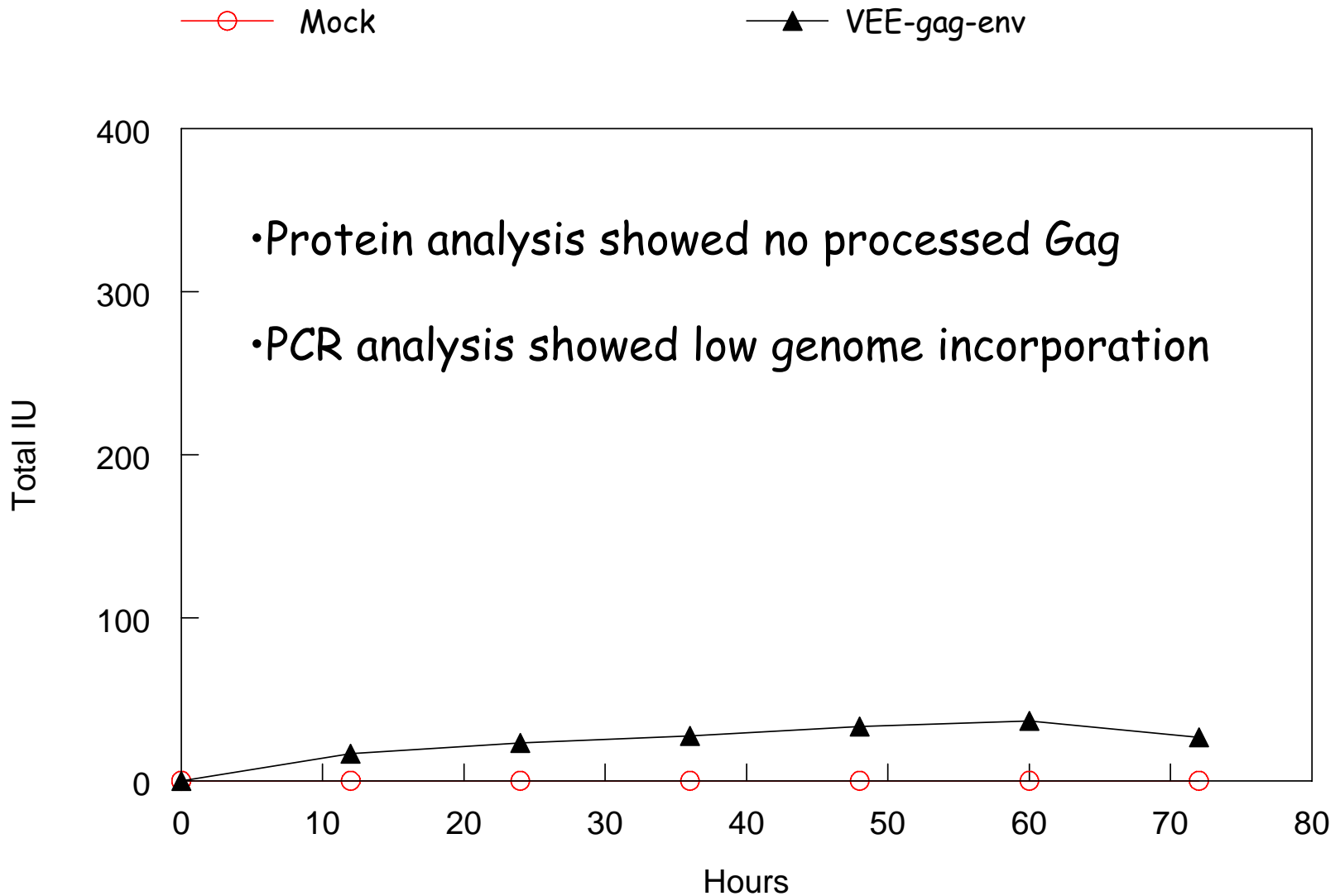


Chimeric particles produced in Vero cells at 14 hours post electroporation

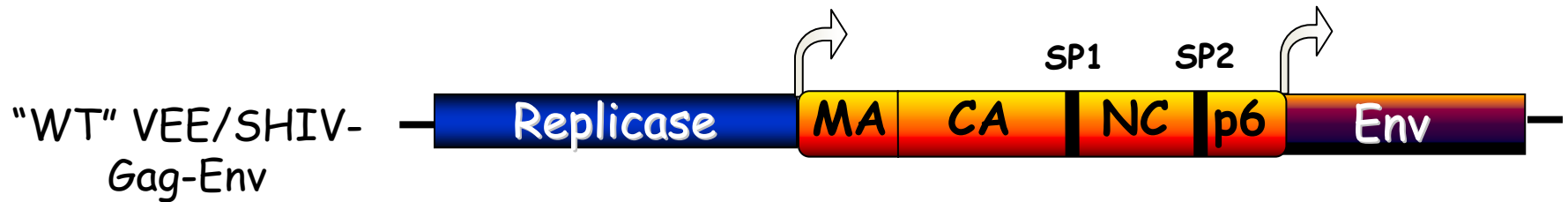


Immunogold labelled chimeric particles from 3T3-CD4-CCR5 cells at 14 hours