Comparative Analysis of Measures of Viral Reservoirs in HIV-1 Eradication Studies

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Disclosures: None

ARCHE
amfAR
MAKING AIDS HISTORY

HIV
CARE | Collaboratory of AIDS Researchers for Eradication

CENTER FOR AIDS RESEARCH
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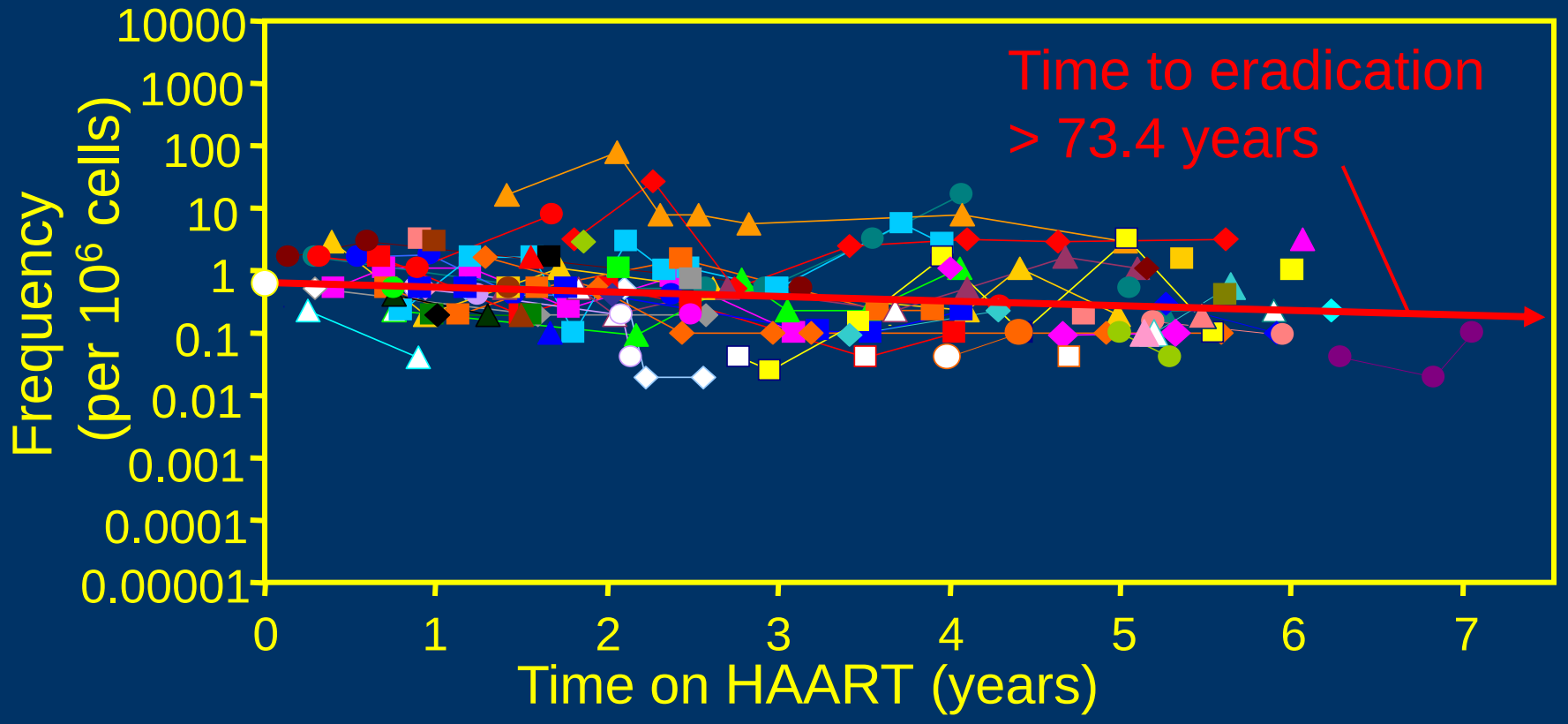
Slow decay of latently infected CD4+ T cells



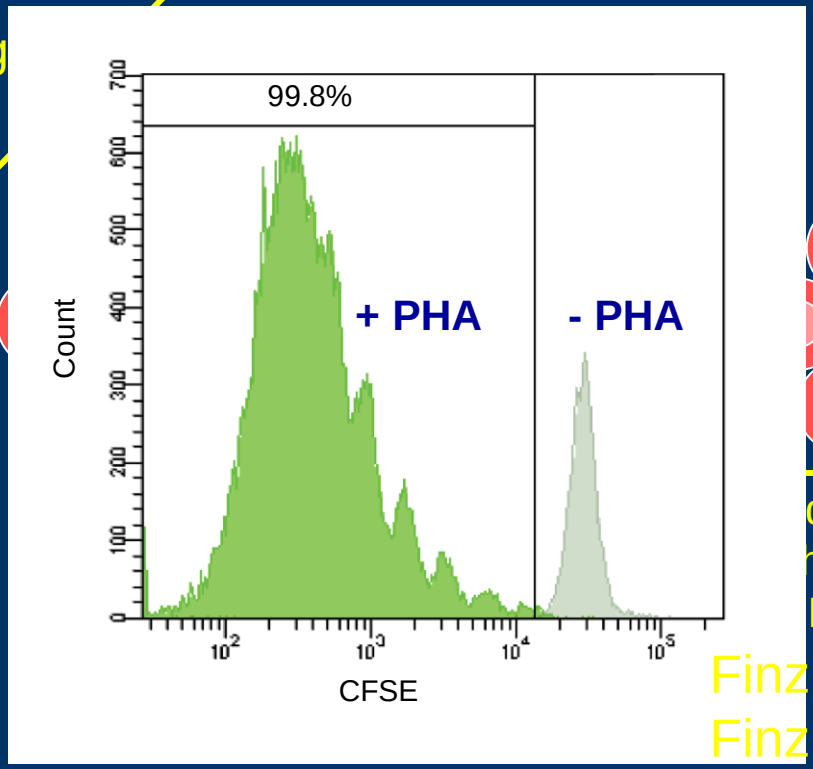
Finzi et al., Nature Med., 1999
Siliciano et al., Nature Med., 2003

Virus culture assay for latent HIV-1 in resting CD4+ T cells

180-200 ml blood

Purified resting CD4+ T cells

5x10^6 | 10^6 | 2x10^5 | 4x10^4 | 8x10^3 | 1.6x10^2 | Negative control

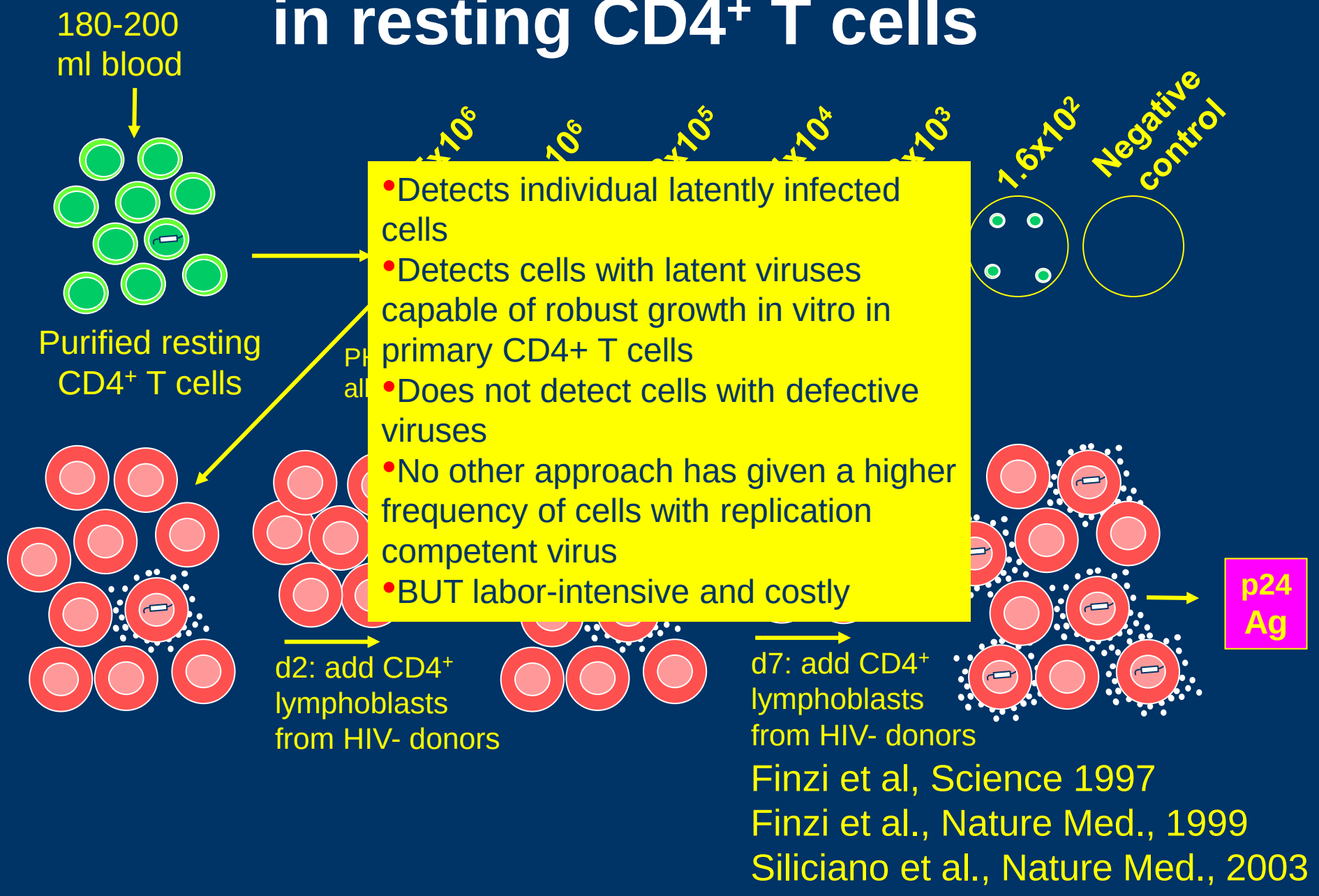


dd CD4+
noblasts
HIV- donors

p24 Ag

Finzi et al, Science 1997
Finzi et al., Nature Med., 1999
Siliciano et al., Nature Med., 2003

