

# EIAV as a Research Model for other Lentiviruses: Lessons learned and to be learnt.

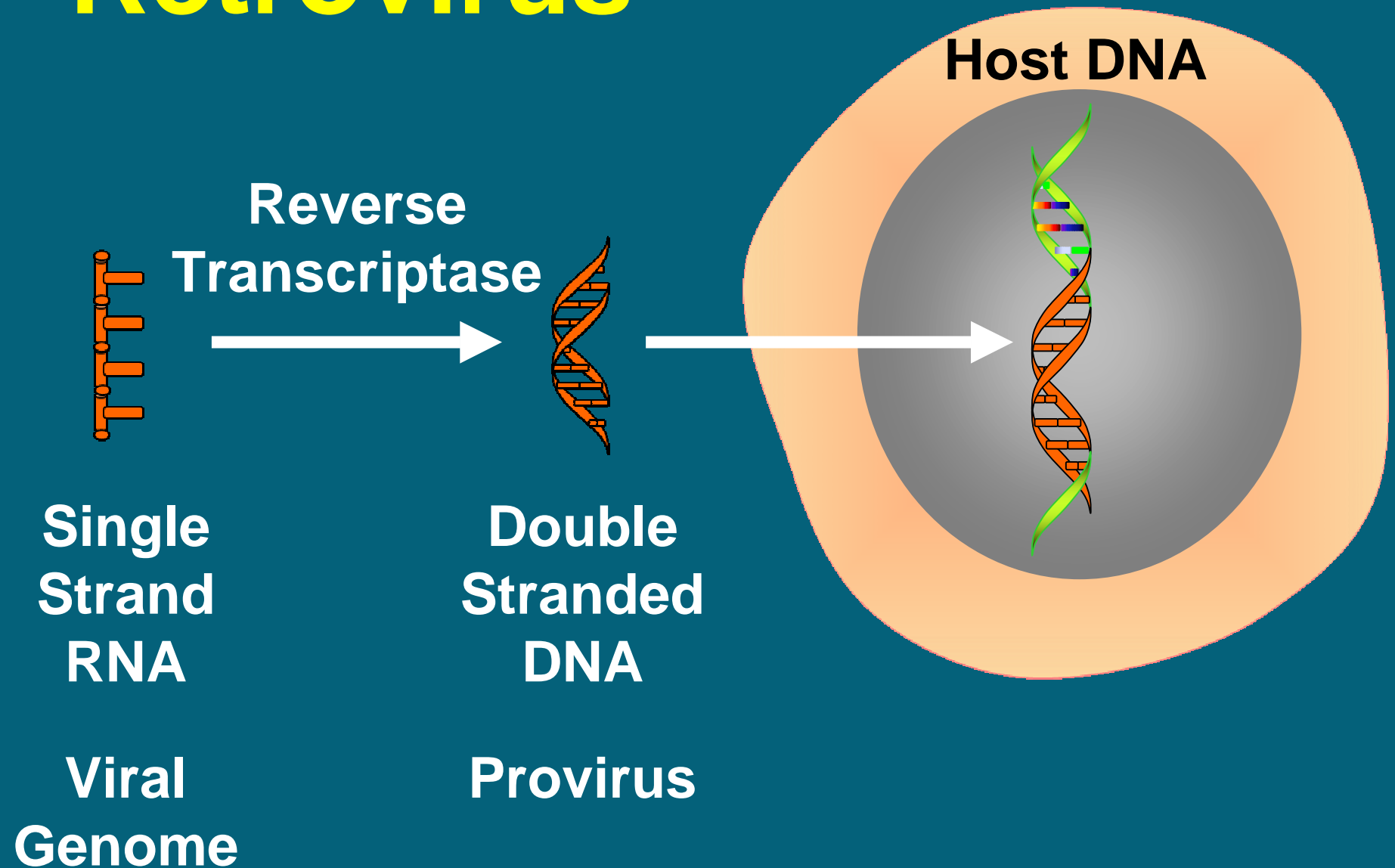


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University of Kentucky

# What is a Lentivirus?

- Family: Retroviridae
- Subfamily: Orthoretrovirinae
- Genus: Lentivirus

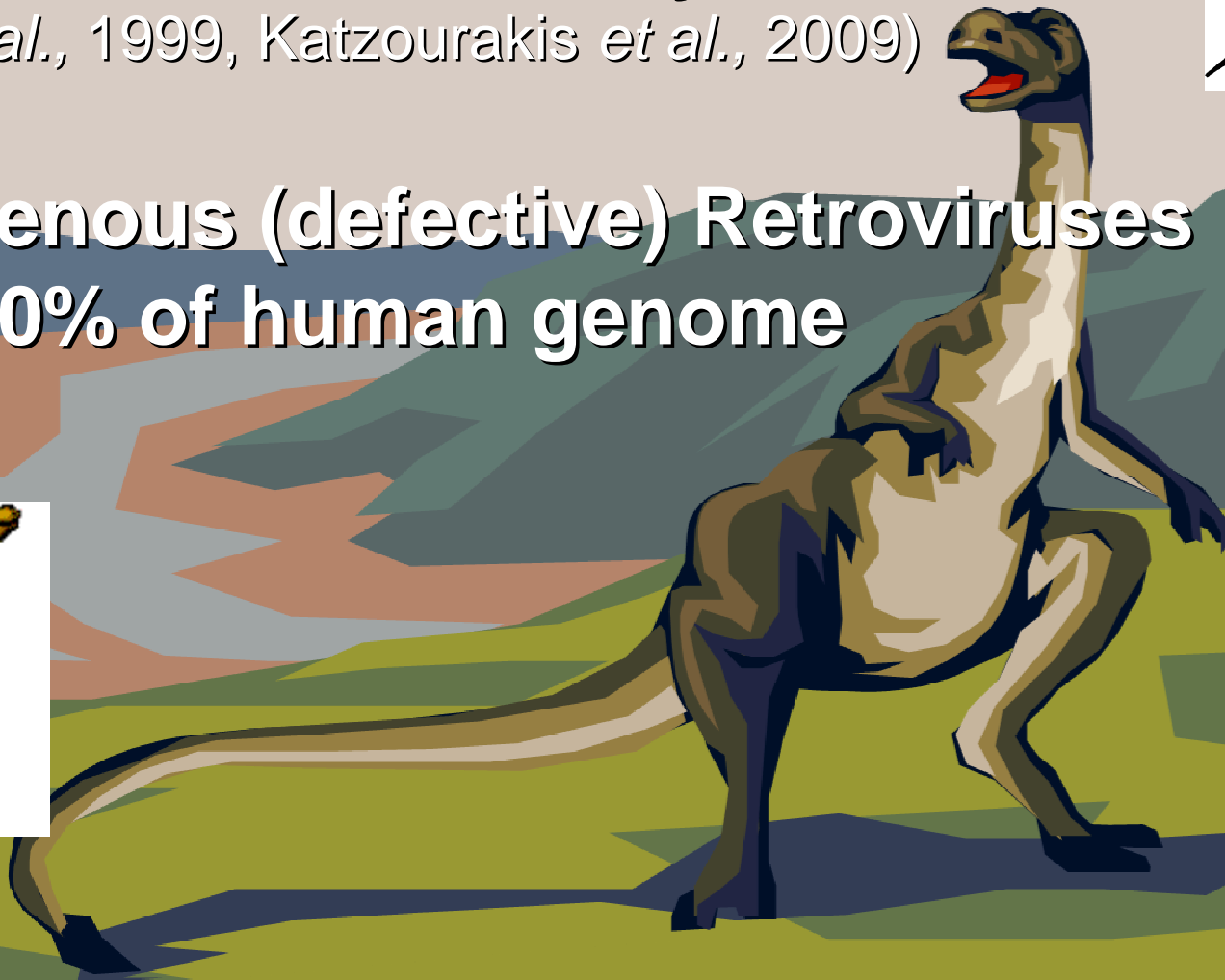
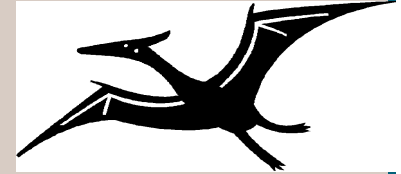
# Retrovirus



# Retroviruses

Ancient  $100 - 150 \times 10^6$  years  
(Benit *et al.*, 1999, Katzourakis *et al.*, 2009)