post infection (Gag-5nm gold, Env-20 nm gold)

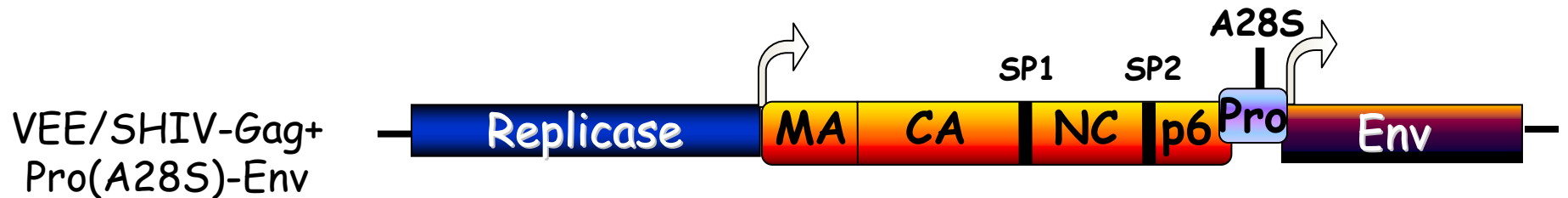
Growth Curve of Pass 1 Chimeric Particles on 3T3-CD4-CCR5



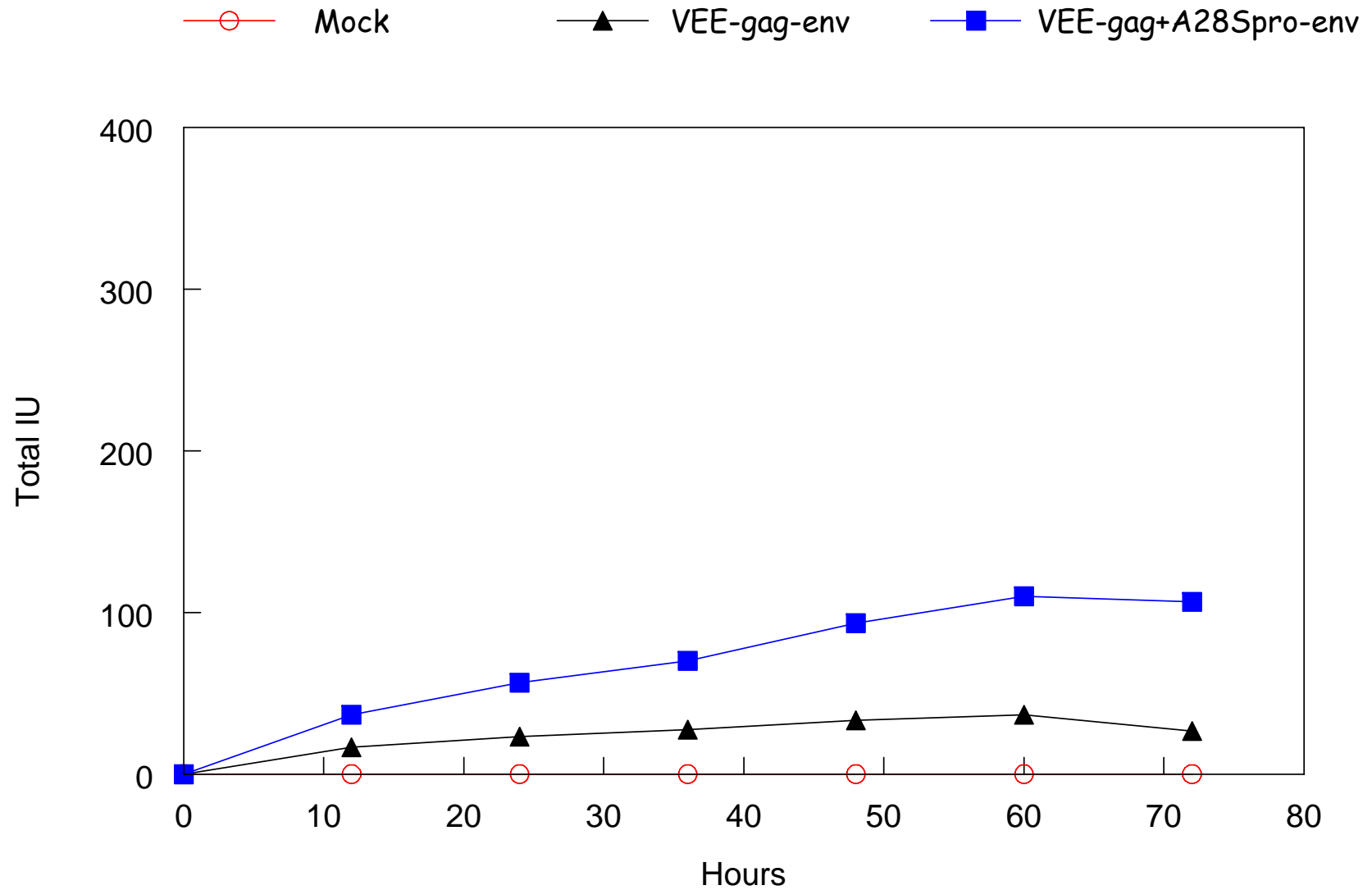
Improving growth of VEE/SHIV chimeric particles



