# VEE Replicon Vaccines in Development

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- HIV Clade C (prophylactic)
- HIV Clade B (therapeutic, UNC)
- Marburg
- Botulinum toxins
- Equine encephalitis viruses
- Influenza

Wyeth

- HSV
- PIV
- RSV
- HPV

Progenics / Cytogen

- PSMA

Duke University

- CEA

NMRI

- Malaria

# Alphaviruses

- *Togaviridae* family
- Alphavirus genus (n=25)
- Enveloped particles; ~60 nm diameter
- Icosahedral nucleocapsid
- Single-stranded RNA genome; ~11.5 kb
  - Positive sense (infectious)
- 3 structural proteins
  - Capsid protein (C)
  - Envelope glycoproteins (E1 and E2)

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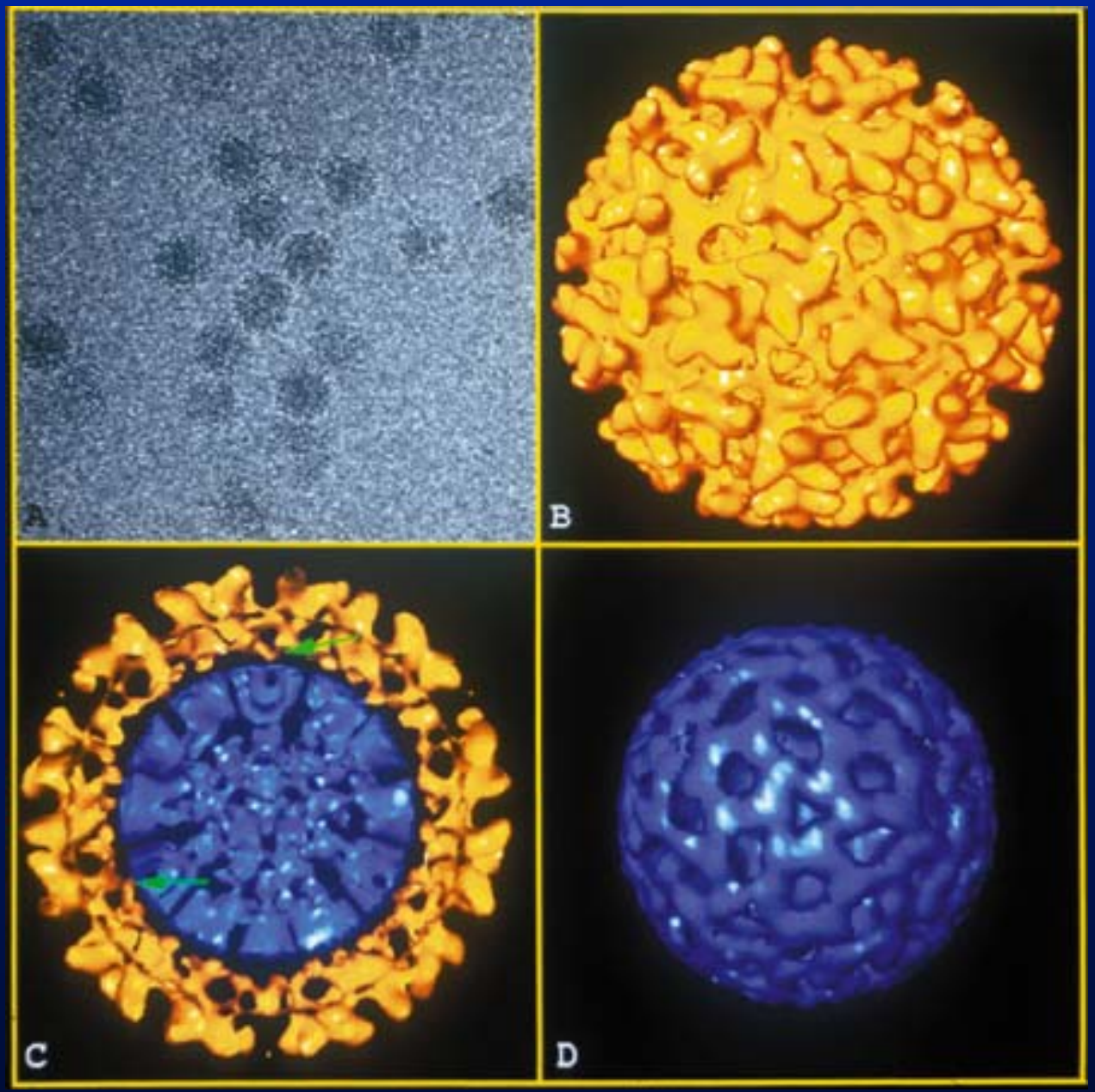
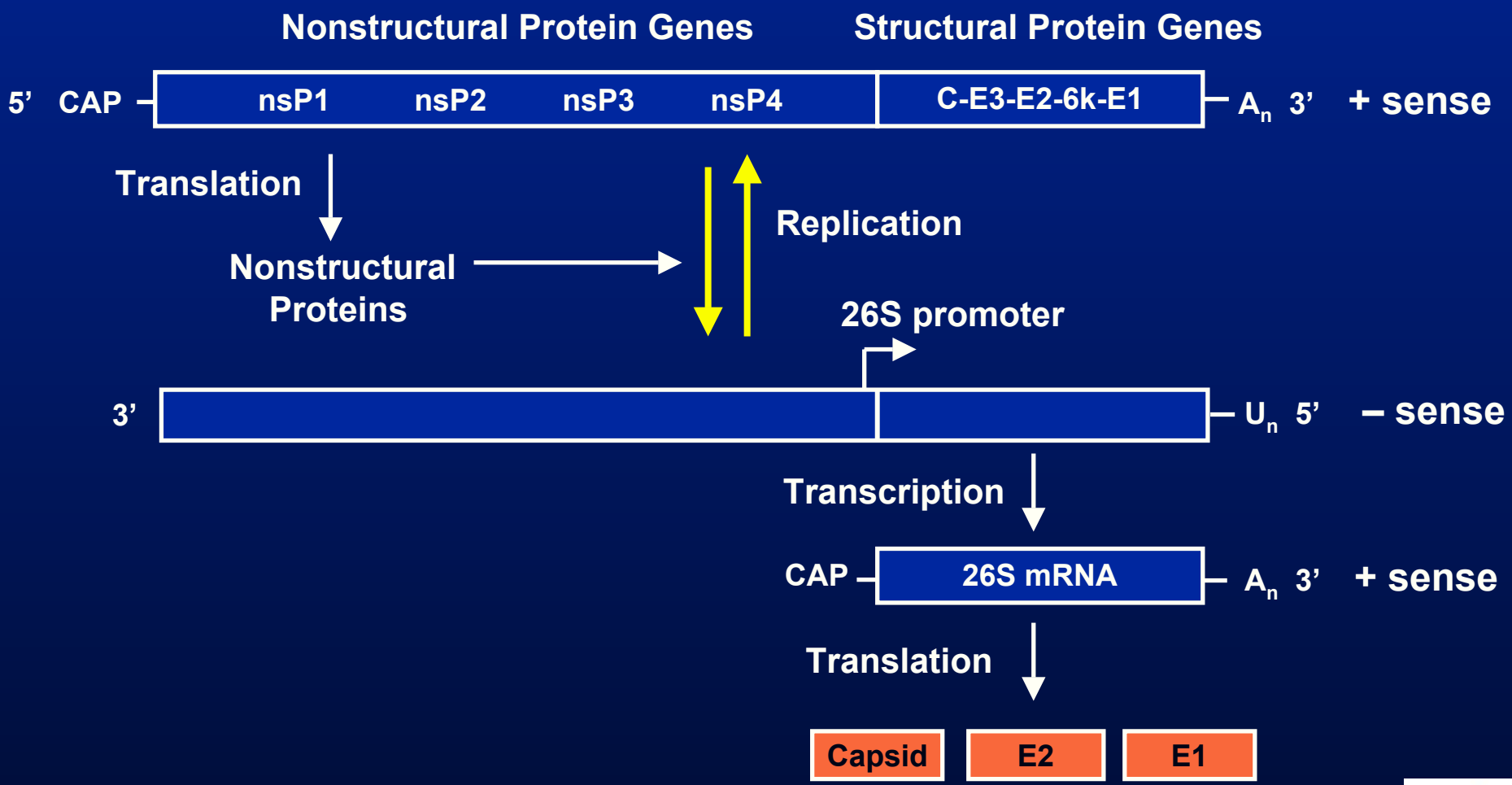
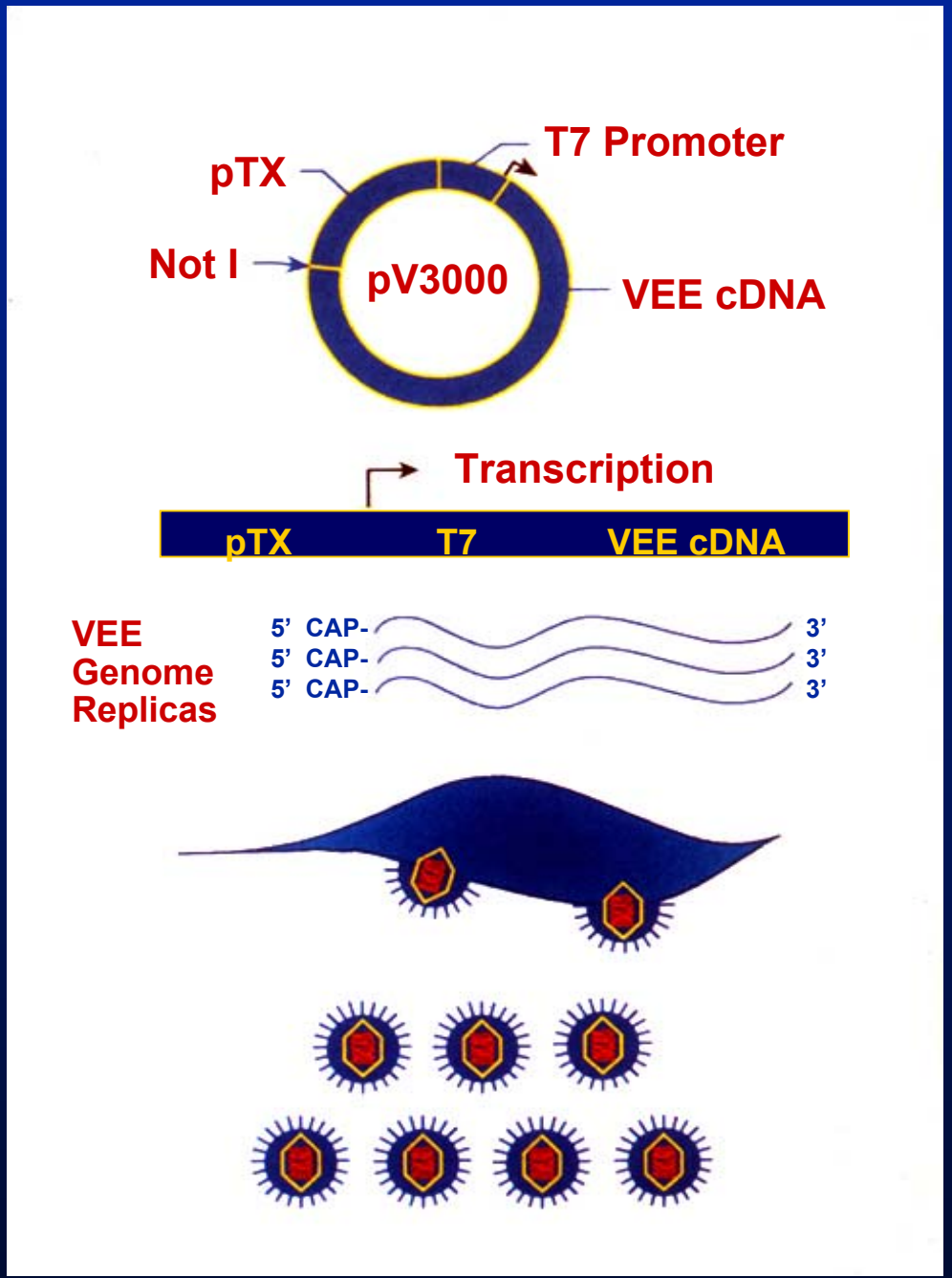