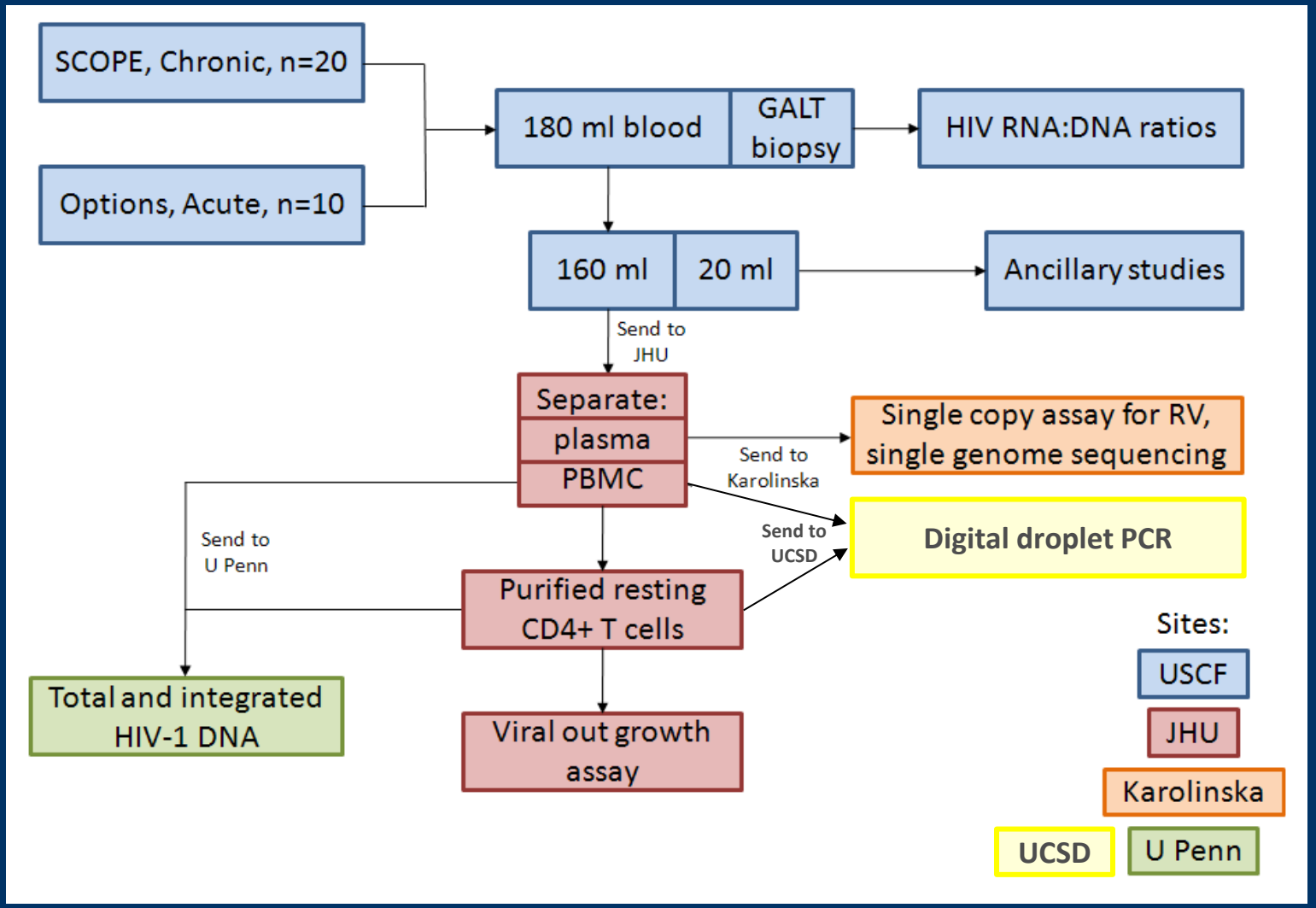
Virus culture assay for latent HIV-1 in resting CD4+ T cells



- Detects individual latently infected cells
- Detects cells with latent viruses capable of robust growth in vitro in primary CD4+ T cells
- Does not detect cells with defective viruses
- No other approach has given a higher frequency of cells with replication competent virus
- BUT labor-intensive and costly

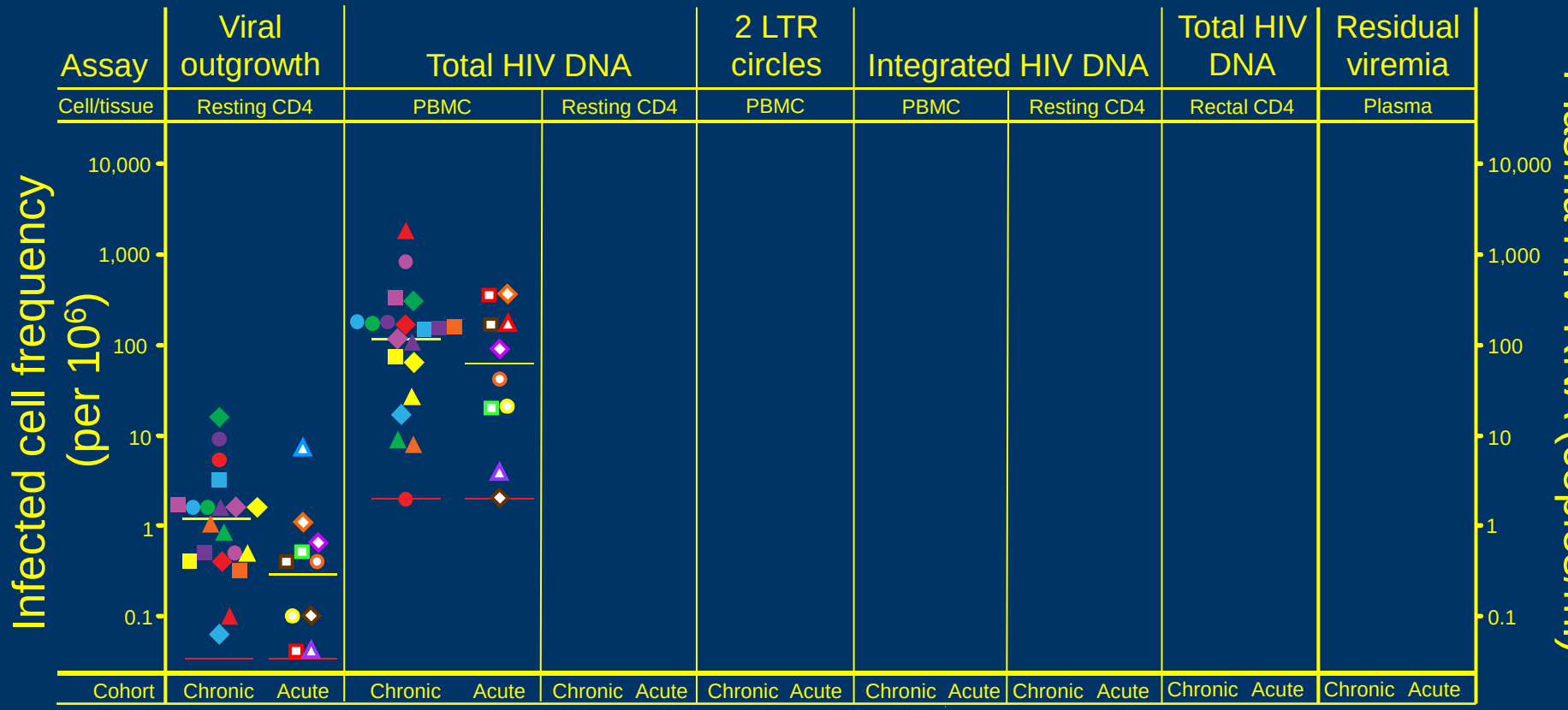
Finzi et al, Science 1997
Finzi et al., Nature Med., 1999
Siliciano et al., Nature Med., 2003