Endogenous (defective) Retroviruses  
= 8 - 10% of human genome



# Retroviruses

LT  
R

GAG

POL

ENV

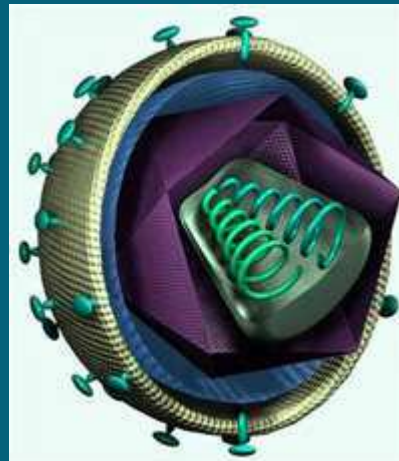
LT  
R



Structural Core  
Proteins  
p15, p26, p11, p9

Replicative  
Enzymes

Envelope Surface  
Glycoproteins



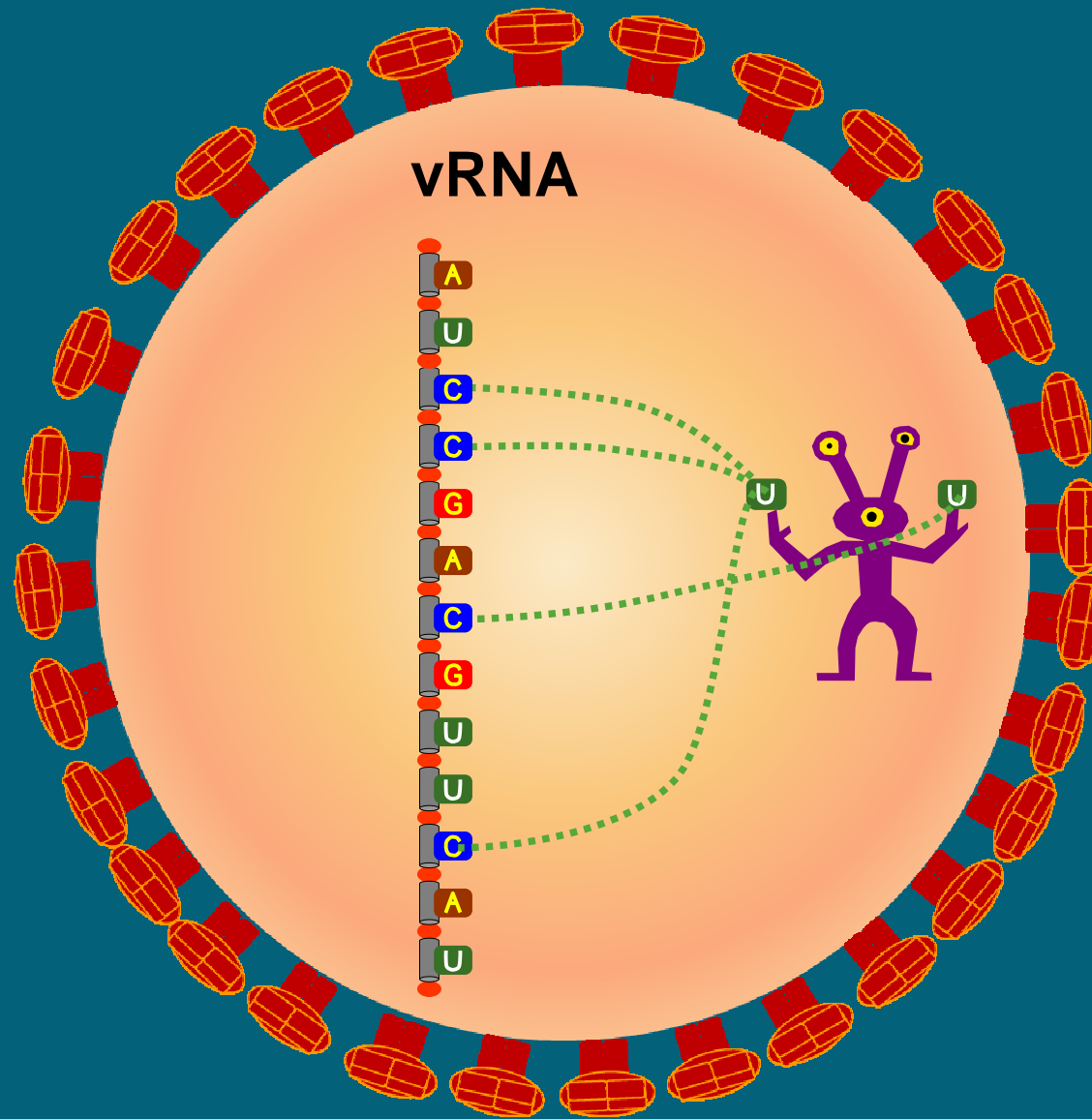
# Host Defenses

**Immune Response:**    Innate  
                                    Adaptive

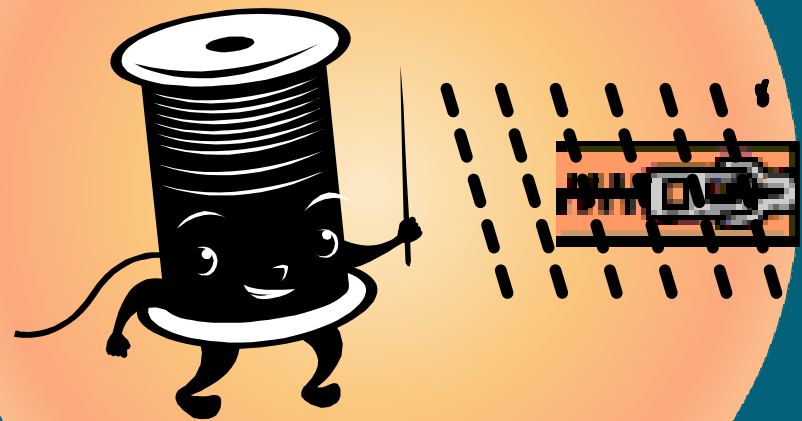
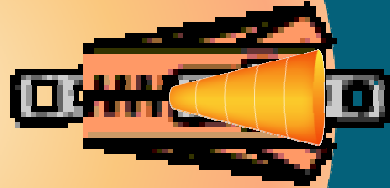
**Retroviral Restriction Factors:**

- **Apolipoprotein  $\beta$  Editing Complex 3  
(APO $\beta$ EC3)**
- **Tripartite Motif-Containing Protein 5 $\alpha$   
(TRIM5 $\alpha$ )**
- **Tetherin**

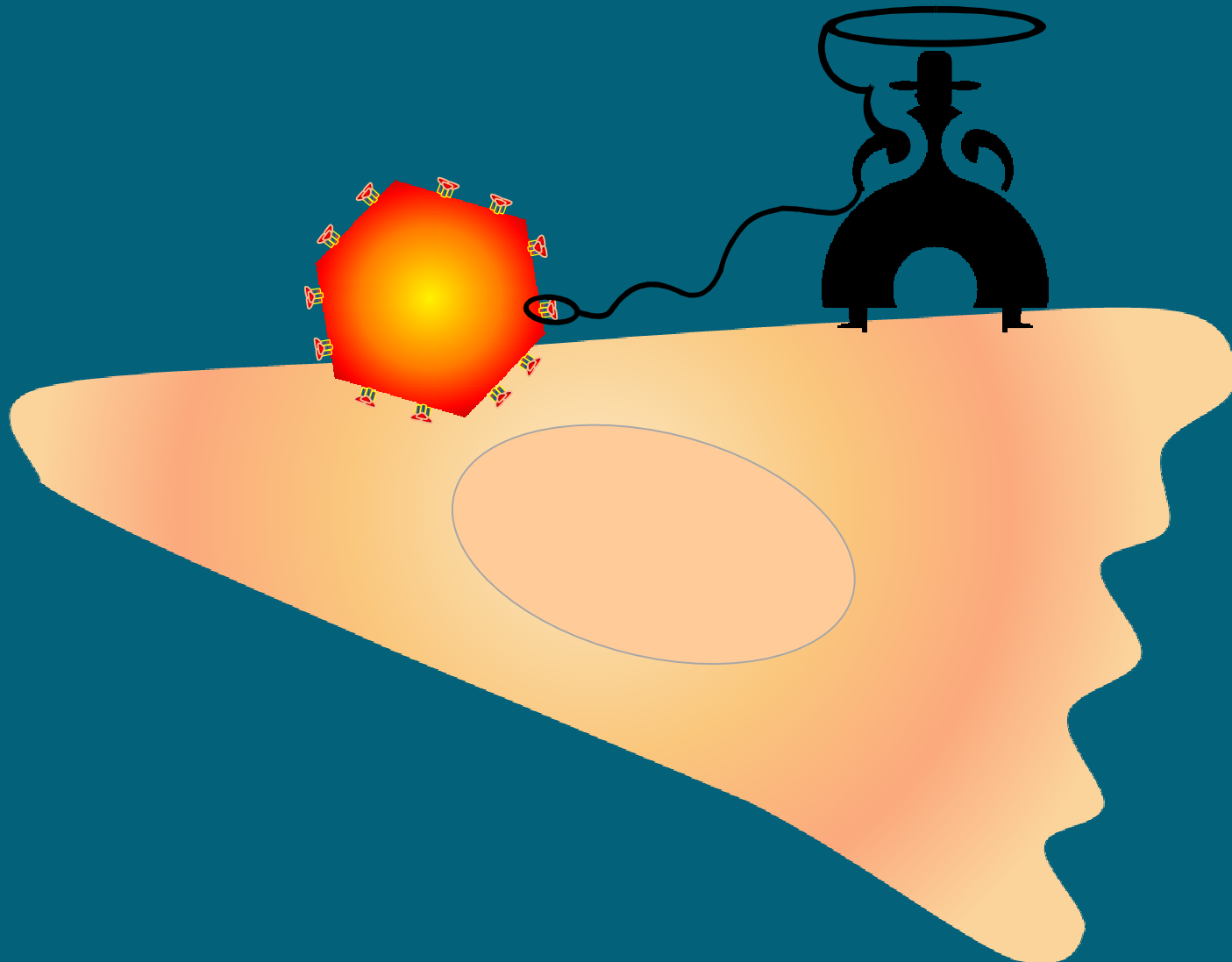
# APOBEC3 (Cytosine Deaminase)