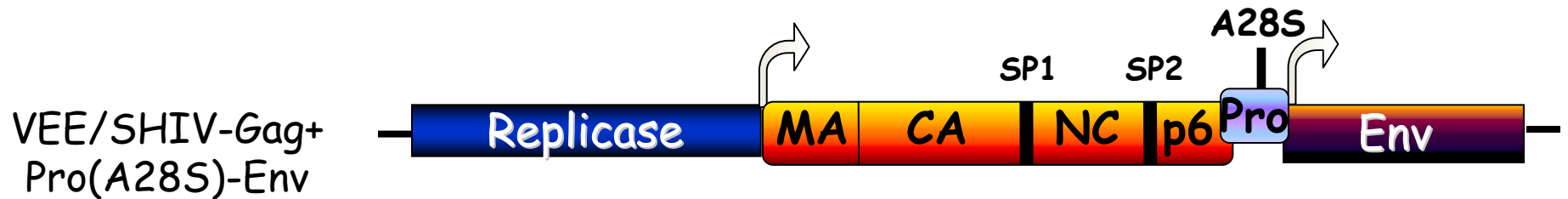
Add low activity protease to process Gag protein properly



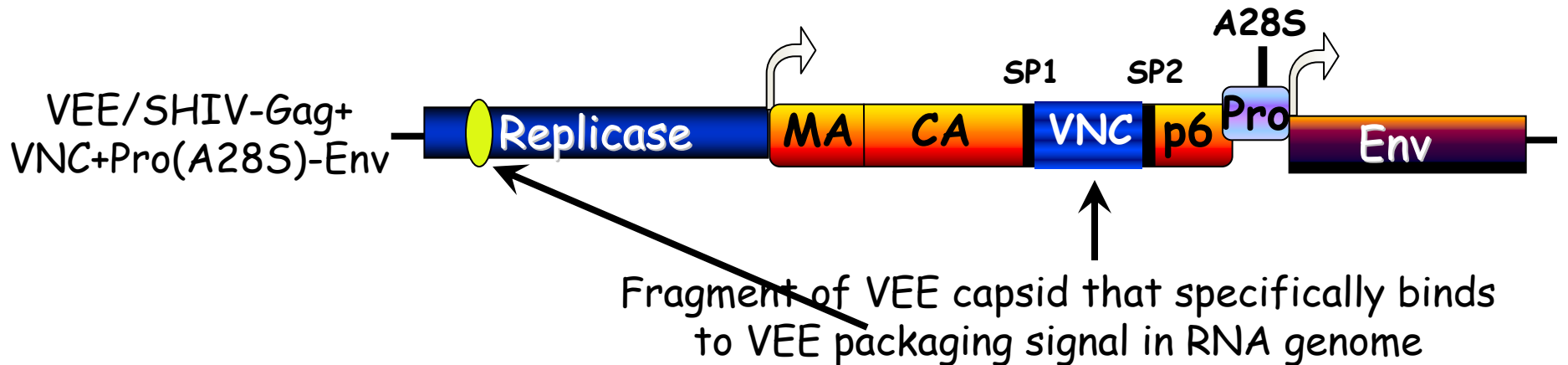
Growth Curve of Pass 1 Protease containing Chimeric Particles on 3T3-CD4-CCR5