Comparison of reservoir assays



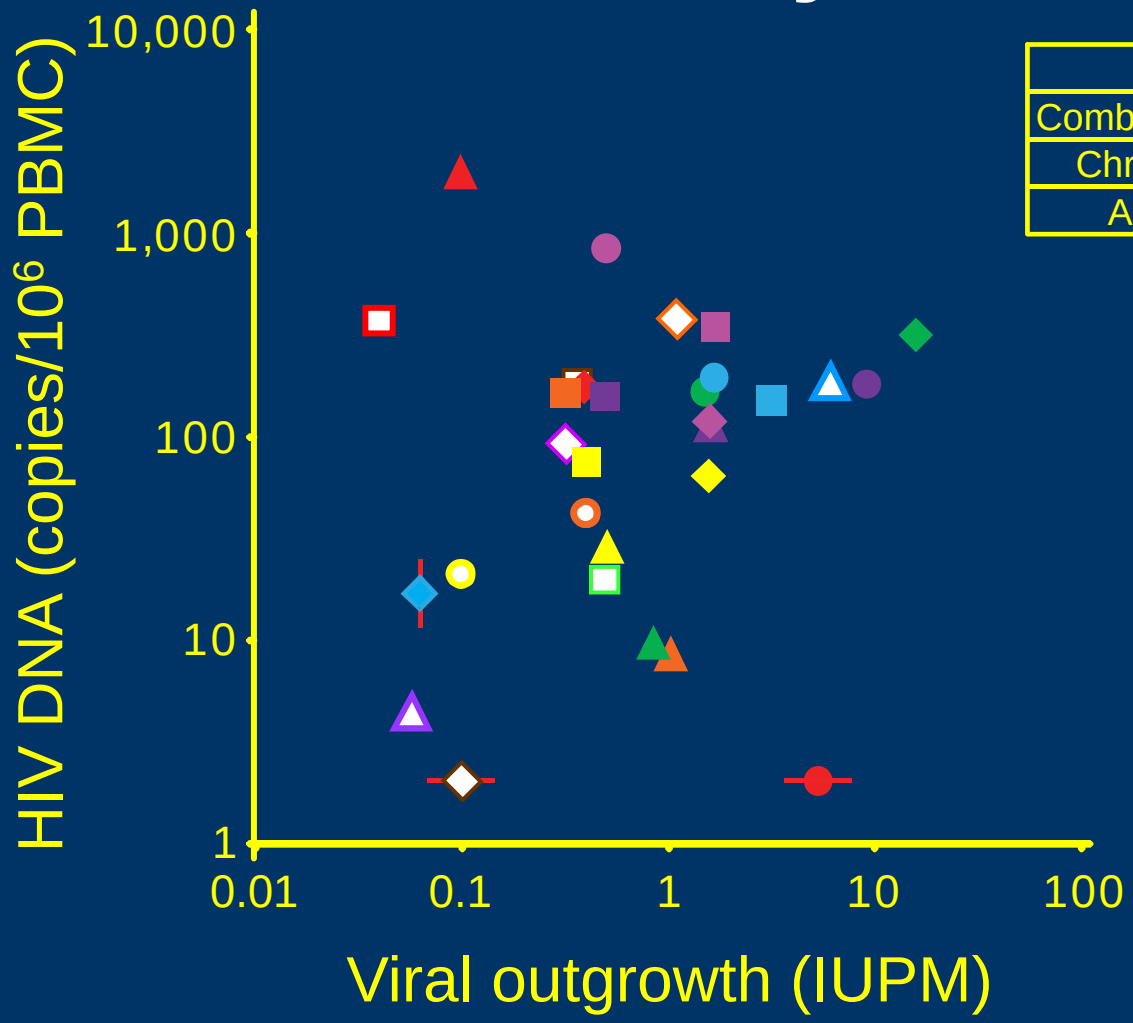
Eriksson et al, PLoS Pathogens, 2013

Assay for persistent HIV in patients on HAART



Eriksson et al, PLoS Pathogens, 2013

Correlation between culture and PCR assays



	r	P	n
Combined	0.20	0.29	30
Chronic	-0.04	0.87	18
Acute	0.46	0.18	20

Eriksson et al, PLoS Pathogens, 2013

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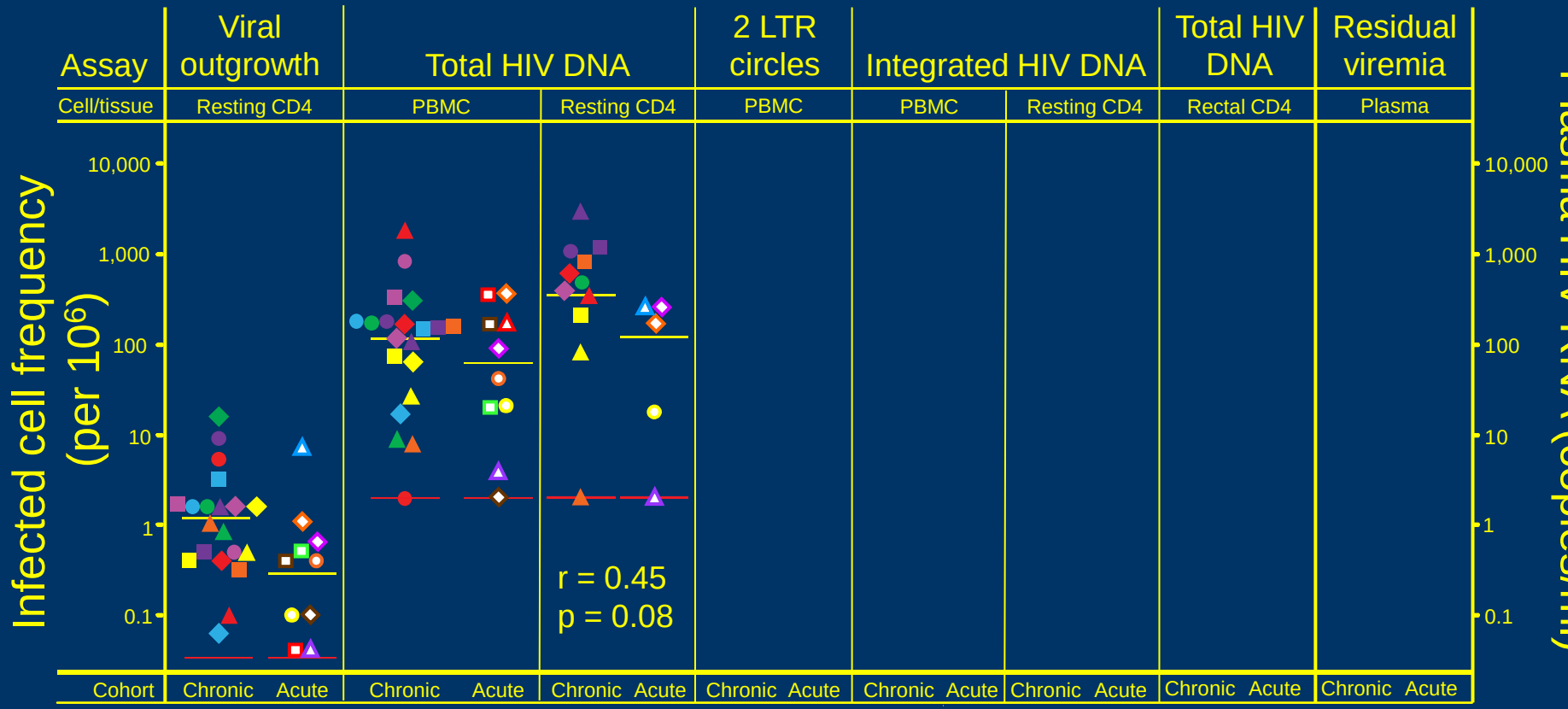
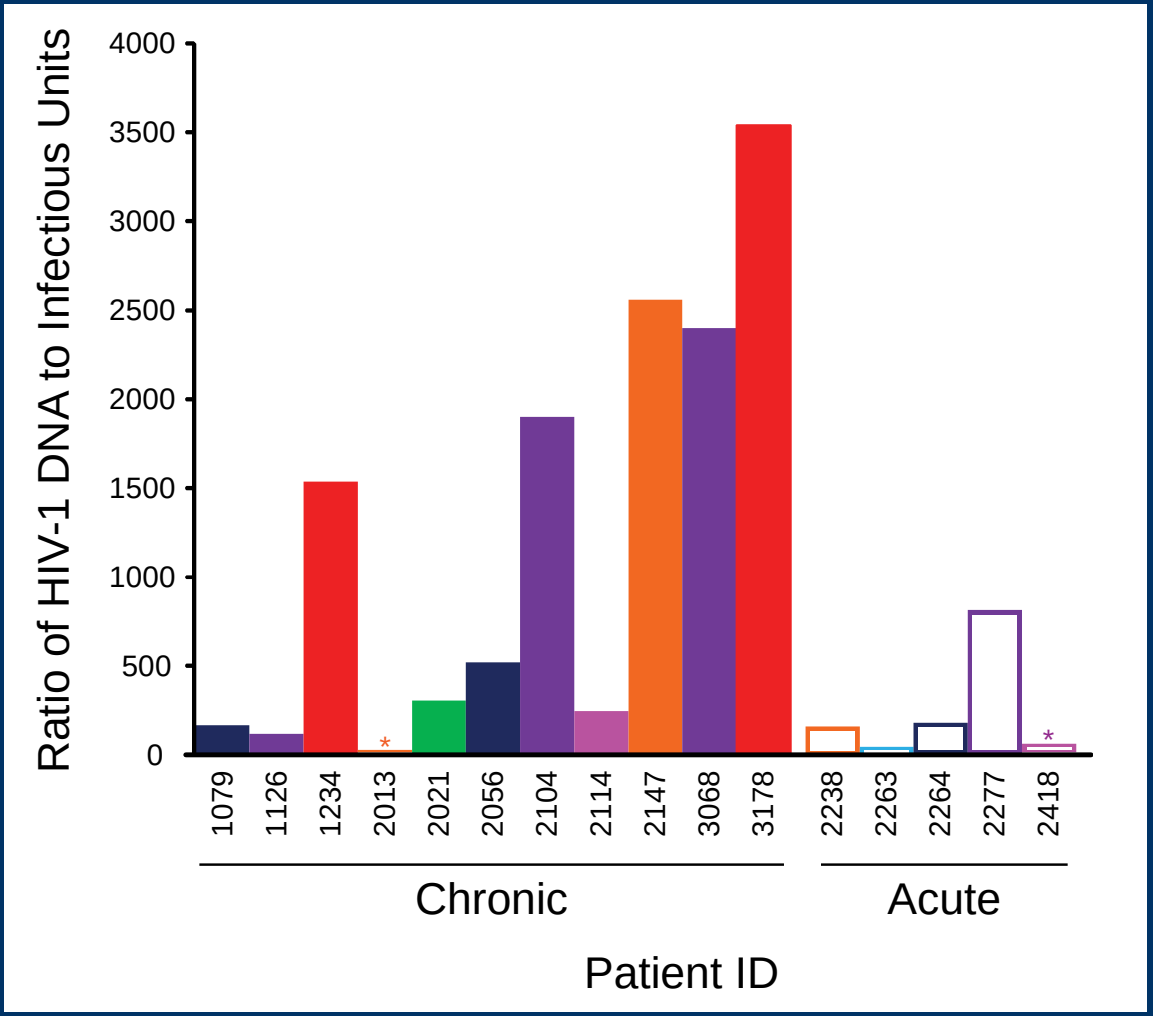