# TRIM 5α



# Tetherin



# Lentiviral Characteristics

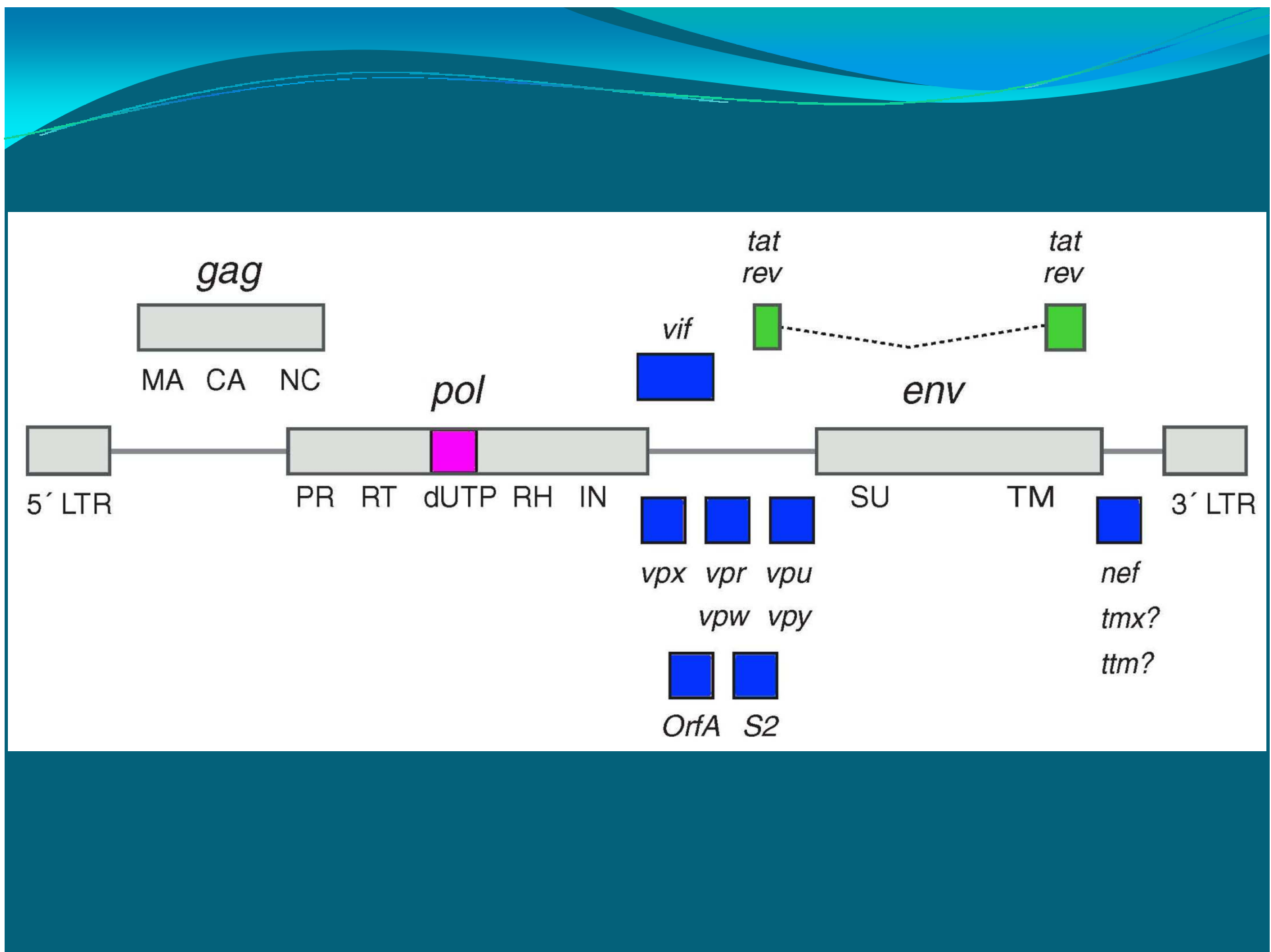
Genetically, Morphologically Distinct  
Infect Non-Dividing Cells

Hostile environment

Low dNTP (SAMHD1) High dUTP

Evolved Additional ORFs to GAG,  
POL, ENV

Complex Retroviruses



# Additional / Ancillary Lentiviral Genes

<b>Tat</b>	Transactivator for replication	<b>Vpu</b>	CD4 ↓ Tetherin ↓
<b>Rev</b>	Export of viral RNA from nucleus	<b>OrfA</b>	CD134 ↓
<b>dUTPase</b>	dUTP → dUMP	<b>nef</b>	CD4 – signaling
<b>S2</b>	Binds cellular proteins Inflammatory cytokine ↑		–intracellular trafficking –cell migration –apoptotic pathways
<b>Vif</b>	APOβEC3 degradation		
<b>Vpr</b>	SAMHD1 degradation		

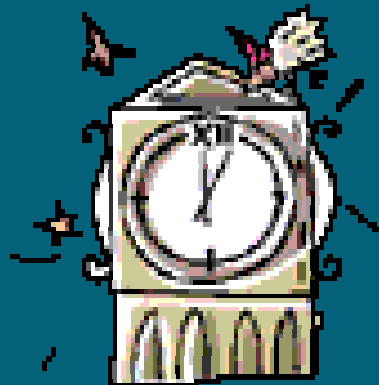
# Timeline of Lentiviral Evolution

## Molecular Clock Estimate

Entire Genus = < 1 million years

Individual Lentiviruses = 100's to 1000's  
years

Human	HIV-1M	1908 – 1933
Gorilla	SIVgov	1818 – 1906
Chimpanzee	SIVcp2	1266 – 1685



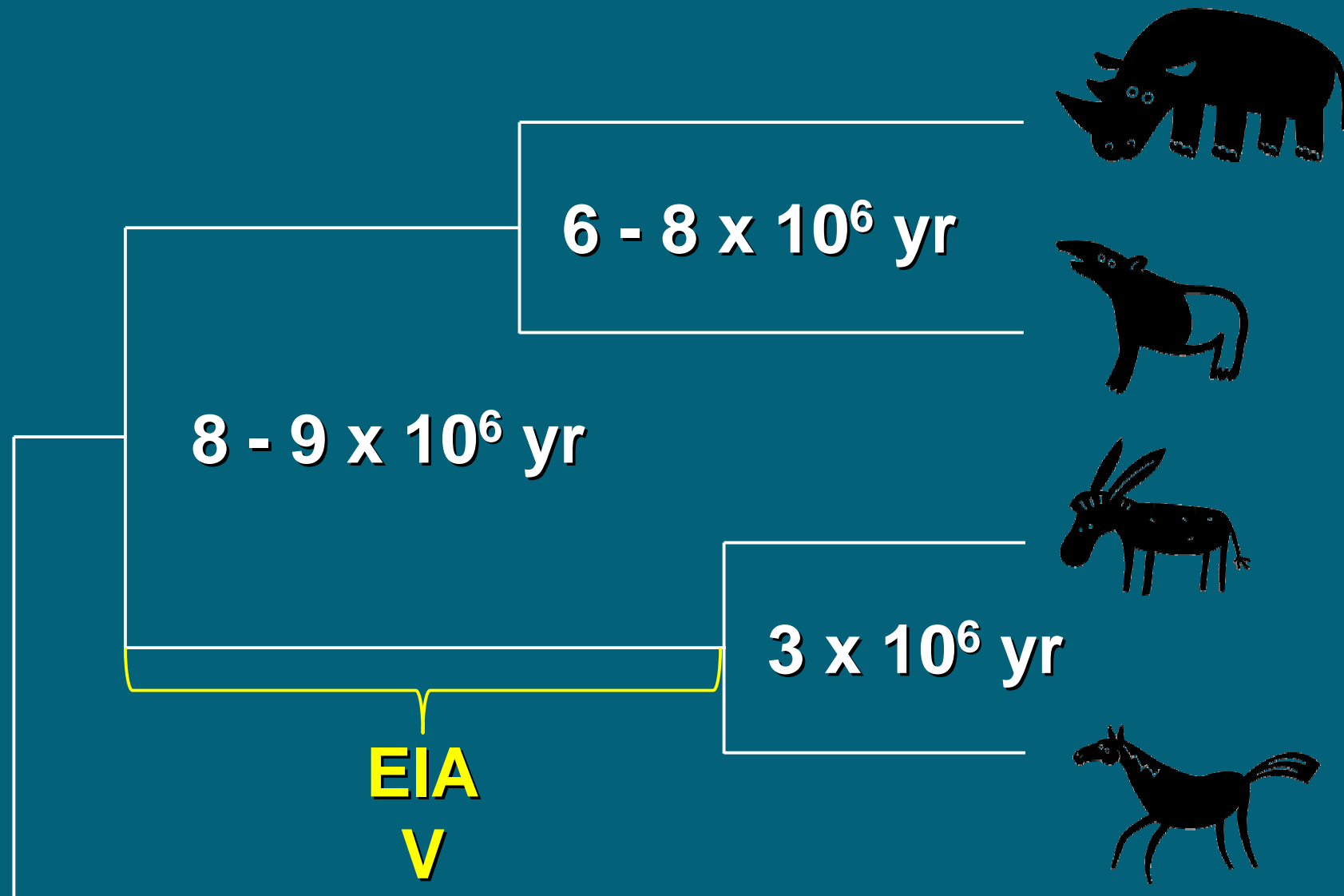
*Wertheim et al.,  
2009*

# Lentivirus Distribution

Retroviruses All Vertebrates



# Perissodactyla



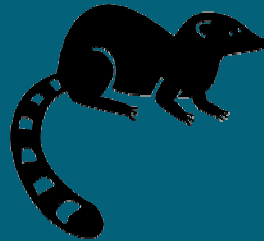
# Revised Timeline of Lentiviral Evolution