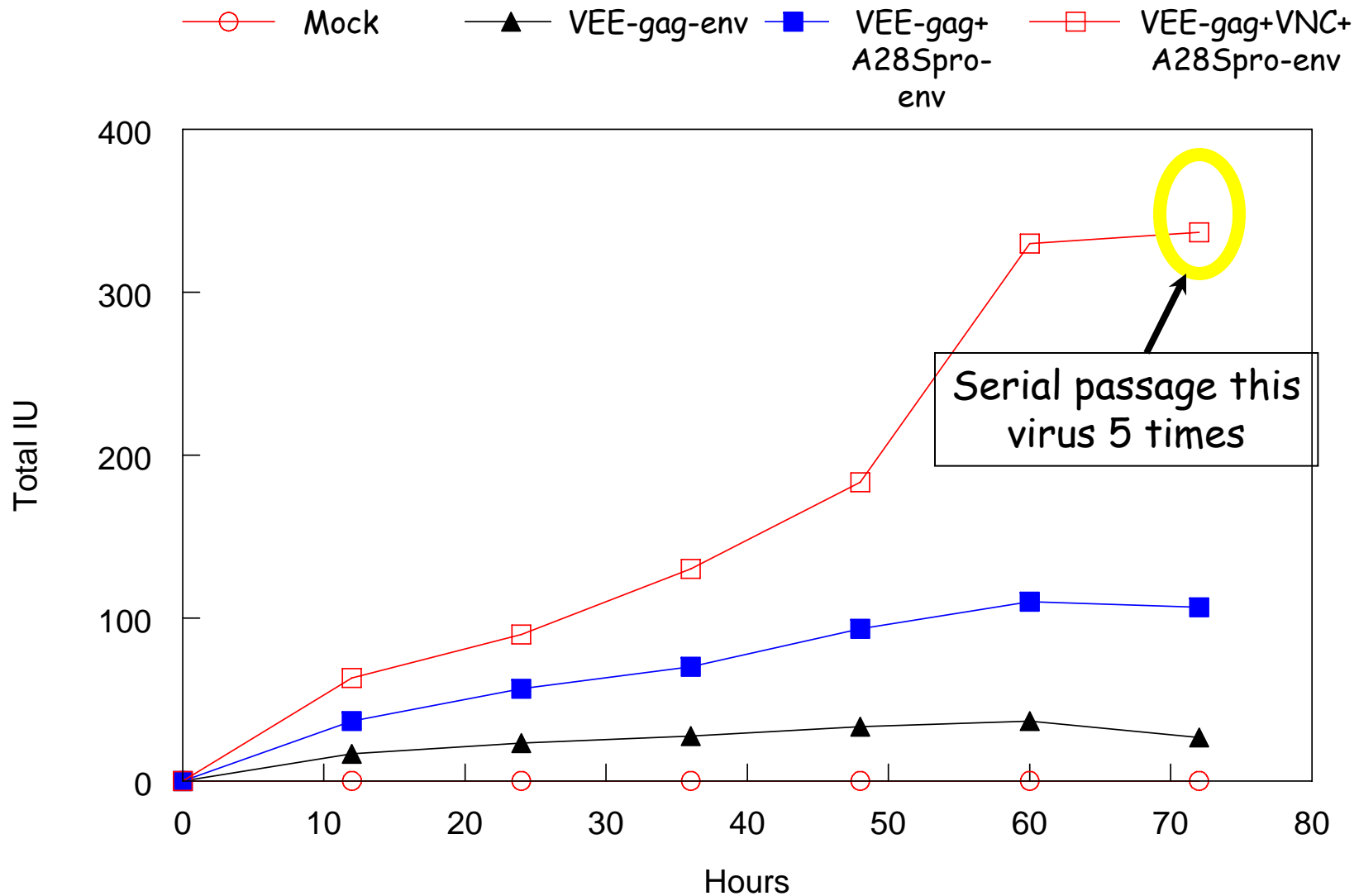
Improving growth of VEE/SHIV chimeric particles