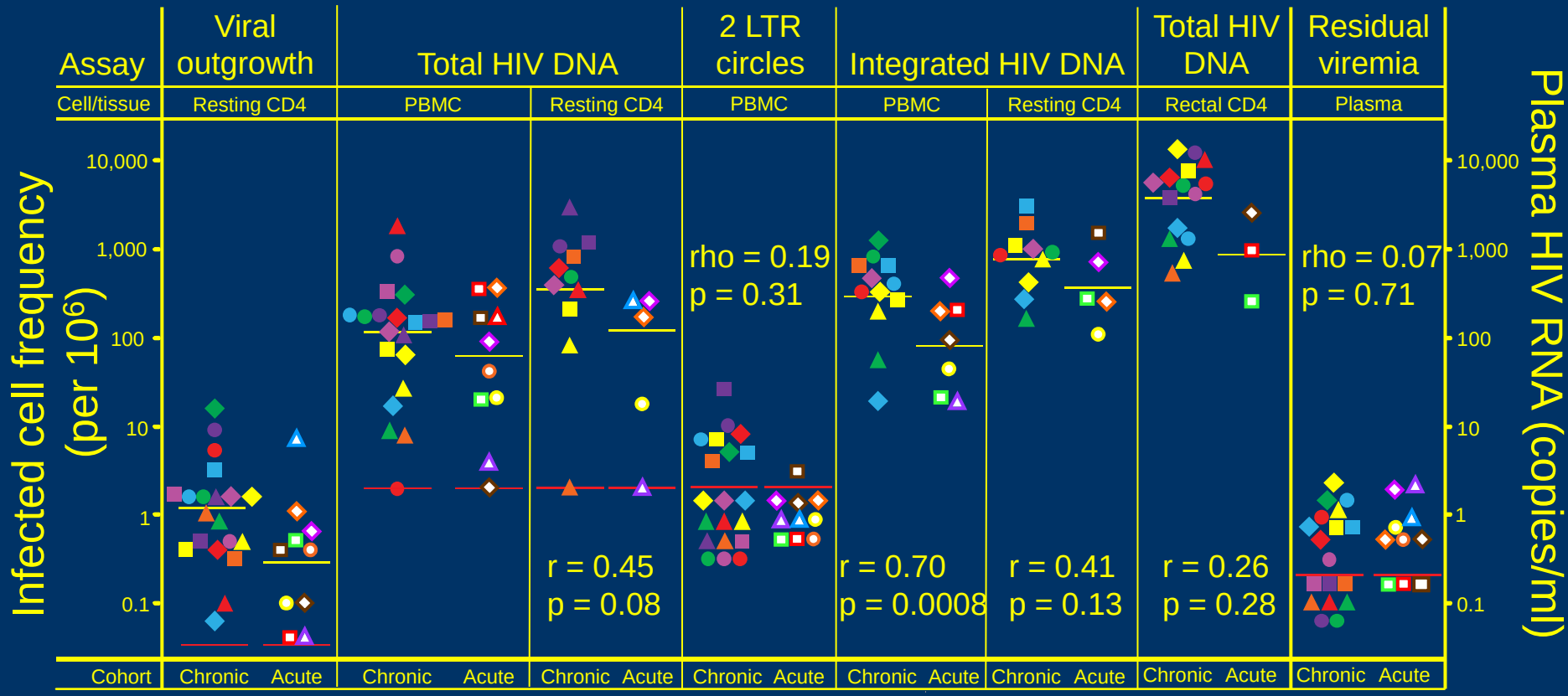
Eriksson et al, PLoS Pathogens, 2013

Ratio of infected cell frequencies by PCR and culture assays



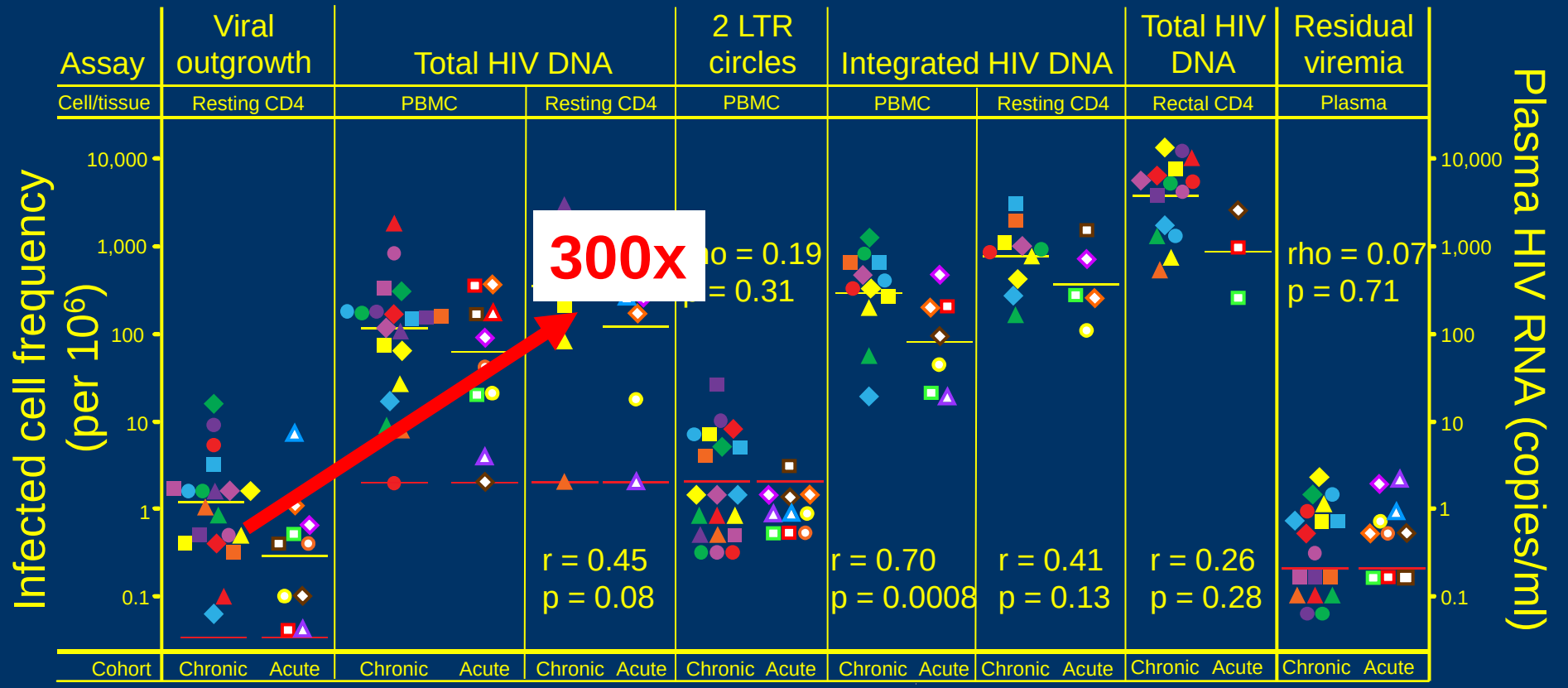
Eriksson et al, PLoS Pathogens, 2013

Assay for persistent HIV in patients on HAART



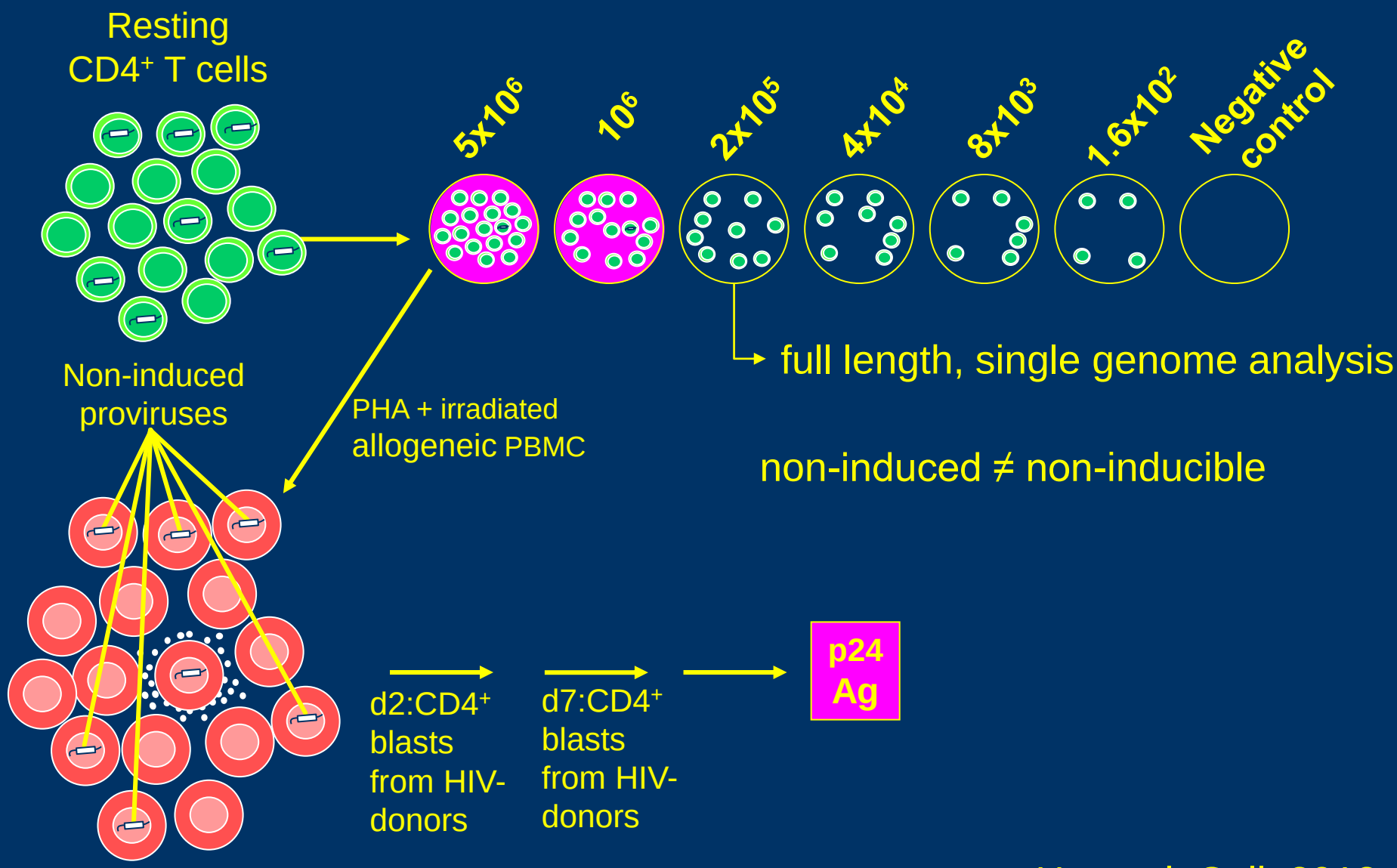
Eriksson et al, PLoS Pathogens, 2013

Which assay should be used?