A
B
C
D

# Alphavirus RNA Replication Strategy

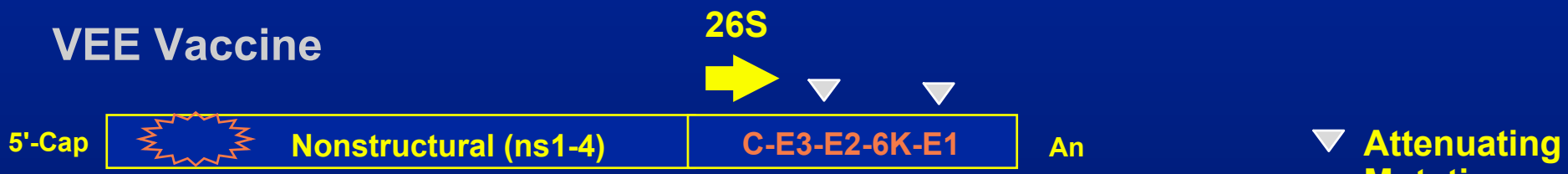


# Basic Genetic System for VEE Virus Mutagenesis

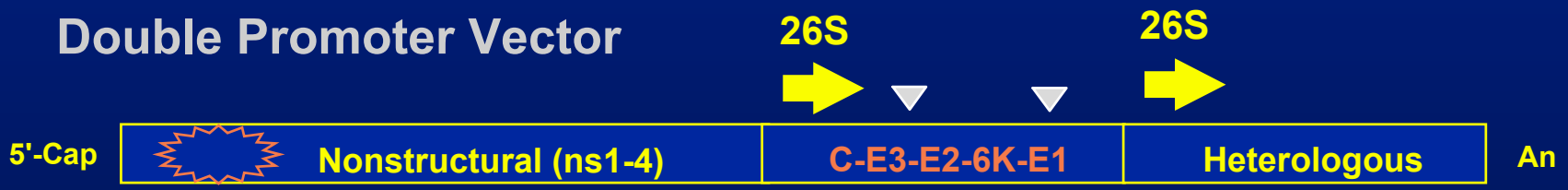


# VEE Vaccines and Vectors

## VEE Vaccine




## Double Promoter Vector




## Replicon Vector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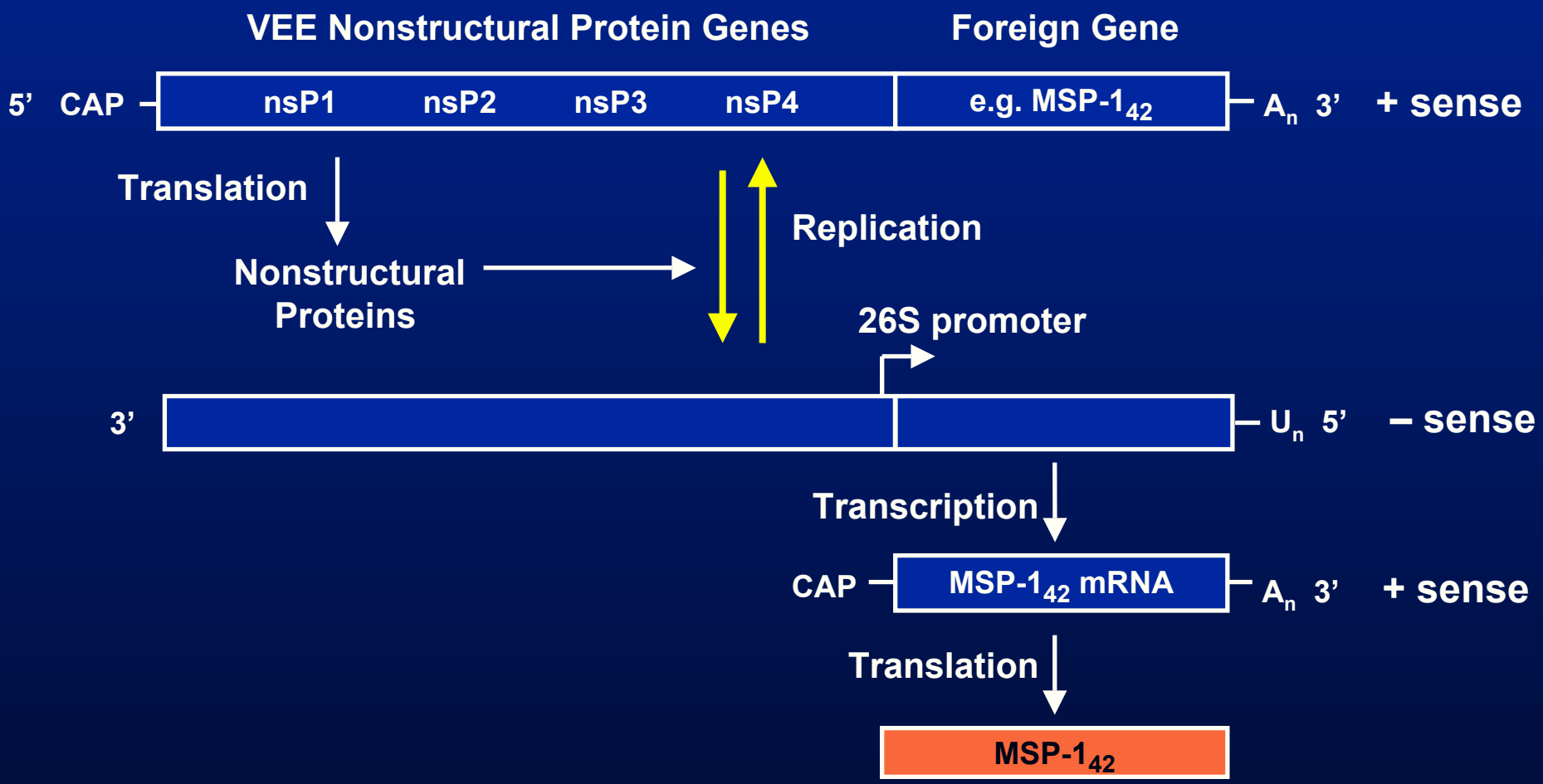
# Expression from Replicon RNAs