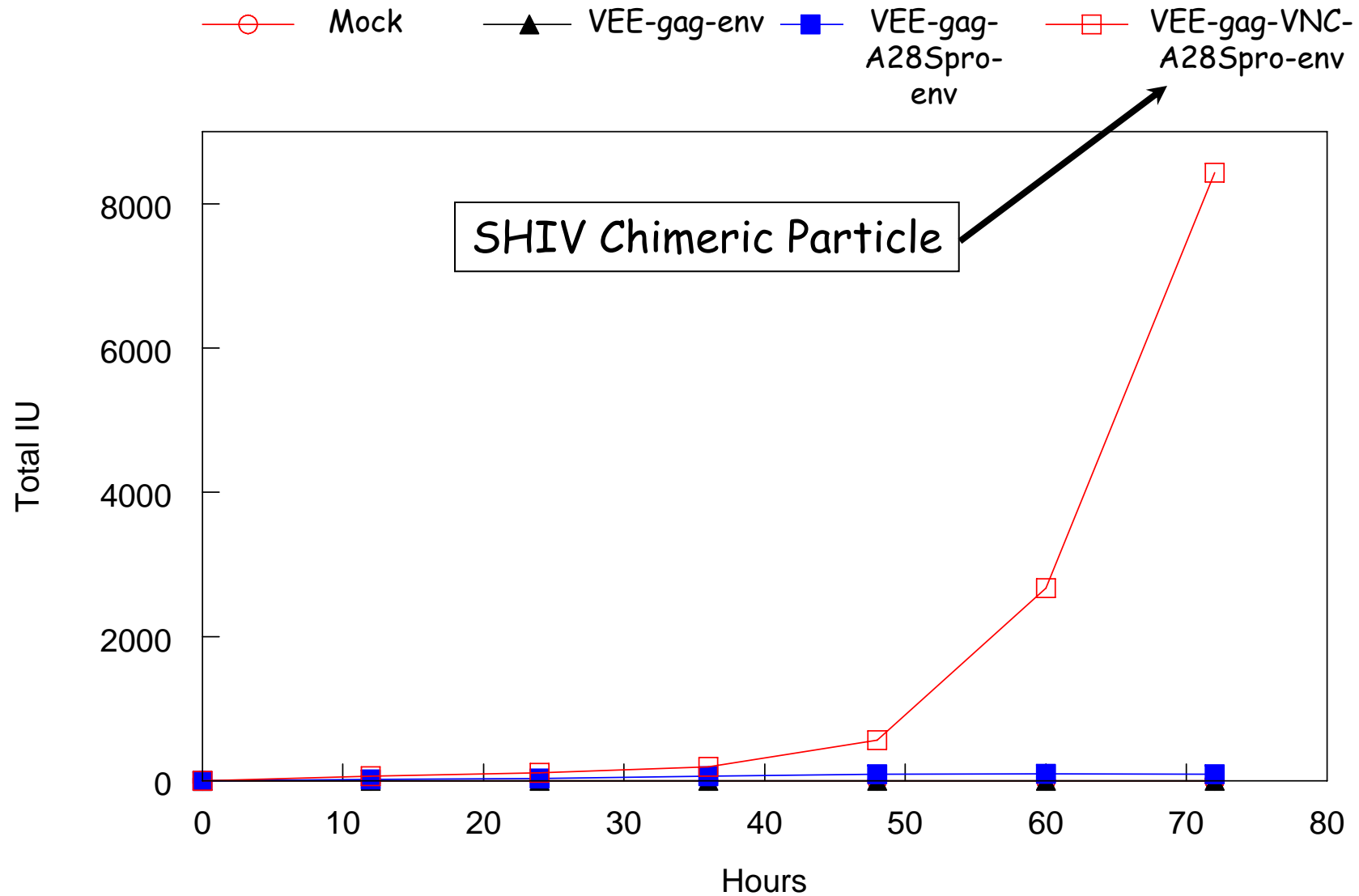
Replace Gag nucleocapsid domain with the specific RNA binding domain of VEE capsid