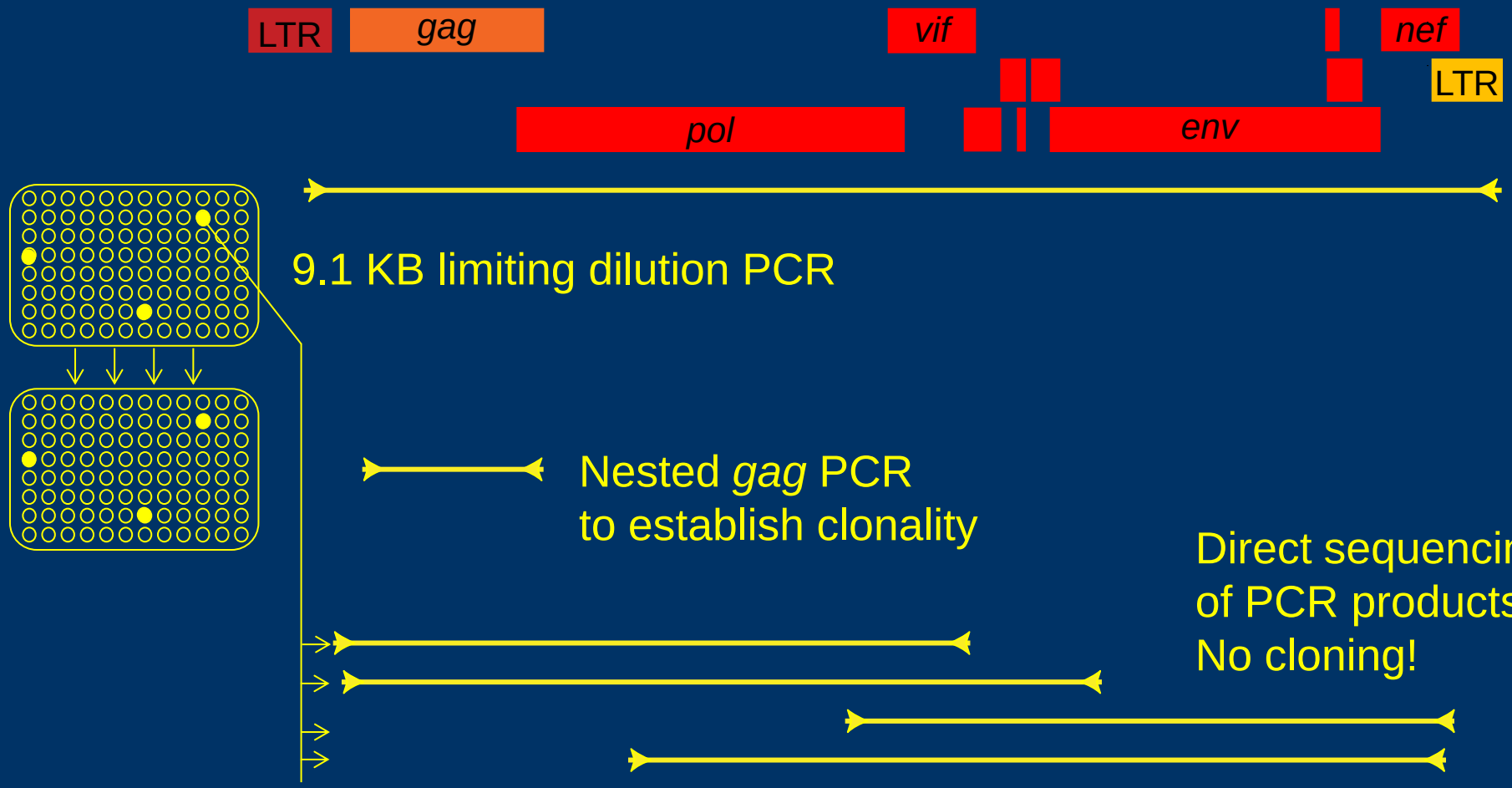
Eriksson et al, PLoS Pathogens, 2013

Non-induced proviruses



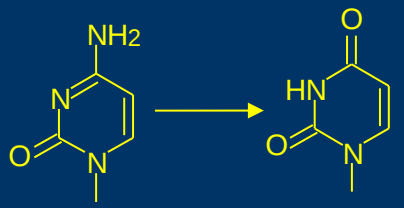
Ho et al, Cell, 2013

Clonal analysis of non-induced proviruses



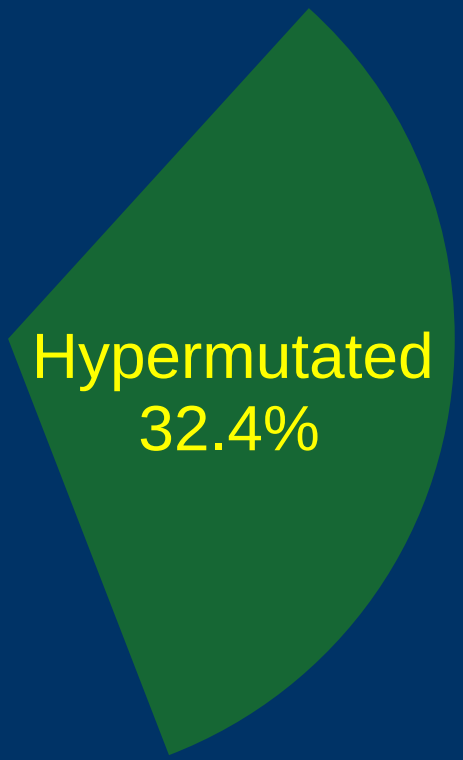
Ho et al, Cell, 2013

Non-induced proviral clones (n=213)



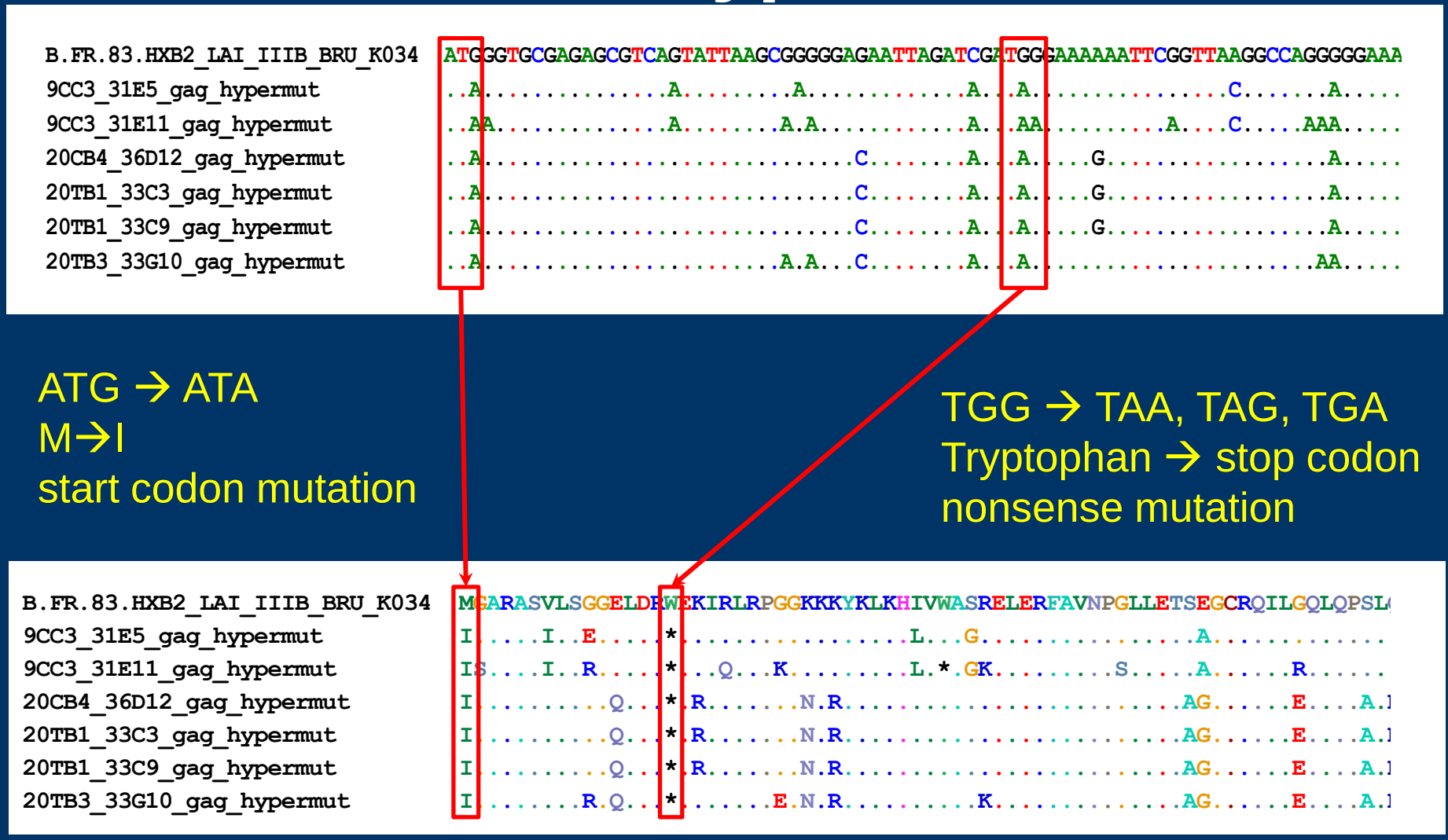
TGG → TAG

Trp Stop



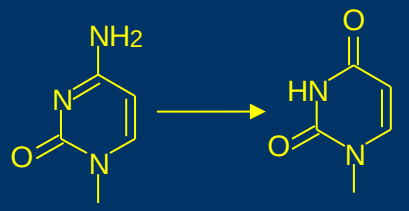
Ho et al, Cell, 2013

32.4% of non-induced proviruses have G→A hypermutation



Ho et al, Cell, 2013

Non-induced proviral clones (n=213)