# Recombinant VEE Replicons

- **Orthomyxoviruses**
  - A/PR/8/34 (H1N1) HA, NA
  - A/HK/156/97 (H5N1) HA
- **Filoviruses**
  - Ebola GP, NP, sGP VP24, 30, 35, 40
  - Marburg GP, NP, VP24, 30, 35, 40
- **Arenaviruses**
  - Lassa N, GPc
- **Flaviviruses**
  - Dengue NS1, CpreM/E
  - RSSE preM/E
  - CE preM/E
- **Poxviruses**
  - Vaccinia L1R, D8L, A33
- **Caliciviruses**
  - Norwalk / Capsid
- **Arteriviruses**
  - EAV $G_L$ and M
- **Bunyaviruses**
  - RVFV M Seg
  - CCHF M Seg
  - SFFS M seg, N
  - Hantaan N
- **Alphaviruses**
  - VEE GP, PE2, 6K-E1
  - VEE IE and VEE IIIA GP
  - EEE GP
  - WEE GP
- **Lentiviruses**
  - HIV gag, pol, gp 160
  - SIV gag, gp 160, gp140t,
  - EI1A gp20
- **Herpesviruses**
  - HSV gD
  - CMV pp65, IE1, gB
- **Multiple**
  - Ebola GP / Lassa GP
- **Prokaryotic**
  - Anthrax Protective Ag
  - BotNT Hc (A,B,C,E,F,G)
  - BotNTA (H, Hb, Hc-1, Hc-2)
  - SEB, SEA (mutated)
  - Plague F1, V, F1-V
- **Reporters**
  - GFP
  - Beta-galactosidase
  - CAT
- **Tumor Antigens**
  - Her 2/neu
  - HPV 16 E7
  - CEA
- **Therapeutic**
  - Canine Factor IX
- **Malaria**
  - falciparum (n = 7)
  - yoelii (n = 5)
  - Knowlesi (n= 6)

# VEE Replicon Packaging System

## Replicon RNA

Packaging Signal

26S promoter

5’ nsP1 nsP2 nsP3 nsP4 Foreign 3’

## Split Helper

26S

5’ Capsid 3’

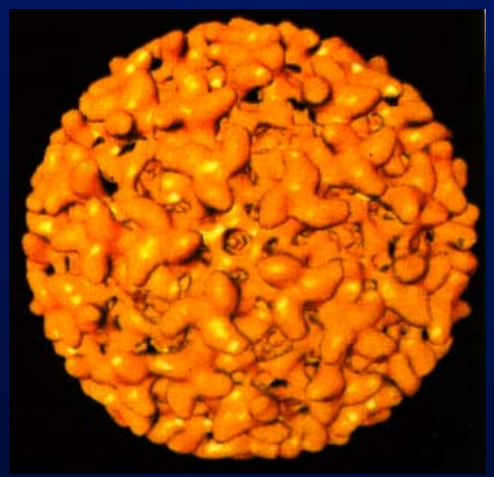
Capsid protein

26S

Attenuating mutations

5’ glycoprotein 3’

Glyco-proteins



Packaged replicon

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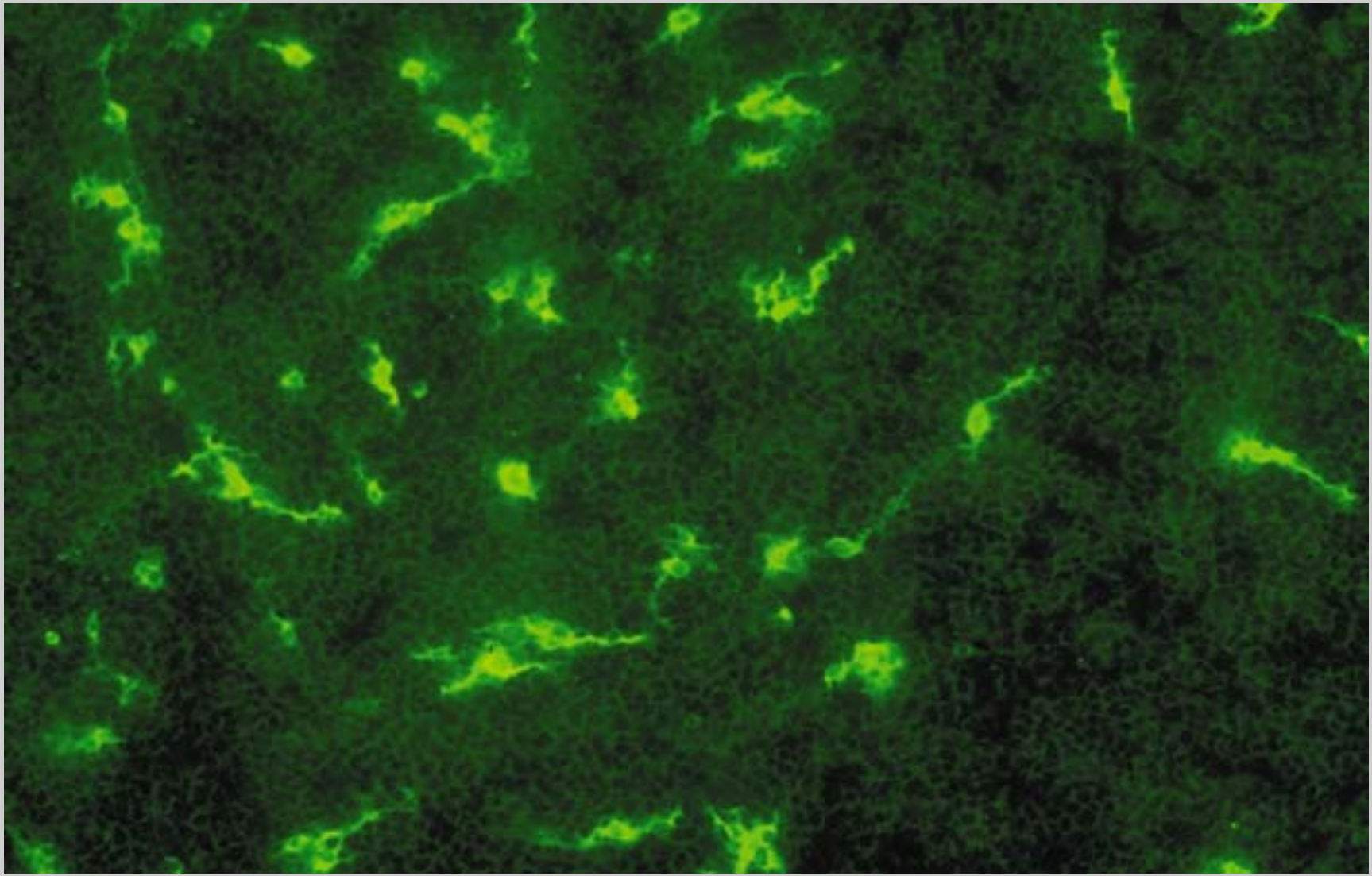
# BHK cells Infected with VRP Expressing Ebola GP