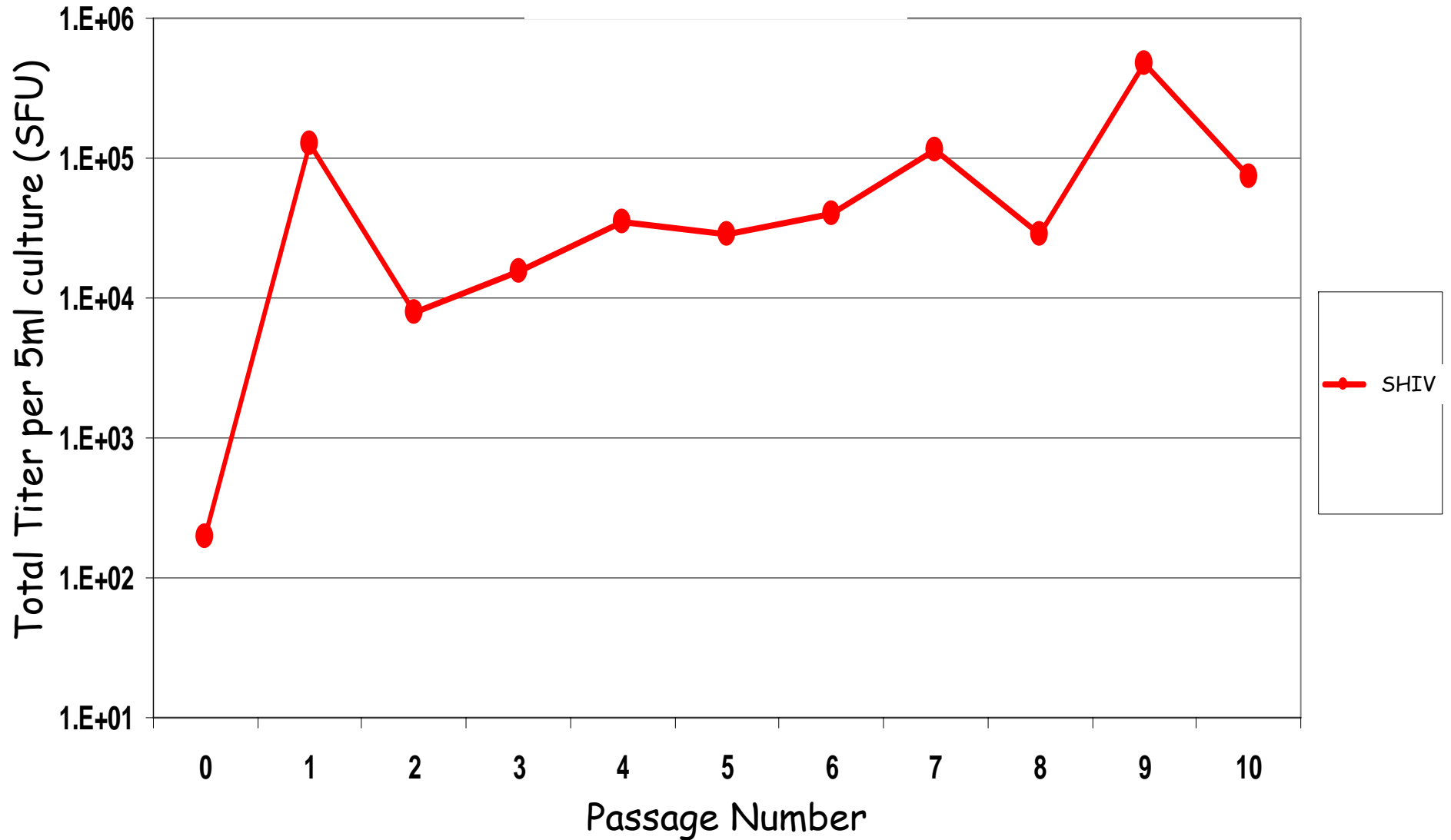
Growth Curve of Pass 1 Improved Chimeric Particles on 3T3-CD4-CCR5