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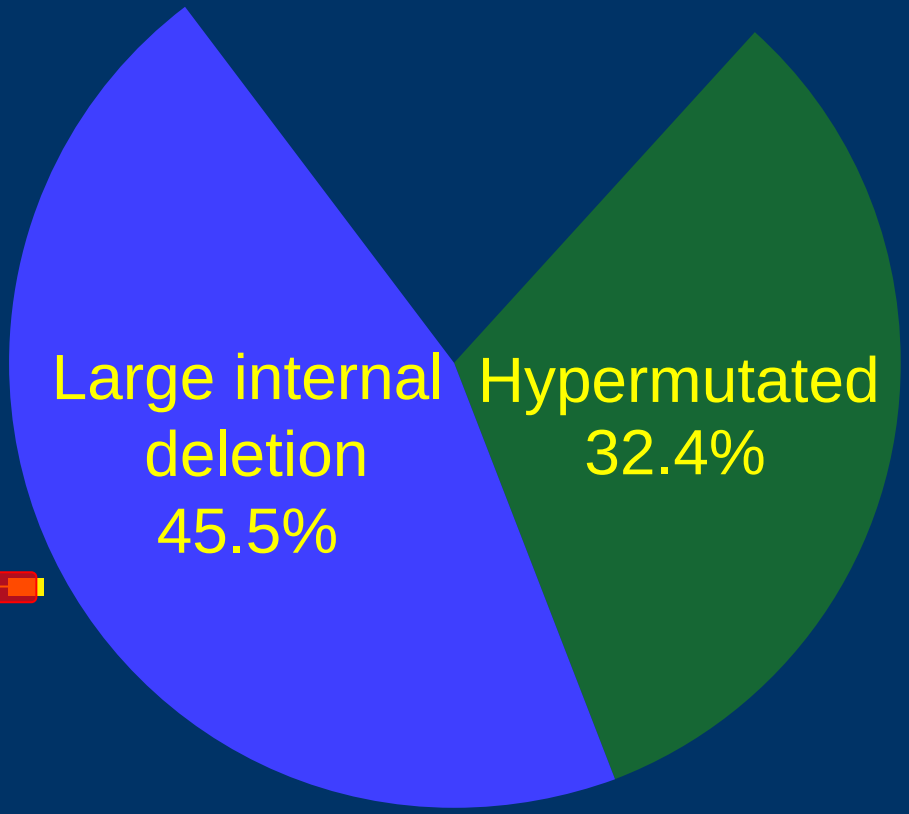
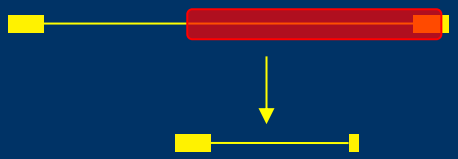
Trp Stop





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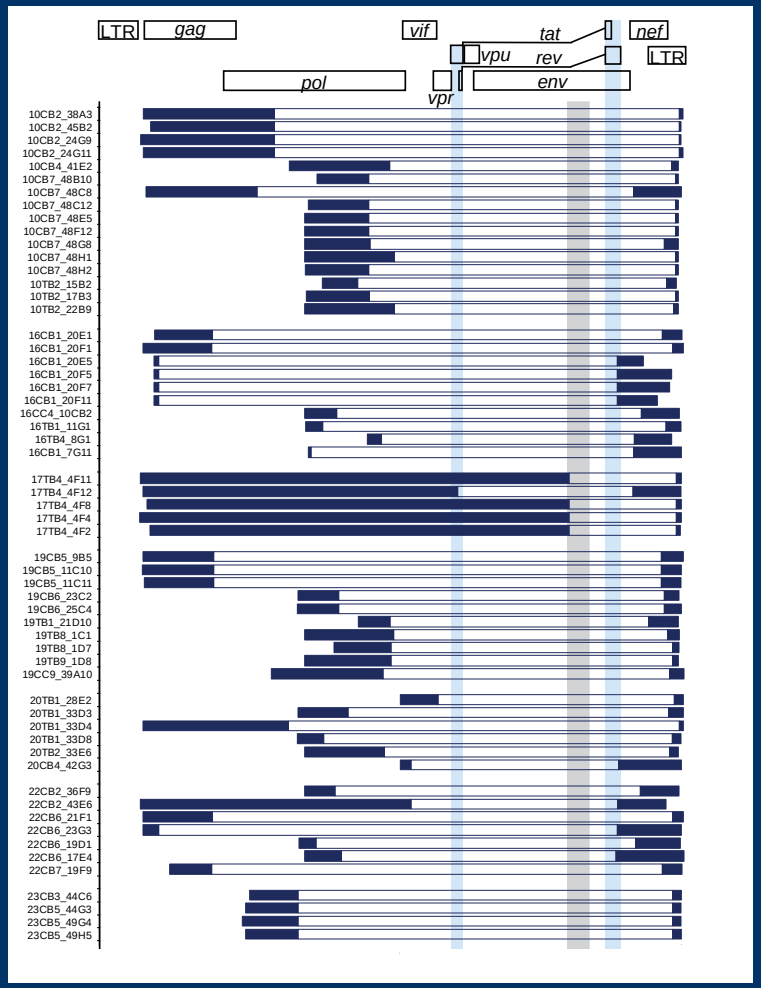
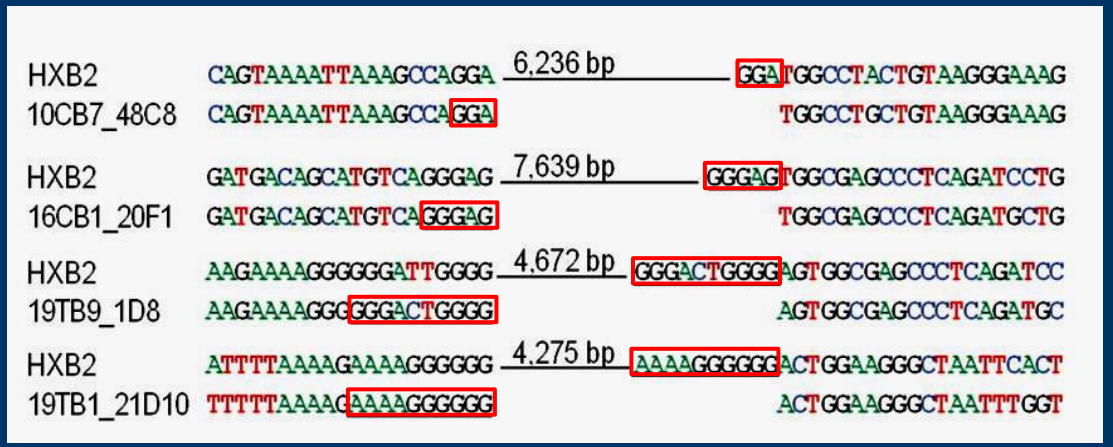
45.5% of non-induced proviruses have large internal deletions





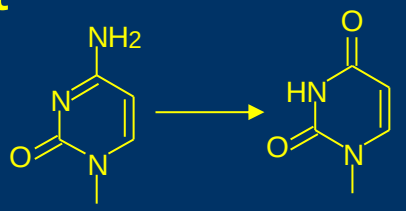
Ho et al, Cell, 2013

Non-induced proviral clones (n=213)