# Efficacy of VRP Vaccines

| Agent | Gene | Animal | % Protection Vaccinated | % Protection Control | Reference |
|---|---|---|---|---|---|
| Marburg | GP | Guinea pig | 100 | 0 | Hevey *et al.*, 1998 |
| | | Cynomolgus | 100 | 0 | Hevey *et al.*, 1998 |
| Lassa | GP | Guinea pig | 100 | 0 | Pushko *et al.*, 2001 |
| | NP | Guinea pig | 100 | 0 | Pushko *et al.*, 2001 |
| Influenza | H5 HK97 | Chick | 100 | 0 | Schultz-Cherry *et al.*, 2000 |
| | H1 PR8 | Mouse | 100 | 50 | Pushko *et al.*, 1997 |
| Ebola | GP | Mouse | 90 | 0 | Pushko *et al.*, 2000 |
| | NP | Mouse | 100 | 0 | Pushko *et al.*, 2000 |
| | NP | Mouse | >75 | <5 | Wilson & Hart, 2001 |
| EAV | $G_L$/M | Horse | 100 | 0 | Balasuriya *et al.*, 2002 |
| HPV 16 | E7 | Mouse | 100 | 0 | Velders et al., 2001 |
| SIV | Gag/env | Rhesus | 100 | 50 | Davis *et al.*, 2000 |
| C. *botulinum* | Hc(A,B,C,D,G) | Mouse | 100 | 0 | Lee *et al.*, 2001 |
| S. *Aureus* | SEB*mut* | Mouse | 75 | 0 | Lee *et al.*, 2002 |

* Morbidity 100%  **Protection from HPV16 $E7^+$ C3 tumor cell outgrowth

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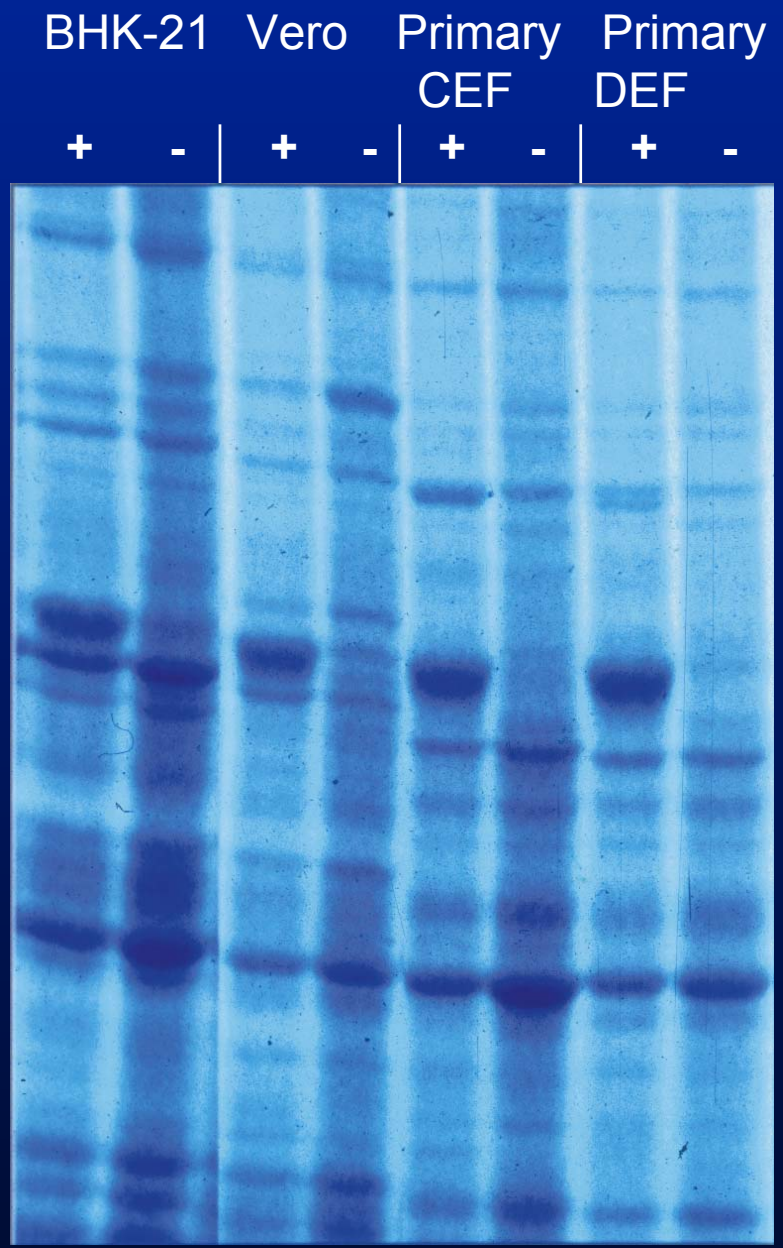
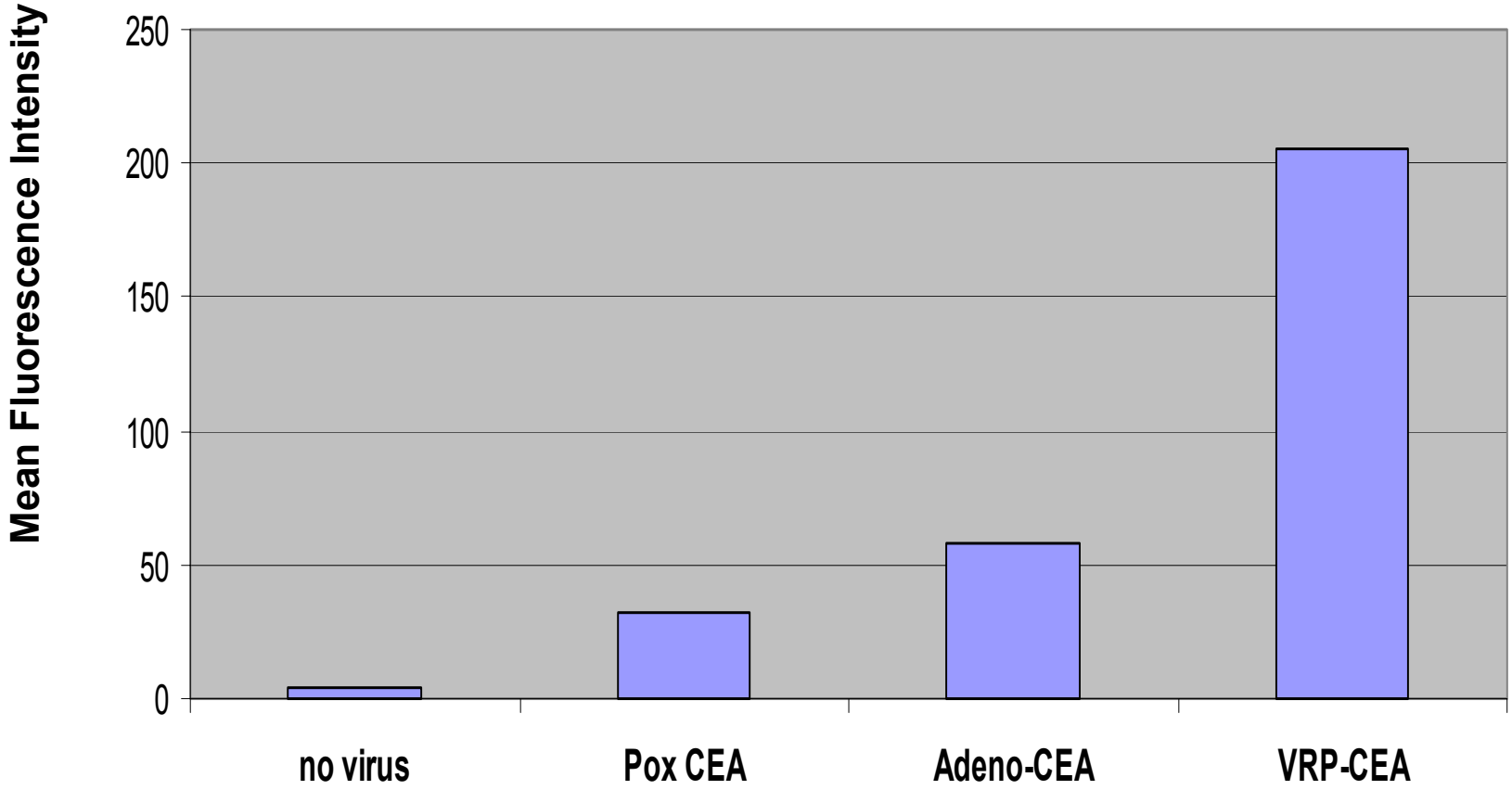
# VRP Expression Levels In Cell Culture