Defective



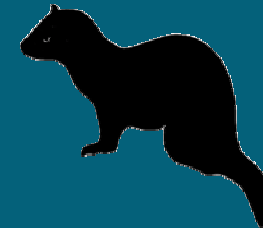
**RELIK**  
 $12 \times 10^6$  yr

Endogenous



**PSIV**  
 $4.2 \times 10^6$   
yr

Lentivirus



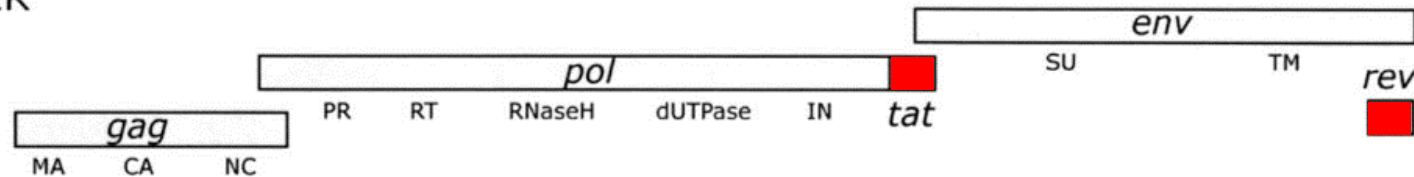
**ELVmpf**  
 $12 \times 10^6$  yr

# Endogenous Lentiviruses

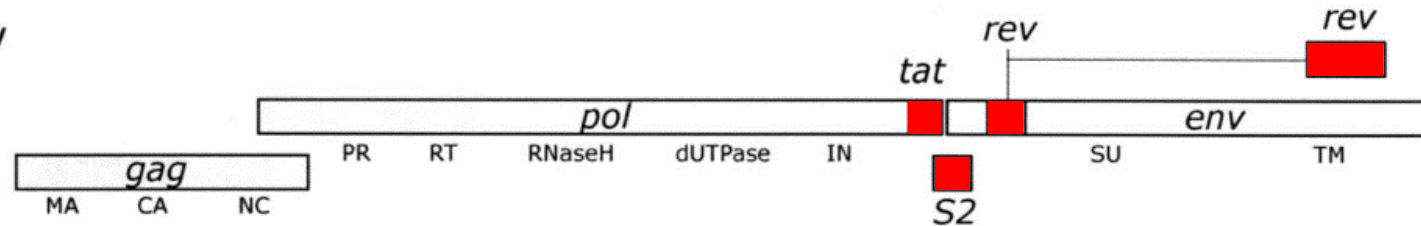
- Older
- Expand Host Range
  - Lagomorph
  - Prosimian
  - Carnivore
- Extinction

# Lentiviral Genome Organization

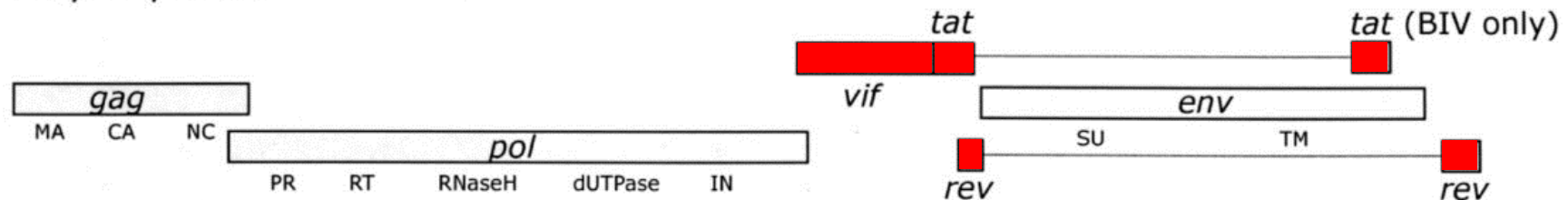
## RELIK



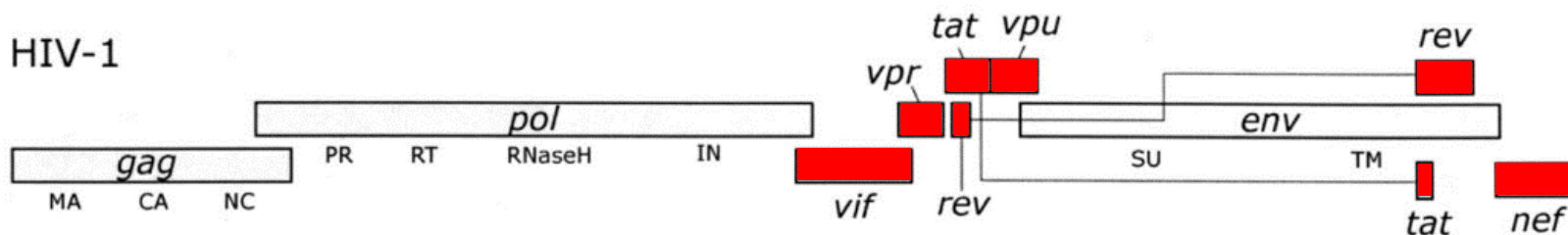
## EIAV



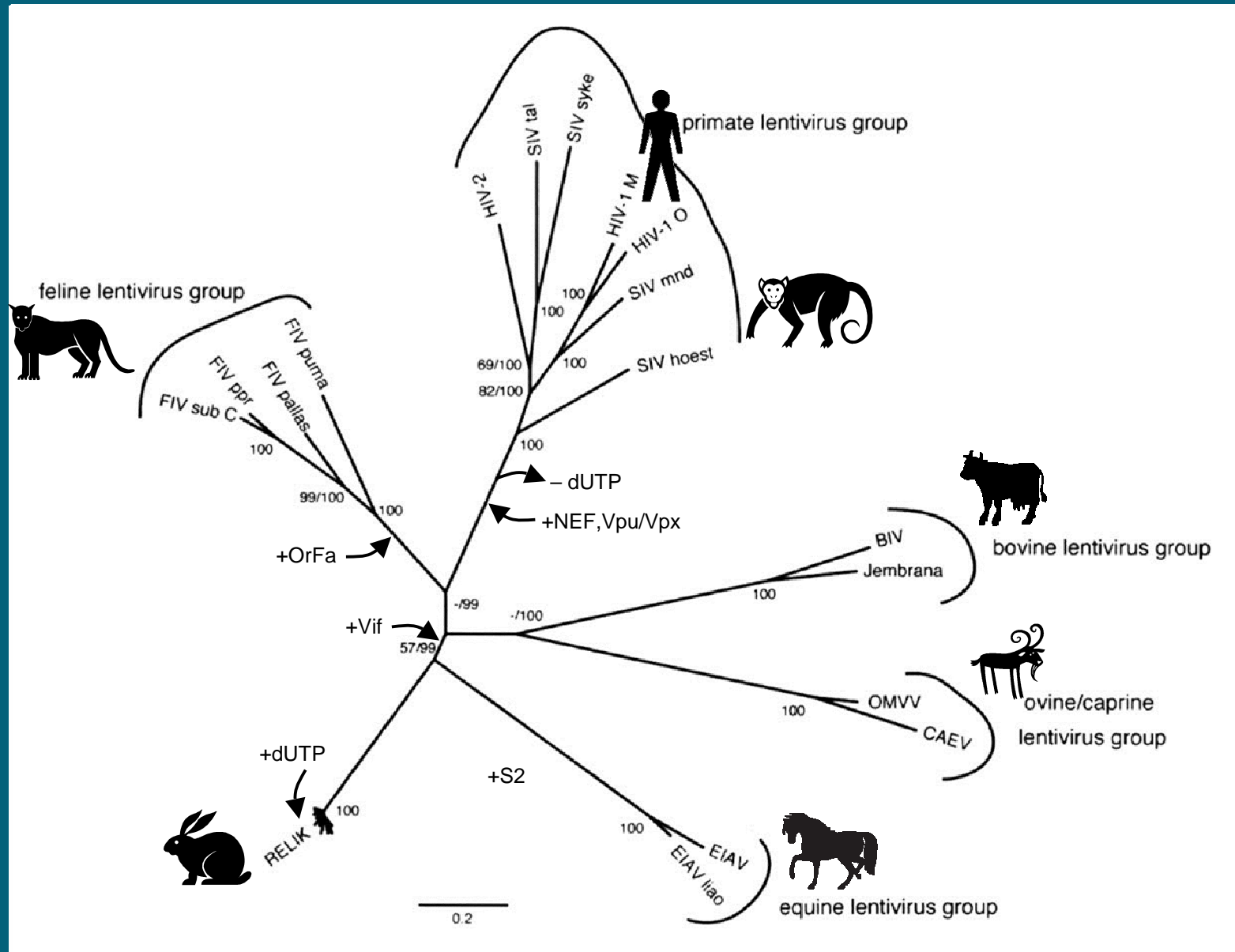
## FIV/BIV/OMVV



## HIV-1



# Lentiviral Phylogeny ?



# EIAV

- Infects ALL Equidae
- Persistent Infection – NOT eliminated by host responses
- Simplest genome organization of any extant Lentivirus
  - dUTP
  - TAT
  - REV
  - S2
- Only extant Lentivirus - VIF

# EIAV and Retroviral Restriction Factors

**APO $\beta$ EC3** – Horse more genes than any other non-primate species

- Not blocked by EIAV
- Packaged in virions
- ?