Nonsense mutations/
INDELS 3.8%

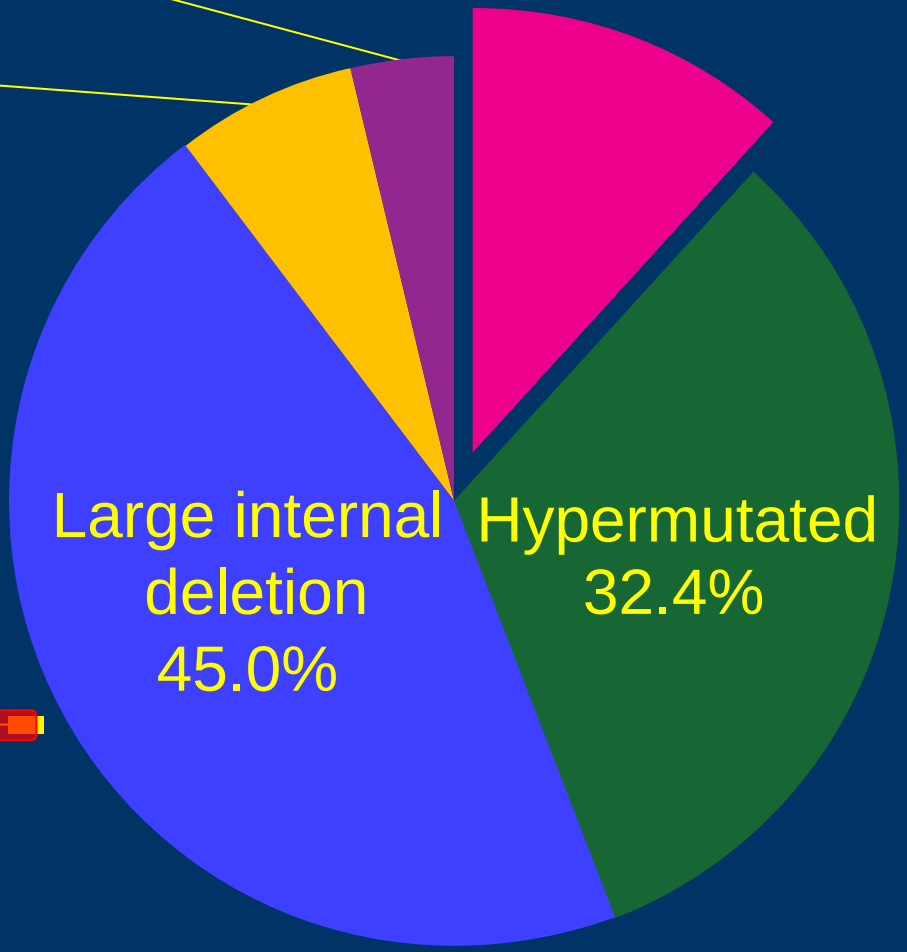
Deletion in ψ/
MSD site 6.5%

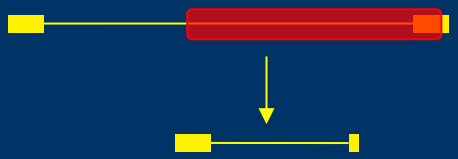
11.7% Intact genome



TGG → TAG

Trp Stop





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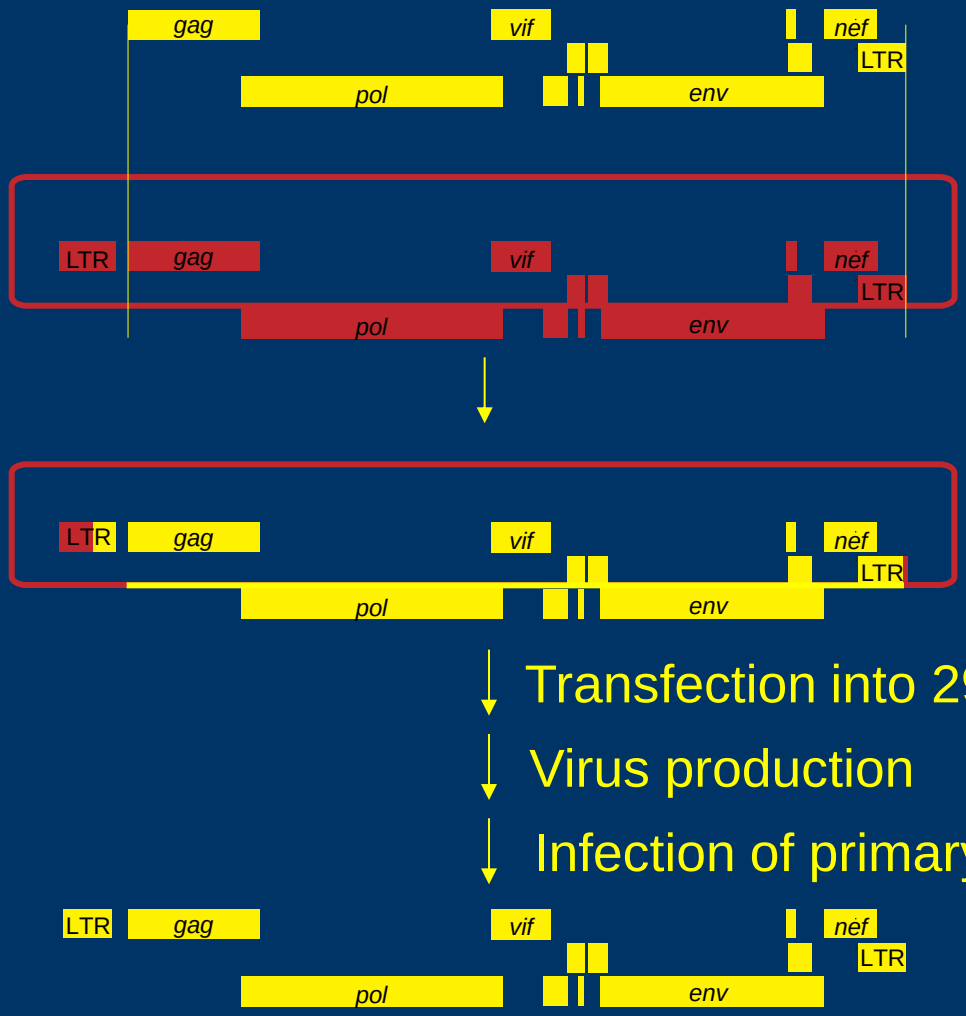
Replication-competence of non- induced proviruses



Non-induced proviral sequence

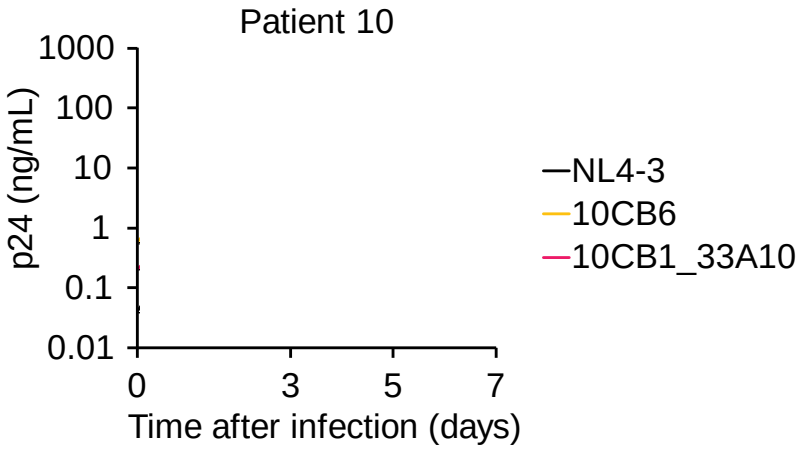
pNL4-3

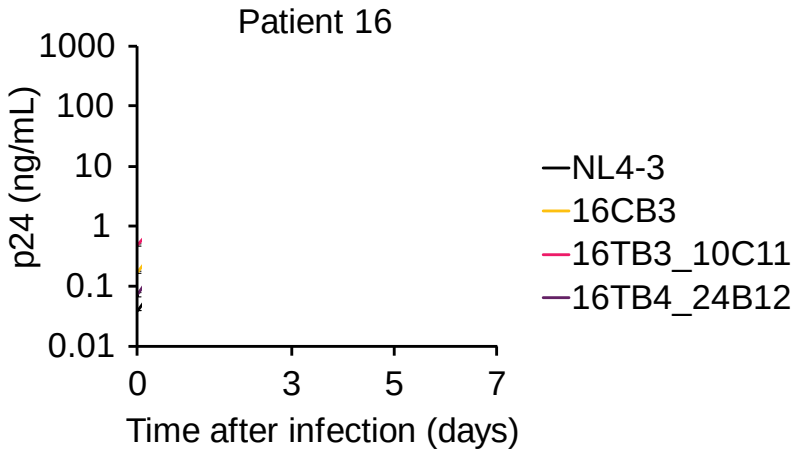
Transfection into 293T cells

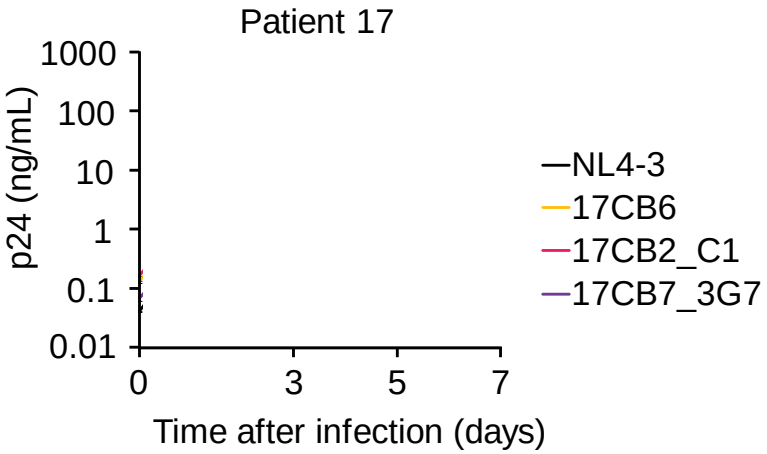
Virus production

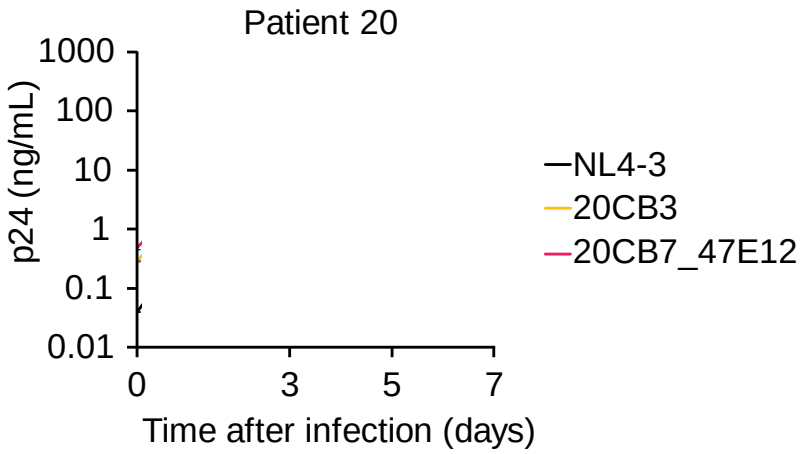
Infection of primary $CD4^+$ T lymphoblasts