- **+** Cells infected with VRP expressing Lassa N gene
- **-** Uninfected cells



# Relative Levels (FACS) of CEA Expressed in Cultured Human Dendritic Cells

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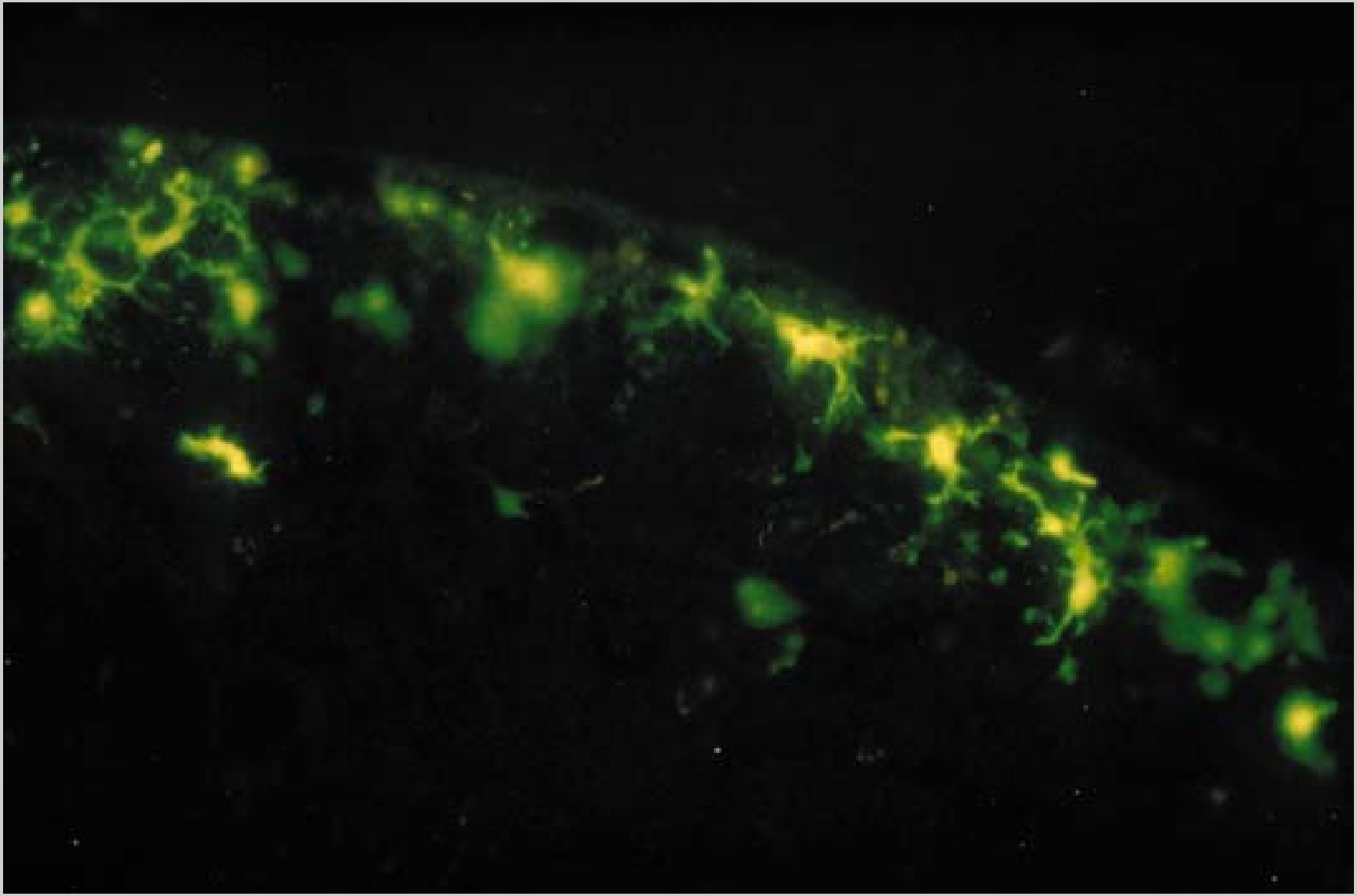
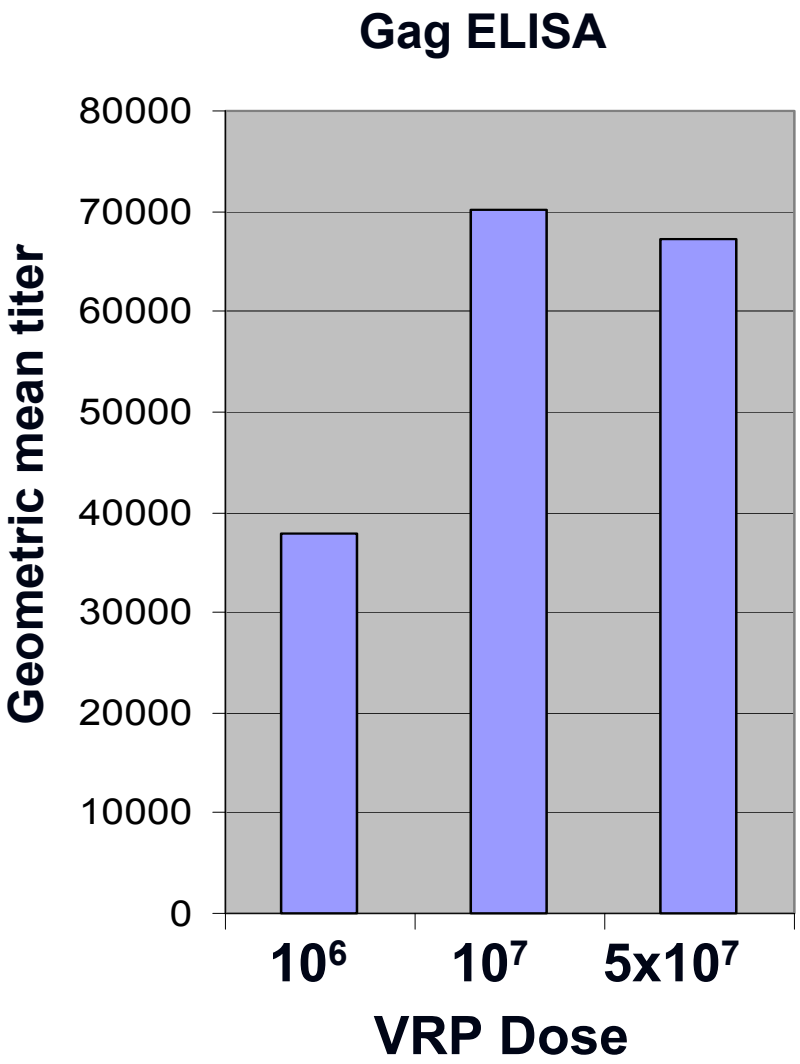
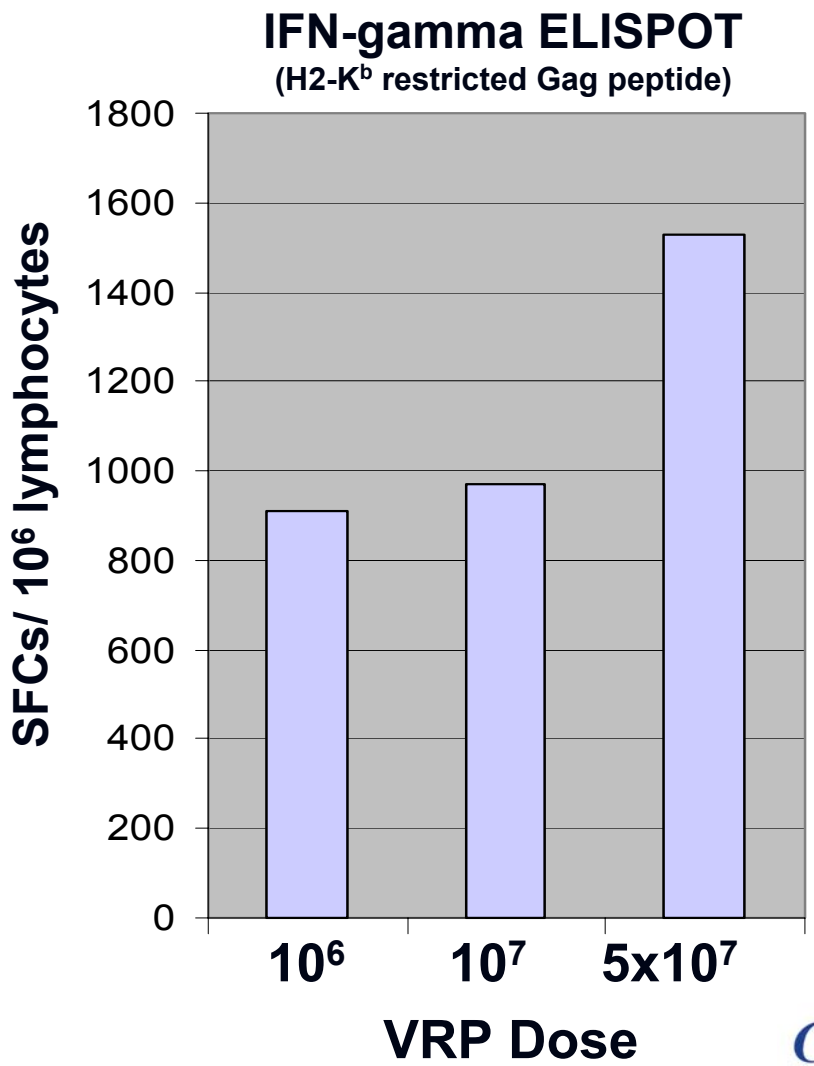
# VRP Target to Dendritic Cells