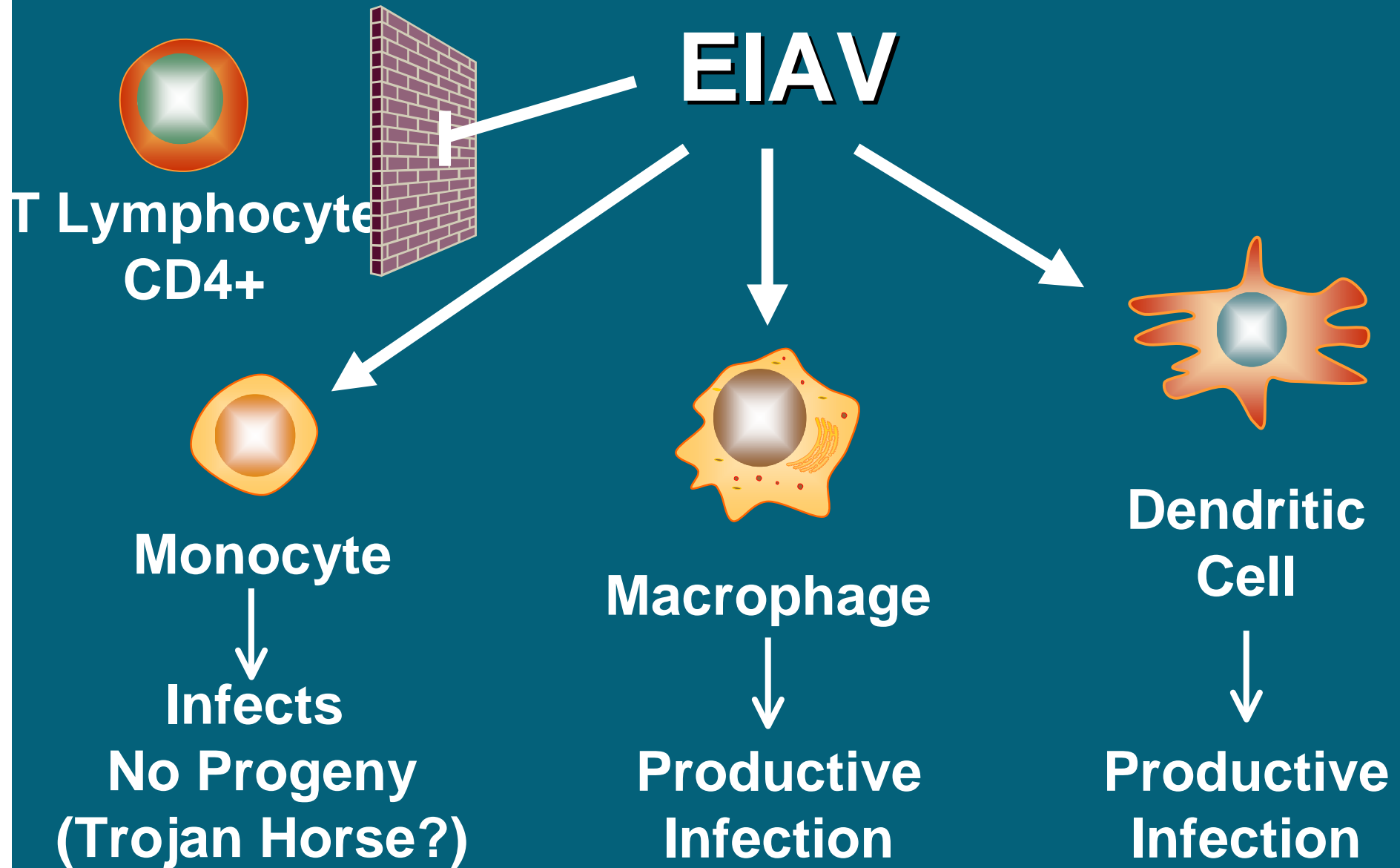
**TRIM5 $\alpha$**  – EIAV p26 resistant?

- Expression in horses?  
( $\Delta$ TRIM5 $\alpha$  in Canidae)

**Tetherin** – EIAV Env resistant?

**Is EIAV a primitive lentivirus?**

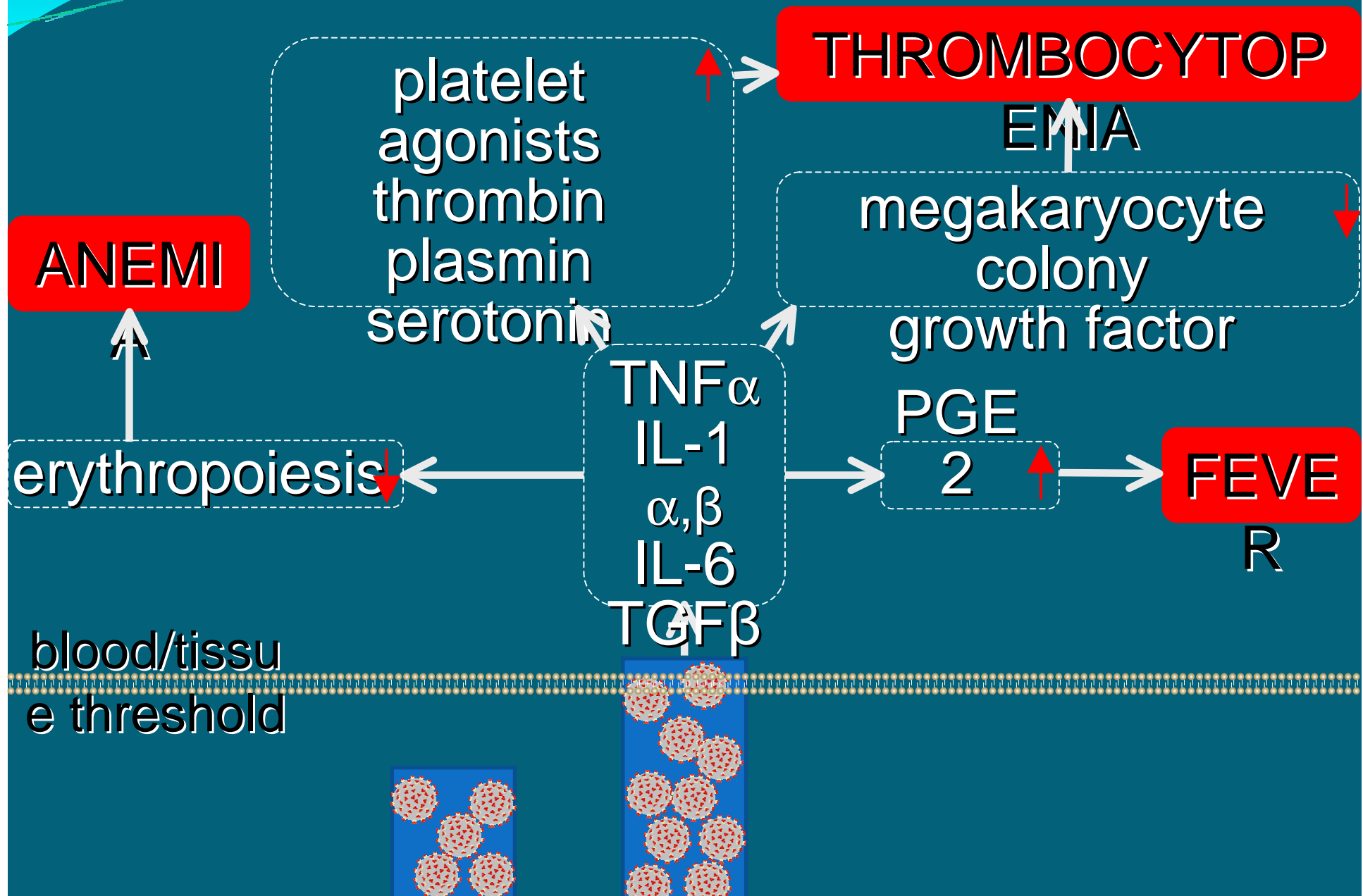
# Host Cell Types



# EIA Clinical Signs

Stage	Signs	Viral Loads
Acute	Fever (>39°C) Thrombocytopenia Lethargy	High
Chronic (12-24 months)	Fever Thrombocytopenia Petechial hemorrhaging Anemia Edema Cachexia	High
Inapparent	None	Low

# Pathogenesis of Acute EIA

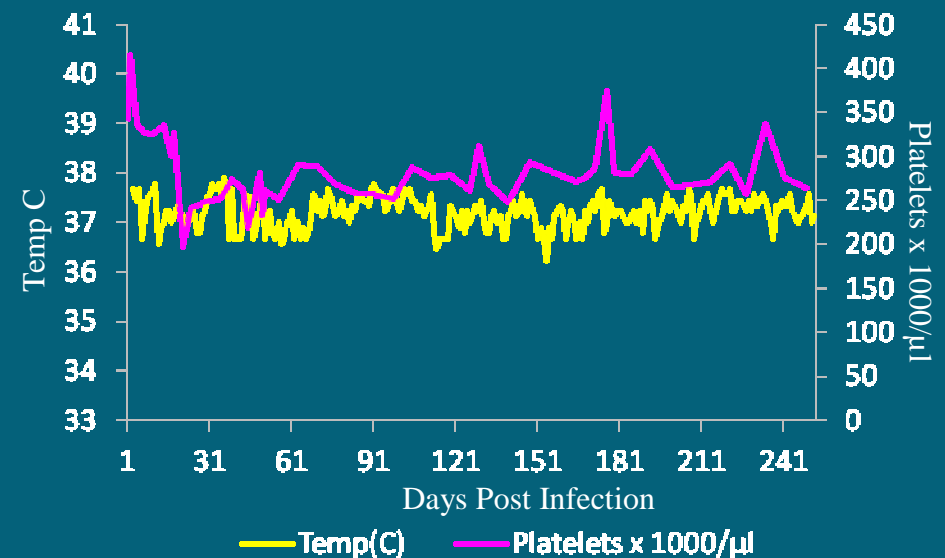
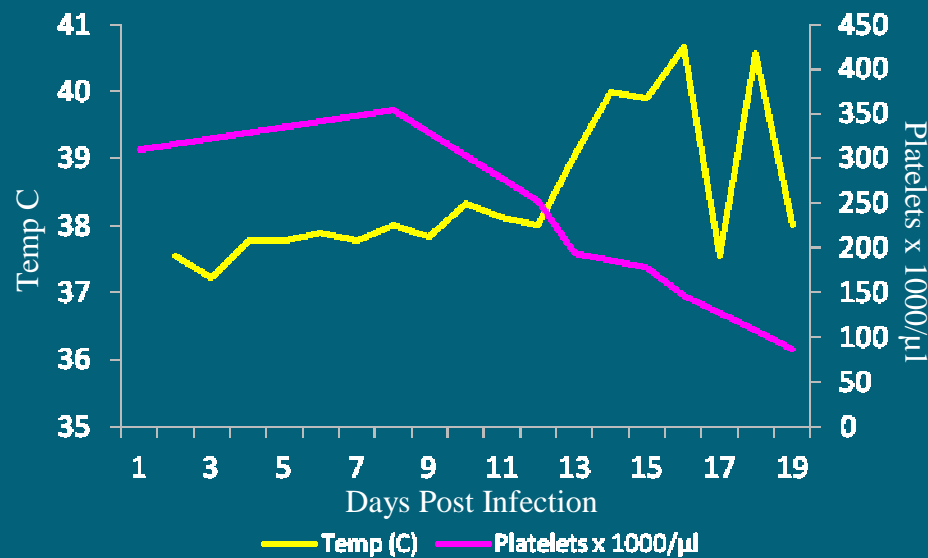


# EIA Clinical Signs

Highly variable  
Sub-clinical – Death  
Individual  
Equid Species



# EIAV Differences between Equid Species



# Host Management of Lentiviral Infections



## Natural:

SIV / African Non-Human

Primates

Subclinical

Viral Replication – High

Immune Control – Ineffective

Limit Pathogenesis, CD4 Depletion

NEF – CD3-TCR↓

## Non-Natural:

HIV / Humans, SIV /

Asian Macaques

AIDS

Viral Replication – High

Immune Control – Limited, Transient

(Exception – elite controllers?)