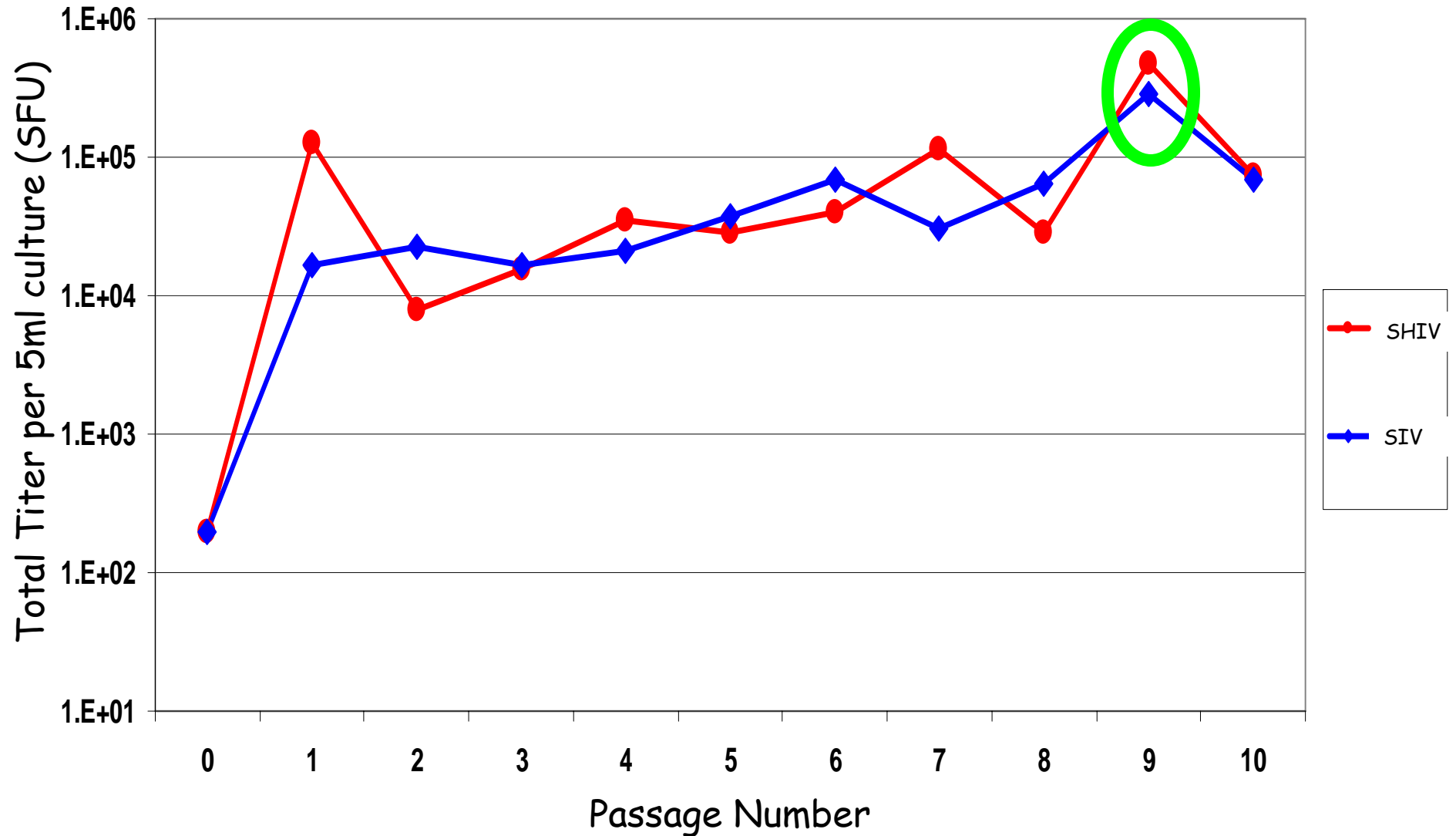
Growth Curve of Pass 5 Improved Chimeric Particles on 3T3-CD4-CCR5



Growth of SHIV Chimeric Particles during serial passage on Ghost cells



Growth of SHIV and SIV Chimeric Particles during serial passage on Ghost cells



Future Directions

- Sequencing of viral genomes after serial passages
- Identification of mutations responsible for improved growth
- Move advantageous mutations into parental infectious clones
- In vivo studies in CD4+/CCR5+ transgenic mice
- Continued development of DNA-launched chimeric particle format
- Production of purified chimeric particles for non-human primate trials in rhesus macaques

Acknowledgments

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