# VRP Immunization Elicits Robust Humoral and Cellular Immune Responses

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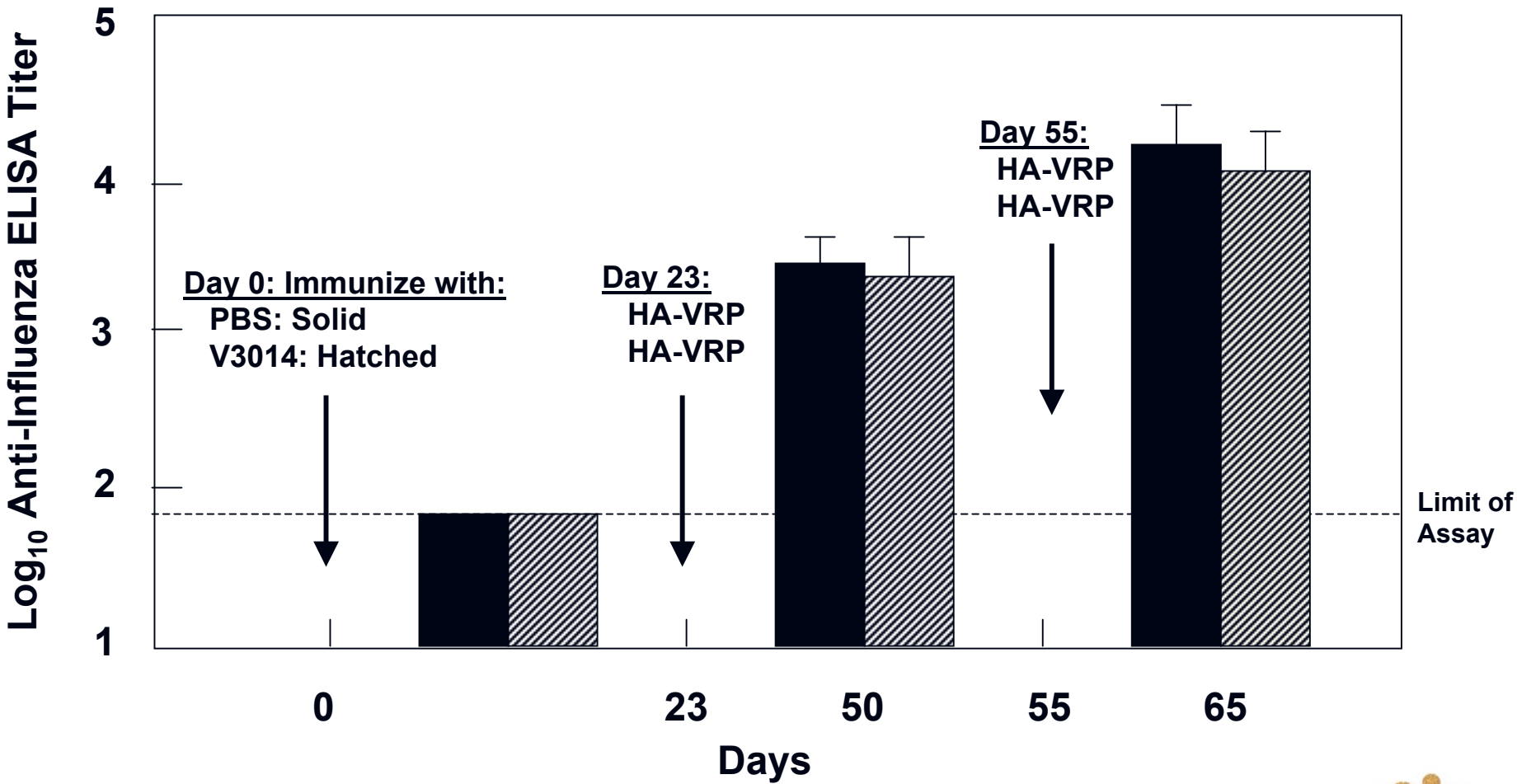
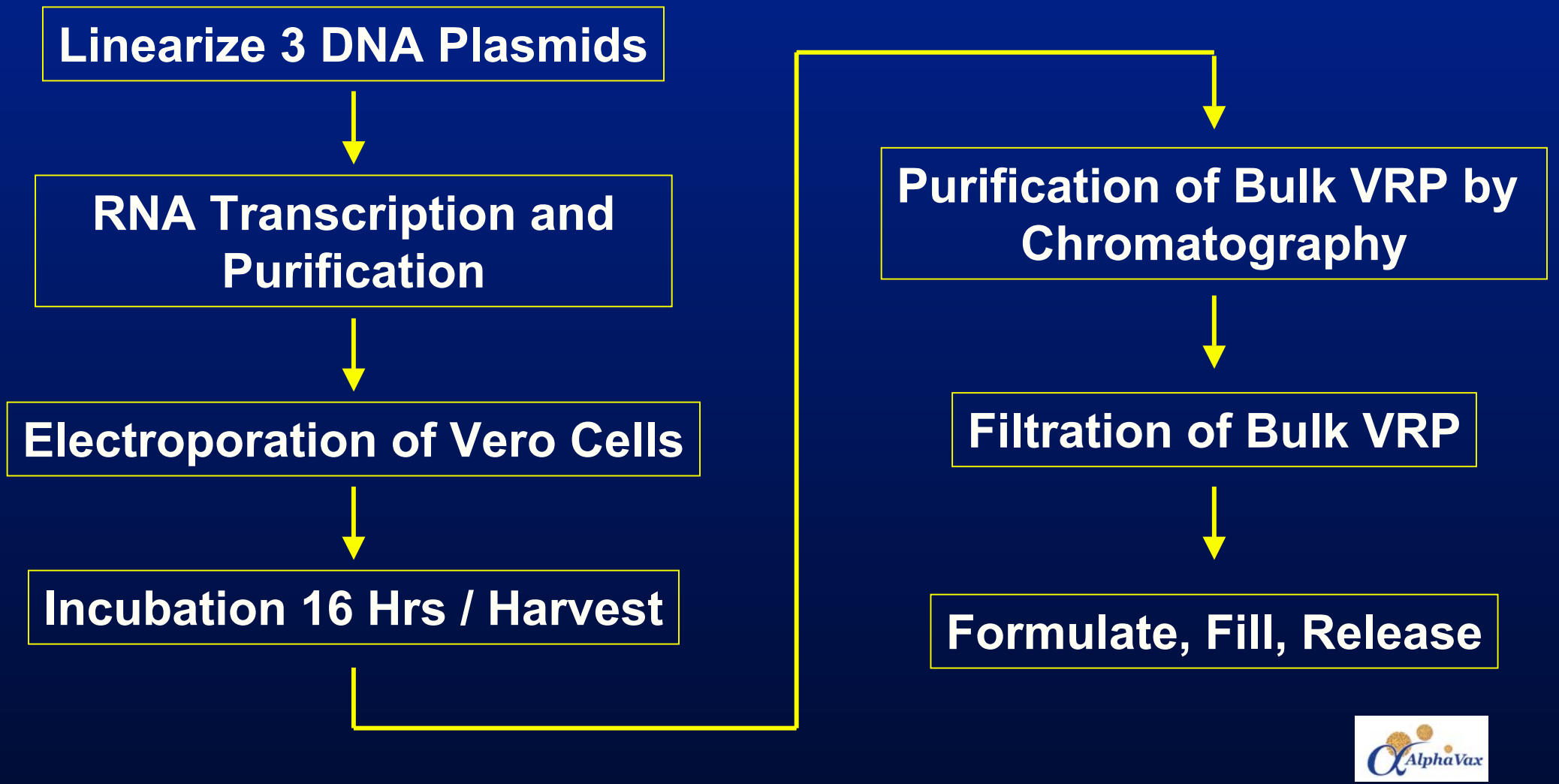
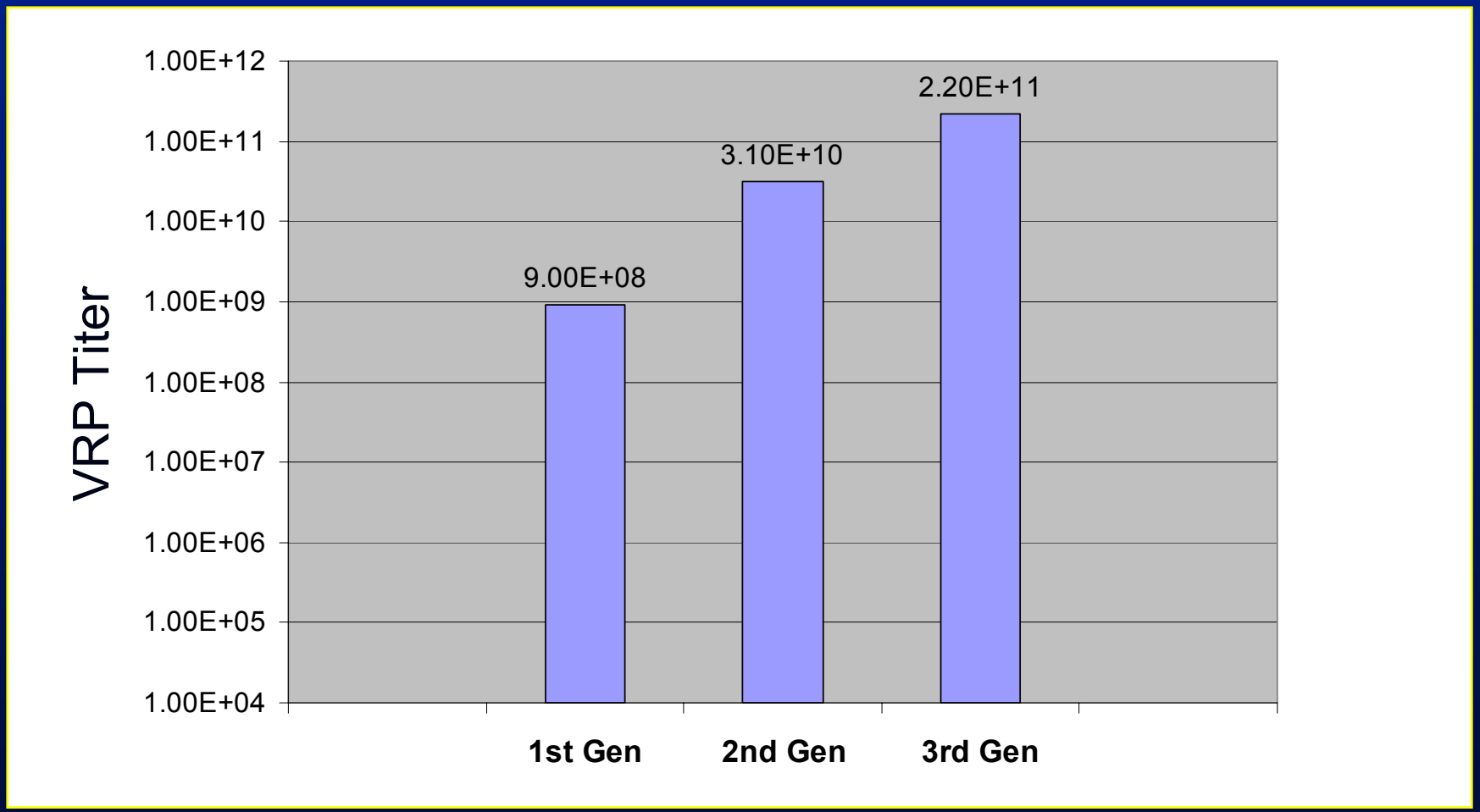
# Immune Response to VRP-HA is Not Diminished in VEE Immune Mice