# EIAV / Horse

Natural Immune Suppression: Viral Load  $\uparrow$

Disease  
Transient  
Strain  
Specific

Long Term  
Cross-  
Reactive

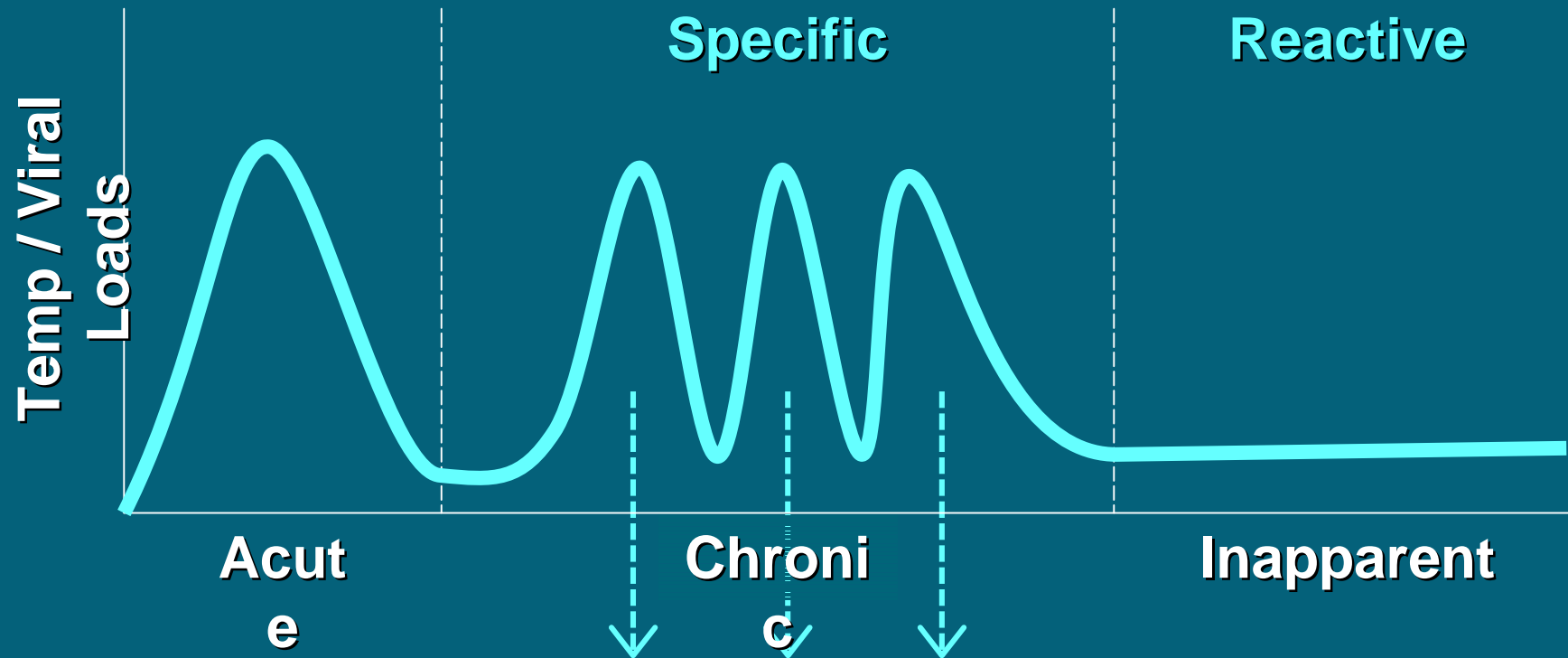
Temp / Viral  
Loads

Acute

Chronic

Inapparent

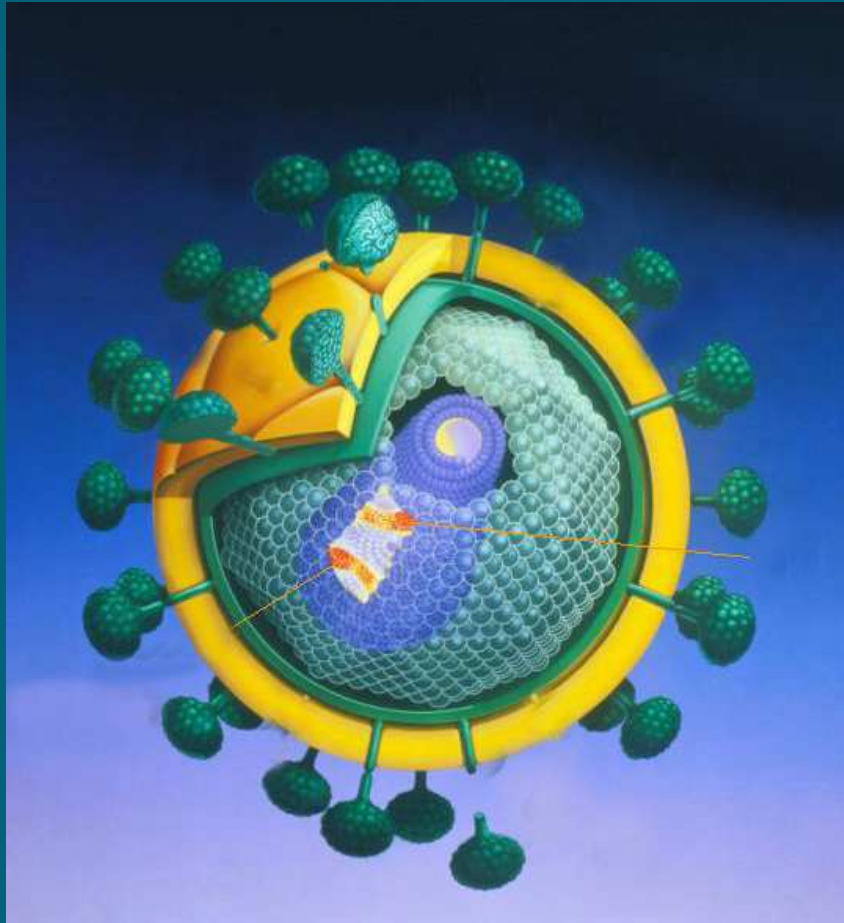
Antigenic  
Variants  
(SILENT)



# Immunological Control of EIAV

- No simple correlate of protection
- Differences between individual horses
- Model for Elite Control of HIV in humans?
- Other mechanisms?

# EIAV SU : A Critical Role



Attachment/Entry  
Neutralizing  
Epitopes

# Variation in SU PND with Time

## SU PND Amino Acid Sequence

	N	S	S	D	S	S	N	P	V	R	V	E	D	V	M
I	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
II	•	•	•	•	•	•	•	S	•	•	•	•	•	•	•
II	•	•	G	•	•	•	•	S	•	•	•	•	•	•	•
I															
I	*	*	*	*	*	*	*	*	*	*	*	*	*	*	•
V															
V	T	•	G	P	•	•	•	S	•	•	G	•	Y	E	T

# $\Delta S2$ EIAV Vaccine

Based on EIAV<sub>UK3</sub> infectious molecular clone (Cook et.al. Virology 313: 588-603, 2003)



Immunize Horses EIAV $\Delta 2$ , challenge >6 months

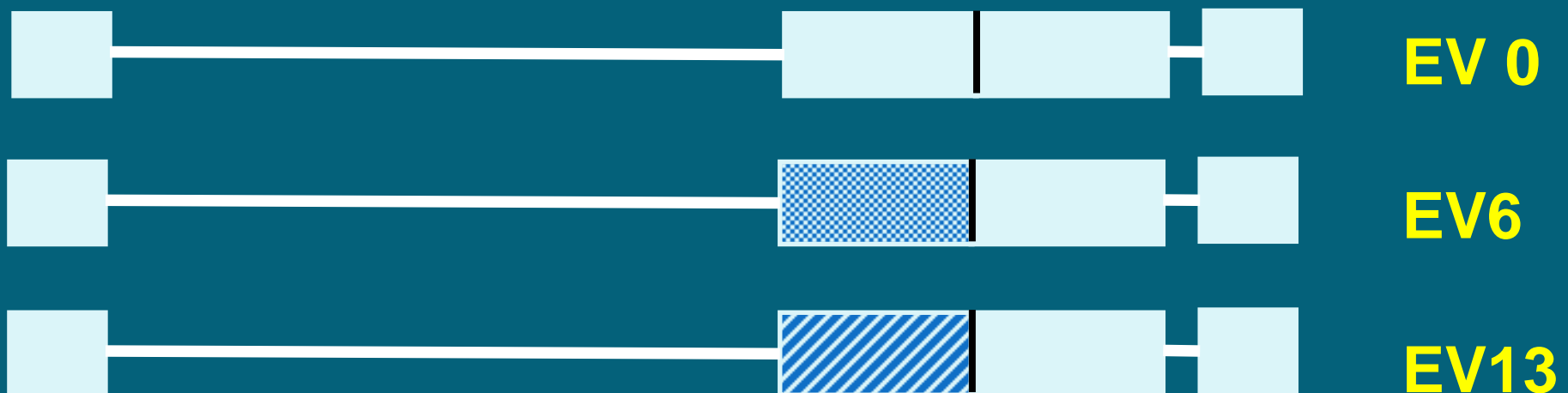
75-100% Protection from INFECTION  
from **HOMOLOGOUS challenge**