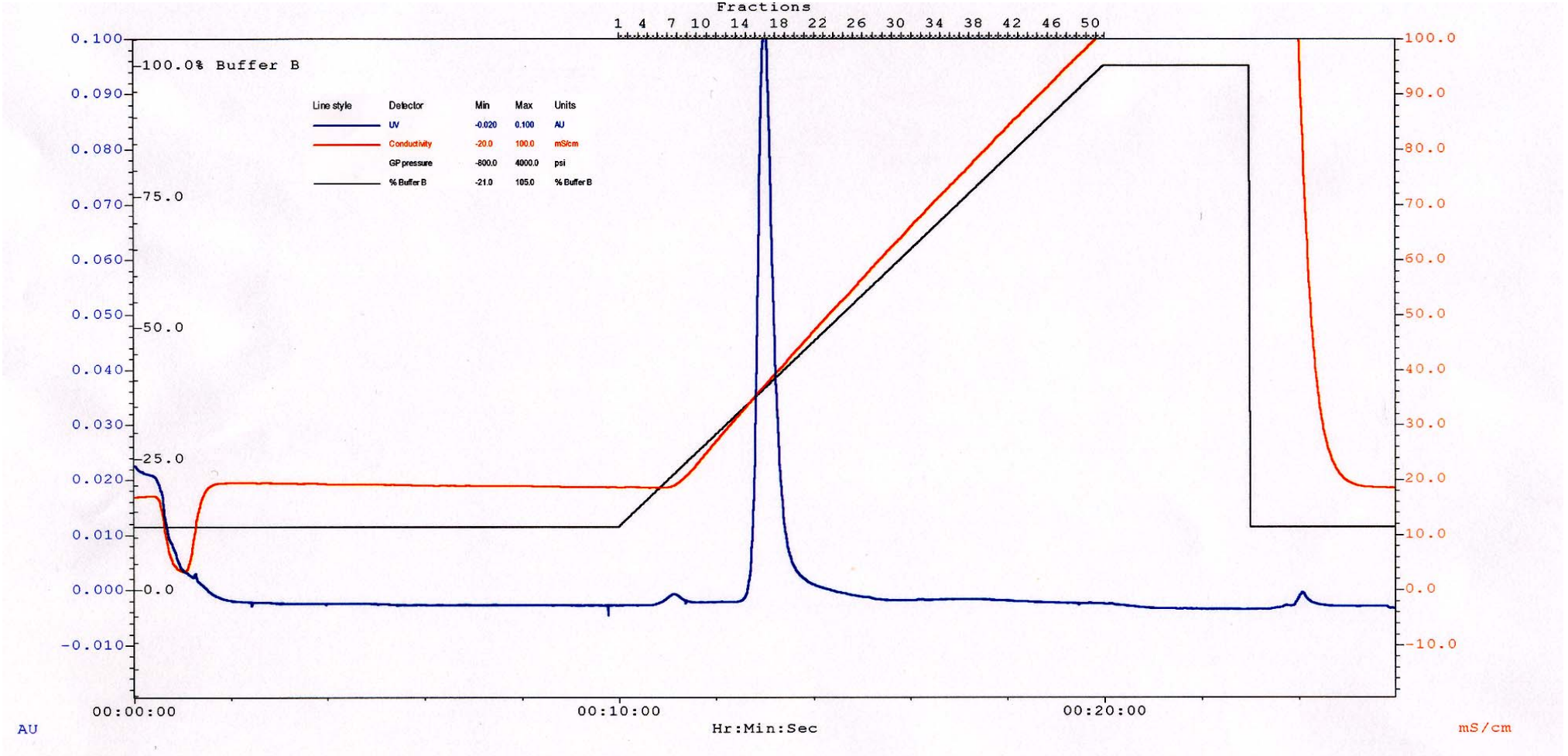
# Production of VRP Vaccines



# Production of VRP Vaccines (VRP*gag*)
## (VRP Yields per $10^8$ Vero Cells)

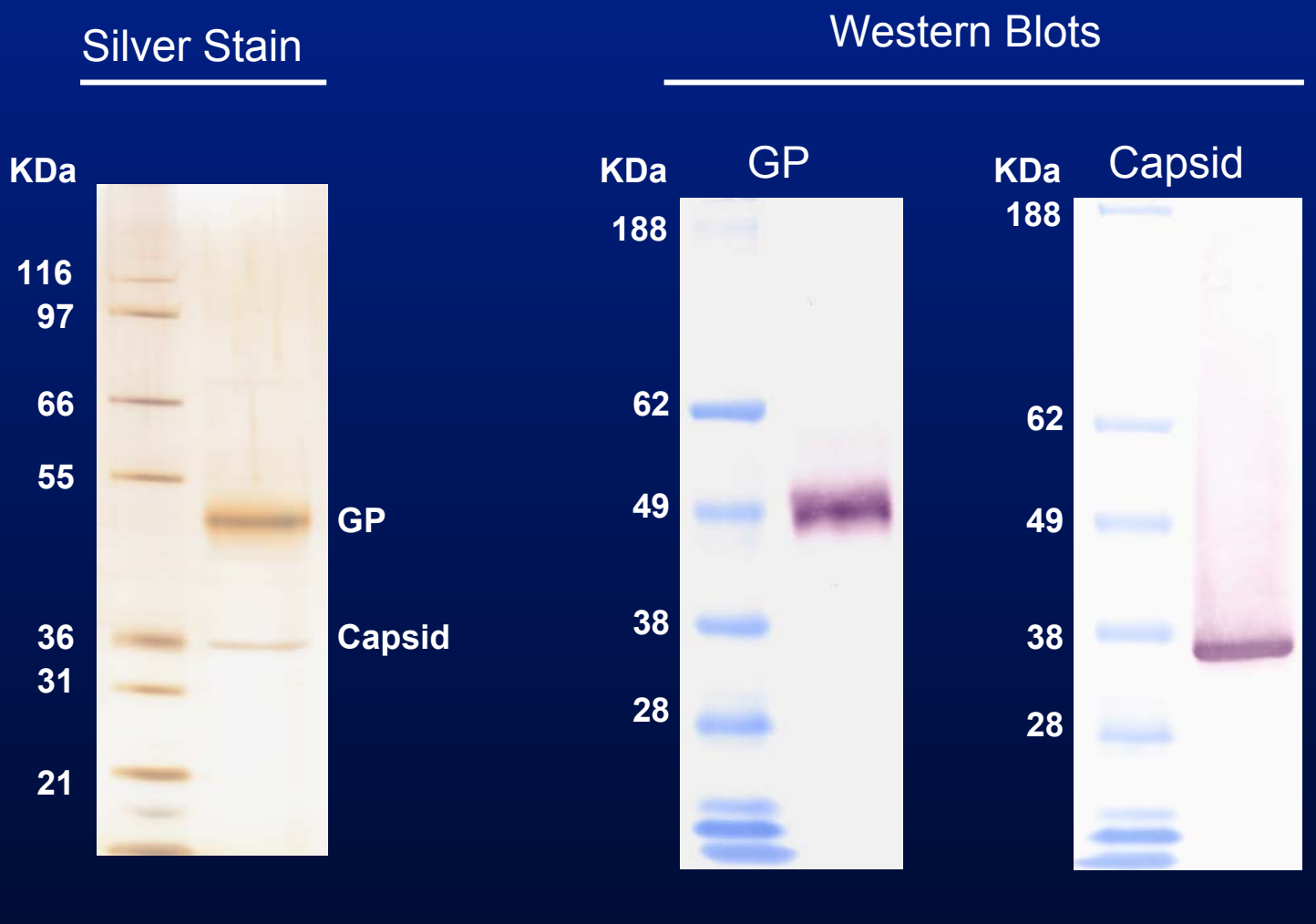


# Purification of VRP Vaccines by Heparin Affinity Chromatography



# Analysis of Affinity-Purified VRP*gag*

**($10^8$ Infectious Units of heparin-purified, unformulated VRP in each lane)**



# Production Capability of VRP Vaccines

- VRP yields are typically 1000 VRP per cell in 16 hours
- 0.5 ml Txn reactions yield RNAs for $>10^{10}$ cells
- Cell Cube at $1/16^{th}$ scale ($2 \times 10^9$ cells) yields $1.6 \times 10^{12}$ VRP*gag*
- Cell Cube at ($5 \times 10^{10}$ cells) expected yield = $2.5 \times 10^{13}$ VRP*gag*
- Single Cell Cube cycle (15-20 days) = 2-5 million primate doses
- VRP produced in serum-free media
- VRP purified by affinity chromatography
- Engineered cell lines are unnecessary for large scale production

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# VEE Replicon Vaccine Vector

- Inherently safe
  - single cycle vector
  - split helper system
  - attenuating mutations
- High expression levels and immunogenicity
- Humoral, mucosal, and cellular effectors
- Polyvalent and sequential immunization effective

- Dendritic cell targeting
- Minimal immunity to vector
- Low seroprevalence to VEE
- Efficacy in animal models
- Cytoplasmic transcription
- Loading capacity >7kb
- Efficient production

AlphaVax

# HVTN Clinical Protocol 040 (AlphaVax VRP*gag*)

- Phase I safety and immunogenicity evaluation of an alphavirus replicon HIV subtype C *gag* vaccine
  - healthy HIV-uninfected adult volunteers
- Plan to enroll 96 healthy volunteers
  - 48 US, 48 South Africa
  - 18-60 y/o, either sex
- Dose escalation ($10^4$, $10^5$, $10^6$, $10^7$)
- 24 subjects per dose level
  - 10 active/2 placebo each country
- 3 subcutaneous injections each subject
  - 0, 1 and 3 months

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# HVTN Clinical Protocol 040 (AlphaVax VRP*gag*)

- Primary endpoint: Safety
  - Local and systemic adverse events
  - Hematology and clinical chemistry
- Secondary endpoint: Immunogenicity
  - Evaluate 2 Weeks after doses 2 and 3, and at Months 6 & 12
  - Humoral response (Gag-specific ELISA, anti-VEE antibody)
  - Cellular response (LPA, $^{51}$Cr-release CTL, ELISPOT)

# HVTN Clinical Protocol 040 (AlphaVax VRP*gag*)

Enrollment

- US cohort 1 (1 x $10^4$ IU)
  - 12 participants, 17 July – 26 Aug 2003
- US cohort 2 (1 x $10^5$ IU)
  - 12 participants, 9 Oct – 16 Dec 2003
- SA cohort 1 (1 x $10^4$ IU)
  - 9 participants, 4 Nov – 25 Nov 2003
  - 3 participants planned for early Jan 2004

# HVTN Clinical Protocol 040
## (AlphaVax VRP*gag*)

Safety

- Vaccine well tolerated at both doses
- No safety concerns identified