# EIAV Heterologous Challenge

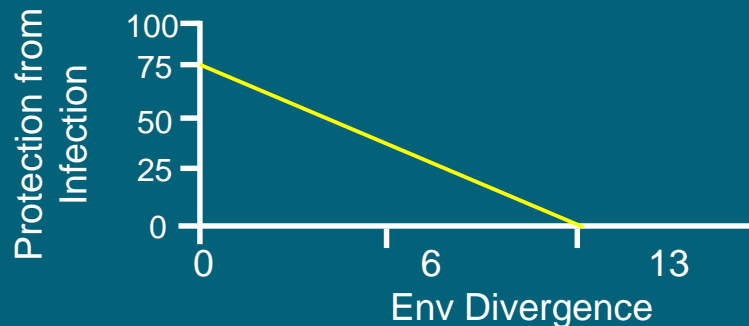
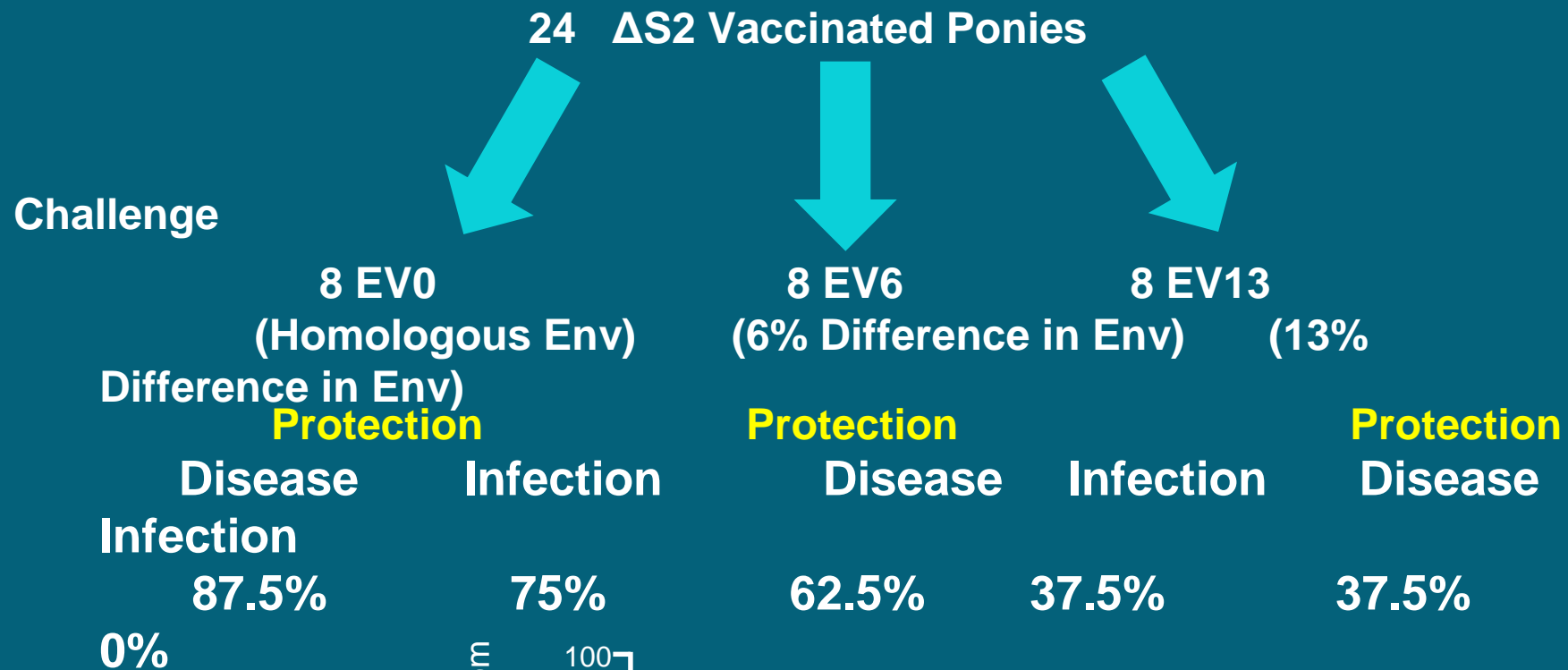
Pony 564 EIAV<sub>PV</sub> = EIAV<sub>UK3</sub> SU = EV0

Febrile episode IV @260dpi (6% divergence = SU =

Inapparent 1219dpi (13% divergence SU = EV13)



# $\Delta$ S2 Vaccine Trial



# Variation in EIAV SU

- Establishment of persistent infection
- Critical role in vaccine efficacy (related strains)
- Limited by structure and function?

# Variation in EIAV SU

		V1											
WYO	MVSIIFYGGIPGGISTPI	ITQQSE--KSKCEENTMFQPY	YNND	SKNSMAESKE--	ARDQE-MNLKEESKEE---	KRRNDWWKIGMFLL	LAGTTGGILWWYEGLPQQHYIGLVAIGGR	[120]					
V70	.....	A.....G.....	D.....	.....	.....	.....	.....	[120]					
V26	.....	A.....G.....	D.....	.....	.....	.....	.....	[120]					
LN	.....	TKSTDTQKGDHVMVY...	DSH.AE...	ARD---T.Y.E.R....	DN---N.....	L.....F.....	QKHS.....T....	[120]					
CHVax	.....C.....V.....	T.STDTQKGDHVMVY...	DSH.EE...	ARD---T.Y.E.R.-D..D----	N.....	L.I...F.....	RQQYSY.....T....	[120]					
PA	.....	A..HQEINTRD.D.AV...	IDGN.GK...	GRD---P.YS.DK.....	DYDEE.GKK.....	L...V...S.....	RVHTTSF.....M...	[120]					
MY2011	.....	SSE..QADTDKRGPMV...	GSN.RR...	EGKQIPPEER.ELFQG..L.K.----	.....	L.....F.....	QEHS....P..V..G	[120]					
		V2											
WYO	LNGSGQSNAIE	NGSFPG	RPFQNYFSYETN	RS	SMHMDNNT	ATLLEAYHREITFIYKSS	TDSDH	QEQY	KKVNL	NSSDSSNSVRVEDVMNTAEY	WGFKWLE	NQTENFKTILVPENEM	[240]
V70	.....	.....	.....	I.....E.-G.....	.....	.....	.....	Q...VSE-----	T.....	.....	L.....	[240]	
V26	.....	.....	.....	I.....E.-D.....	.....	.....	.....	Q...VSE-----	T.....	.....	L.....	[240]	
LN	....MTS.....	.....	T.....	TISR.....	.....	D.Q.V.N..RT..V.....	K.K.Q.Q.RENS.NIIMNCSNNSCE.F...	S.....	AI.....V..	[240]			
CHVax	....MTS.....	.....	S.T.....	IVSR.....	.....	D.Q.V.N..RT..V.....	K.K.Q.Q.EKNSNNIINNCSNNSCE.F...	S.....	AI.....V..	[240]			
PA	....DLT.....	.....	T...R.G...TIYY..D.-...	H.Q.V.Y...T.D....	D...Q..ITENN.GLALTESNSS---	IF.D.E.....	A.....KD..	[240]					
MY2011	....MTS.....	.....	S.....	N.....	KTINI...A...Q...K.GYL..T..V.....	T...Q.FDADKI.NG-----	STTI..ITIIISP.NL...L..KA...V...Q..	[240]					
		V4											
WYO	VNINDTDT	WIPKG	NETWARVKR	PIDILYGIHPIRL	VQPPFFL	VQEK--GIANNSRIGN	SGPTIFLGVL	EDNKG	VVRG-NSTA	NVRRLNINRKDYTGIV	QVPIFYT	NETNITS	N-
V70	....S.....	.....	.....	.....	.....	N--RA.NN..S.....	.....	I...-NS.T.E.NKKE.K.....	N.....	.....	.....	.....	
V26	....S.....	.....	.....	.....	.....	N--RA.NN..S.....	.....	I...-NS.T.E.NKKE.K.....	F.....	N.....	.....	.....	
LN	Q*-S.NN.....	R.....	H..M.L...NR..M.....	FKQN-DTSNN..ILS...	LV...I.....	AAIQ--NGS	TLH.T..K.P.S.F.....	I...NL.GLQ..	.....	.....	.....	.....	
CHVax	Q--QRKN.....	R...KK.....	H..M.L...NR..I.....	FKQN-DTSNN..ILS...	LV...I...N..AAIQ--NGS	TLH.T...L.S.F.....	I...NL.GLQ..	.....	.....	.....	.....	.....	
PA	.E-WGN...R.....	.....	H..A.L.....	TNFNNDSDSNNTV.S...	L.R..I..E...	ISEYSNNN	S.AKKSFQ.P..S.T.....	L.E.HLN-LS..E-	.....	.....	.....	.....	
MY2011	...-ISNS.Y.....	.....	K..H.....	L.....	RKN---NDTNHTLS..QV.....	D...T..KS--EEK	KII.F..K.Q..F.Q.IT.V.N	TLN-SR..Q	[360]				
		V5											
WYO	-NESIISVIMYE	TNQVQYLL	NNNNNSNNYN-----	VQSGFVIGQAHL	ELPRPNKRIRNQSFNQYN	SINN	KTELETWKLVKTSGITPLPISSEANTGLIRHKR	[470]					
V70	...S.....	D.....	N.....	.....	.....	.....	.....	[470]					
V26	-S.S.....	D.....	N.....	.....	.....	.....	.....	[470]					
LN	-GS...I..S.S.N.....	TS.TNSTN.A----	TVS.....	VA...K...LQSPK.AH..T.....	RQ.Q.....	T.....	V....	[470]					
CHVax	-GS...I...S.N.....	TS.TNSTN.A----	NIS.....	VA...K...LQSPK.A..T.....	RQ.Q.....	T.....	V....	[470]					
PA	-GNSTV.I.R..Q.N.....	RG.DTKT,-----	YS.....	T.....	K...E.PR.TY.....	KS.....	V.V...K.....	[470]					
MY2011	K.NKSV.....	SGNI.....	V..EN..I.ITDAIKNYI	IA...K.....	T.G.KQ.N.TR.....	DQG.MRK.T.....	V..SD.S...Y..	[470]					

Dong J-B, Zhu W, Cook RF, Goto Y, Horii Y, Haga T (2012)

# Lesson Learned

- Lentiviruses: complex  $>12 \times 10^6$  YR
- Few mammalian hosts – Extinct
- EIAV : Least complex genome
  - Successful/Persistent
  - Integration
  - MØ host cell
  - Neutralization Ab resistant
  - Antigenic variation

# Lesson Learned

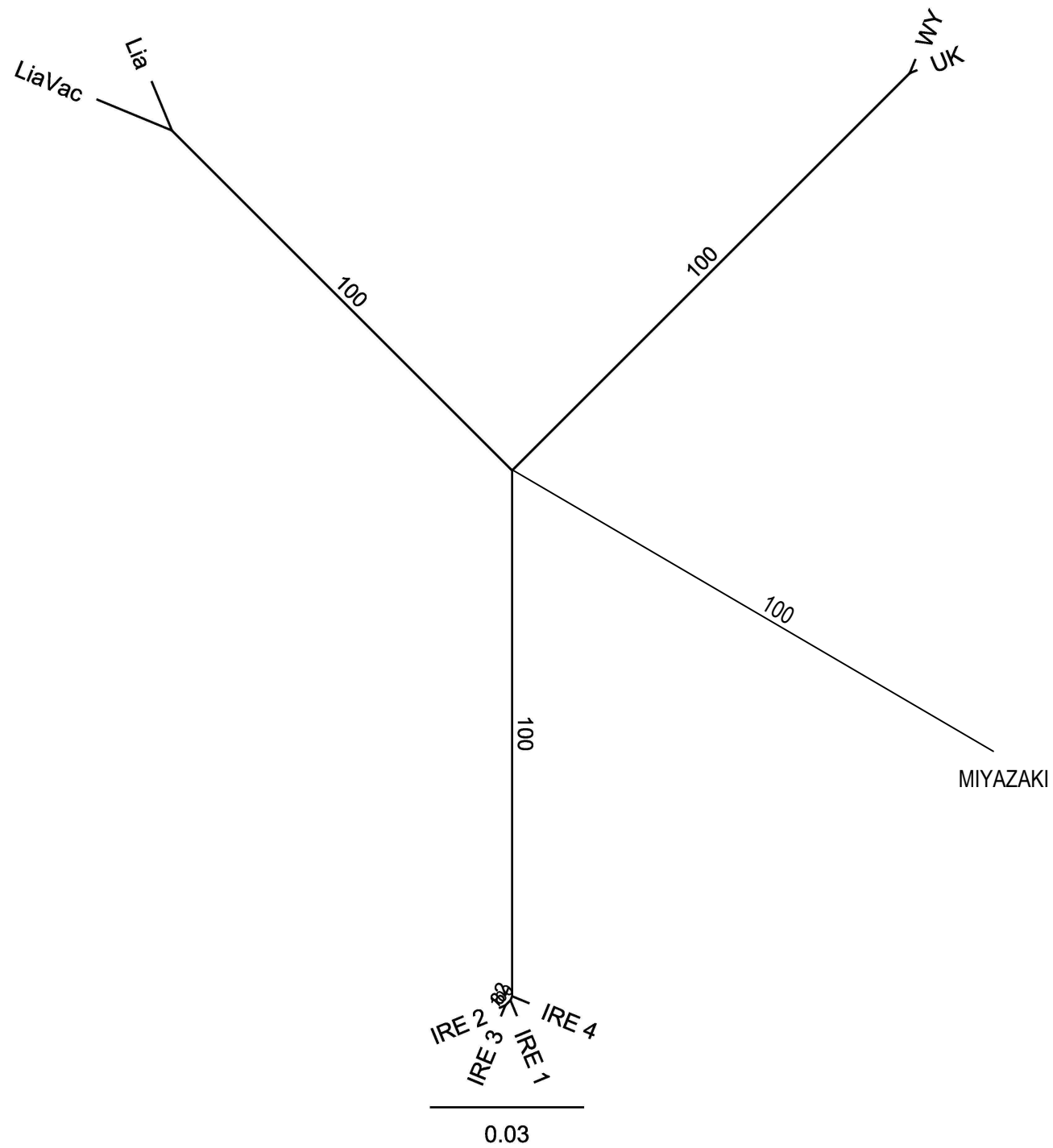
- Disease = High Viral Loads
- Inapparent Carrier = Low Viral Loads
  - Immunological Control
  - Horse = Model for Elite HIV Control?
- Vaccine Efficacy : Variation in SU

# Lessons to be Learned

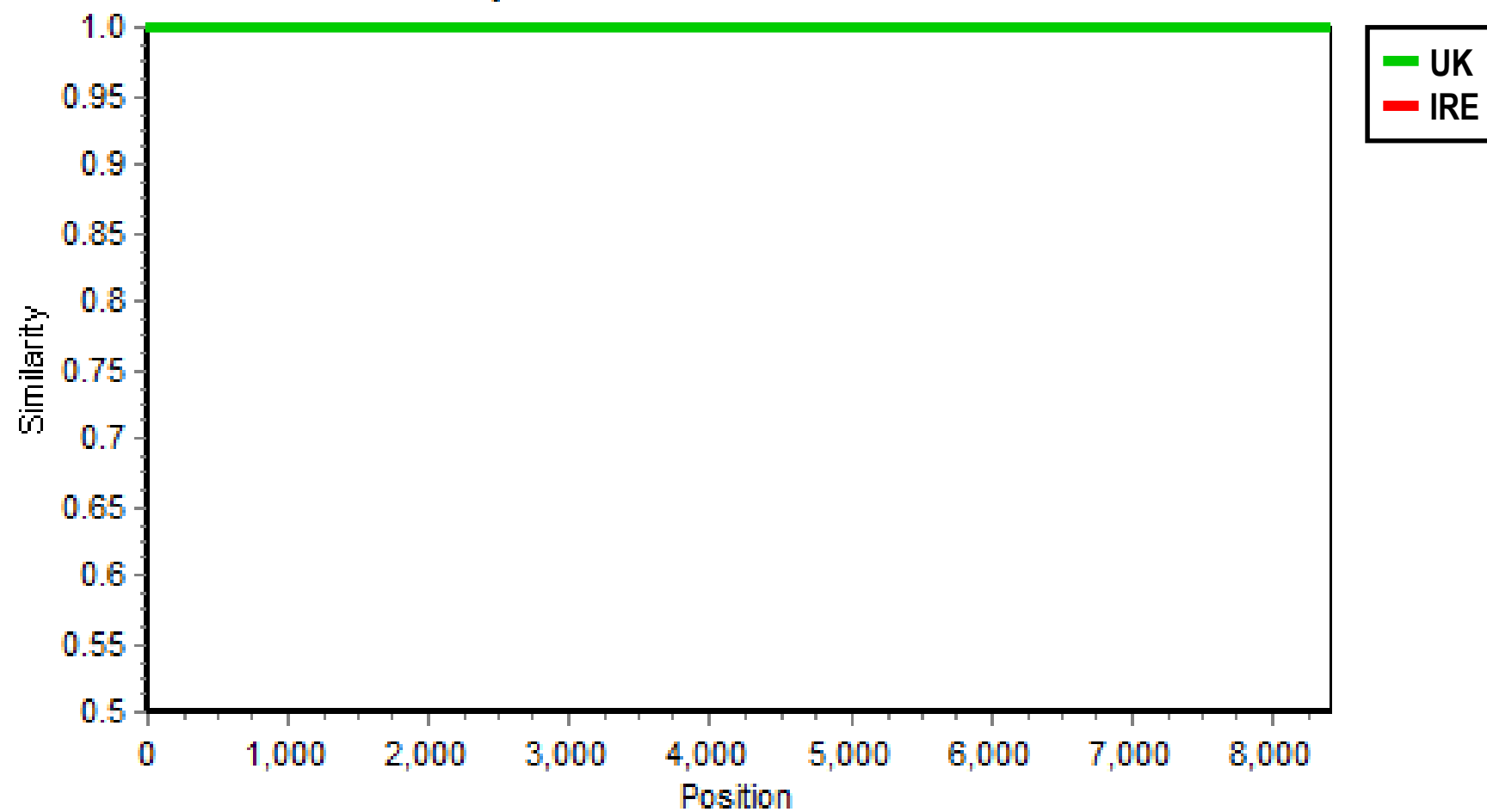
- ? Extent of EIAV Diversity
  - ❖ Molecular Diagnostics
  - ❖ Vaccines
- ? EIAV Persistence Mechanisms
- ? Evasion of Host Restriction factors
  - ❖ NO VIF/VPU

# Lessons to be Learned

- Immunological Control Mechanisms
  - ❖ NO Simple correlations
  - ❖ Other mechanisms
- ? Differences between Horses in Disease and Control
- ? Differences between Equid species



SimPlot - Query: AF016316\_UK  
FileName: D:\My Documents\EIAV IRE\UK vs IRE.fasta



Window: 200 bp, Step: 20 bp, GapStrip: On, Kimura (2-parameter), T/t: 2.0

# Acknowledgements:

Dr. Takeshi Haga, University of Tokyo  
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Gluck Equine Research Center.