Replication of non-induced proviruses clones









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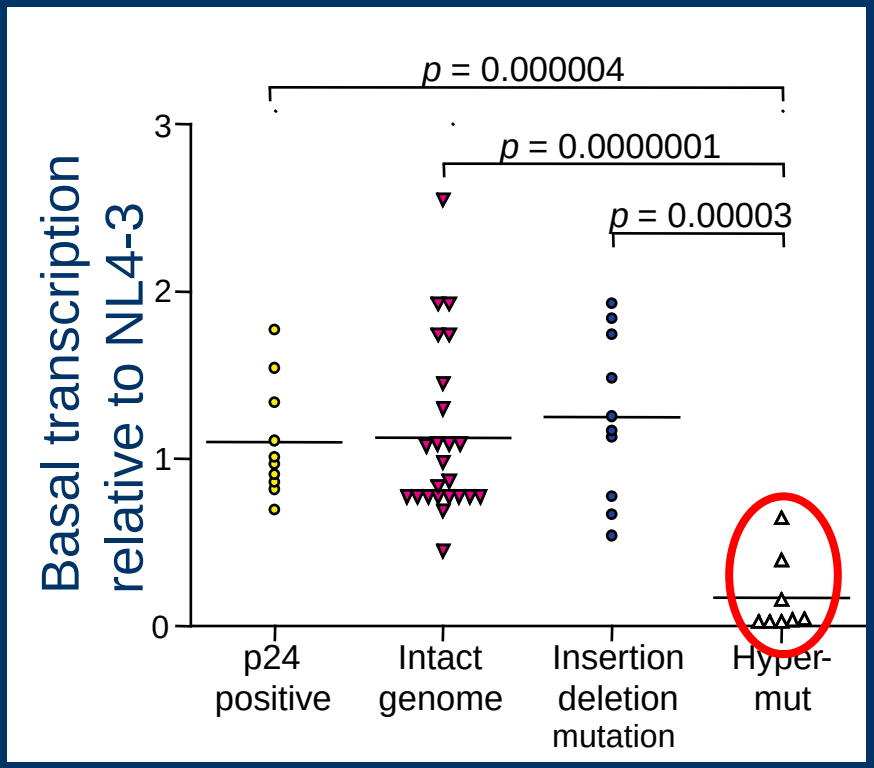
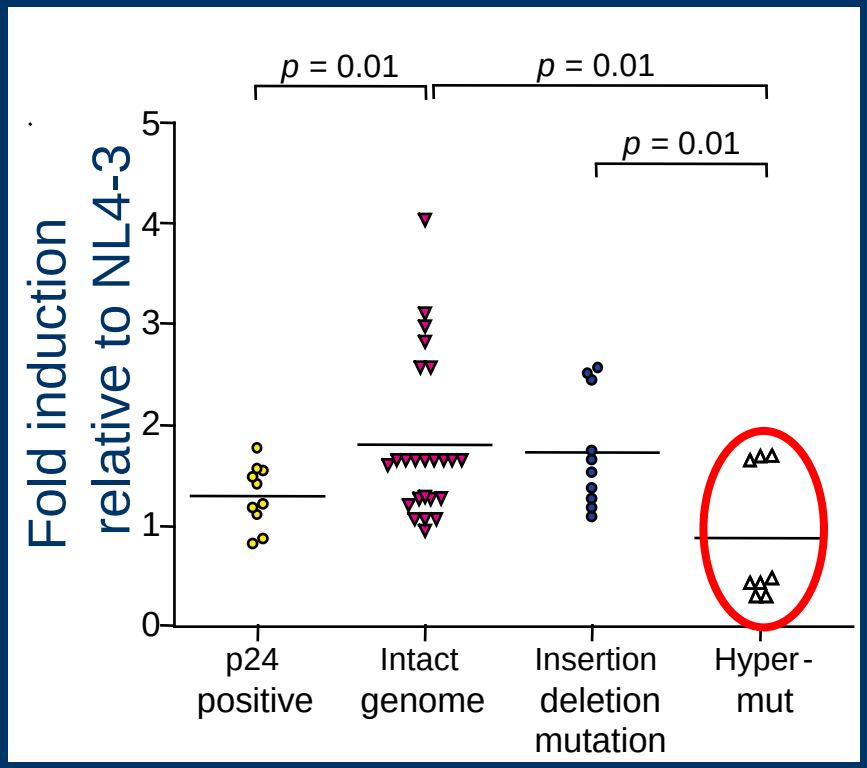
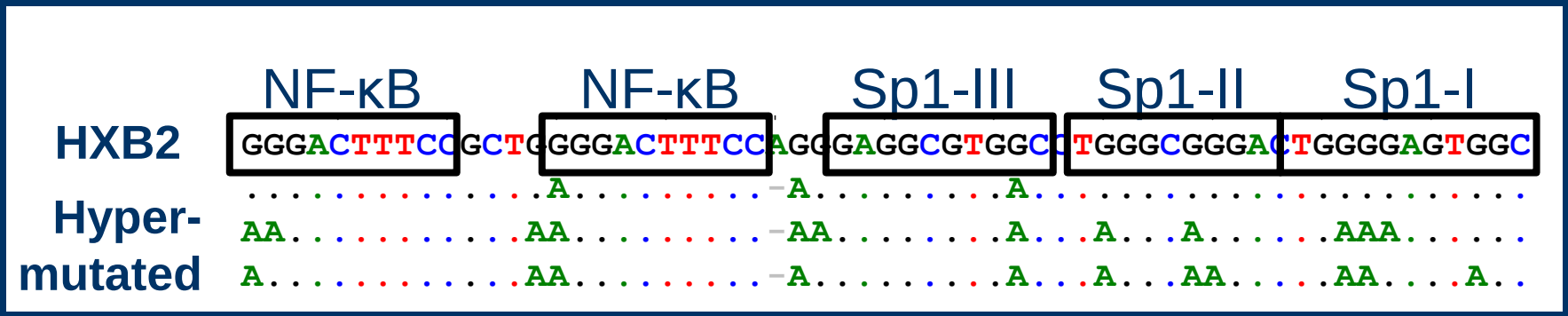
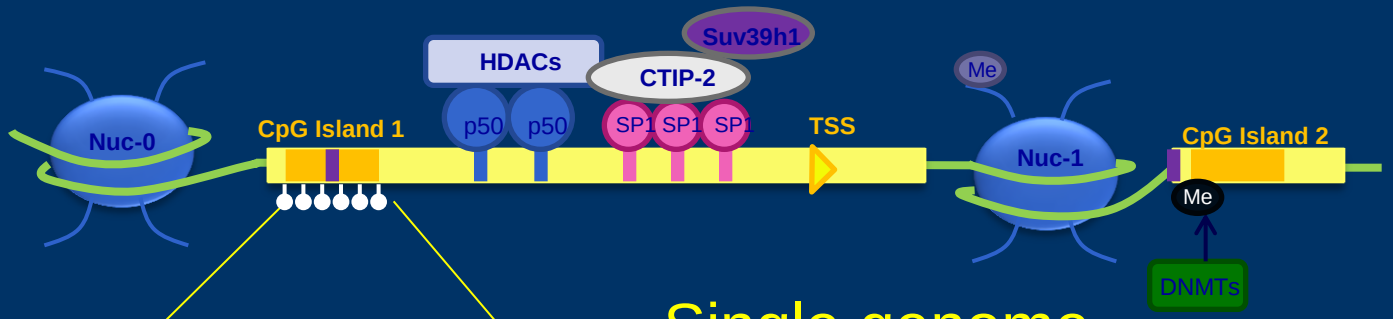
Non-induced proviruses have functional LTRs except for hypermutated clones







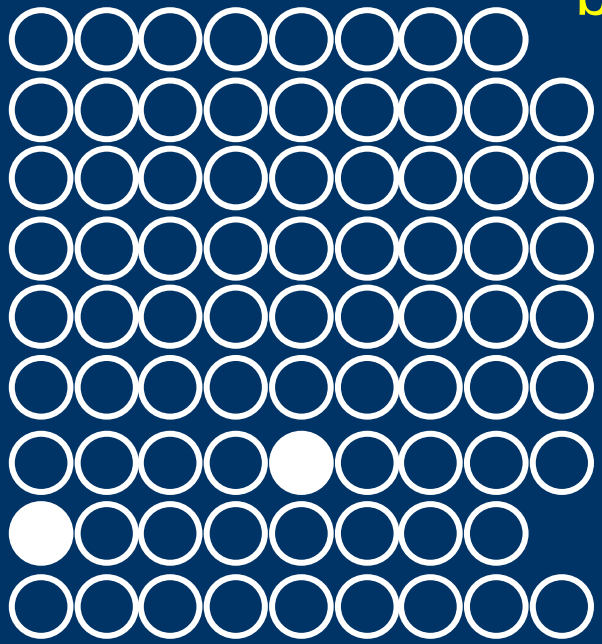
Clonal analysis of DNA methylation



Single genome bisulfite sequencing

Patient 20

Cells from p24 negative co-culture well



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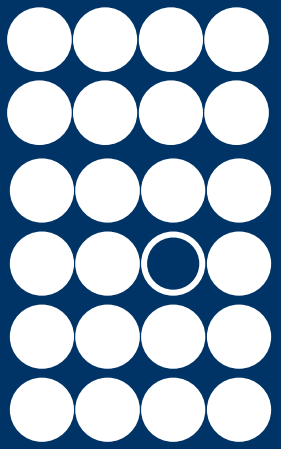

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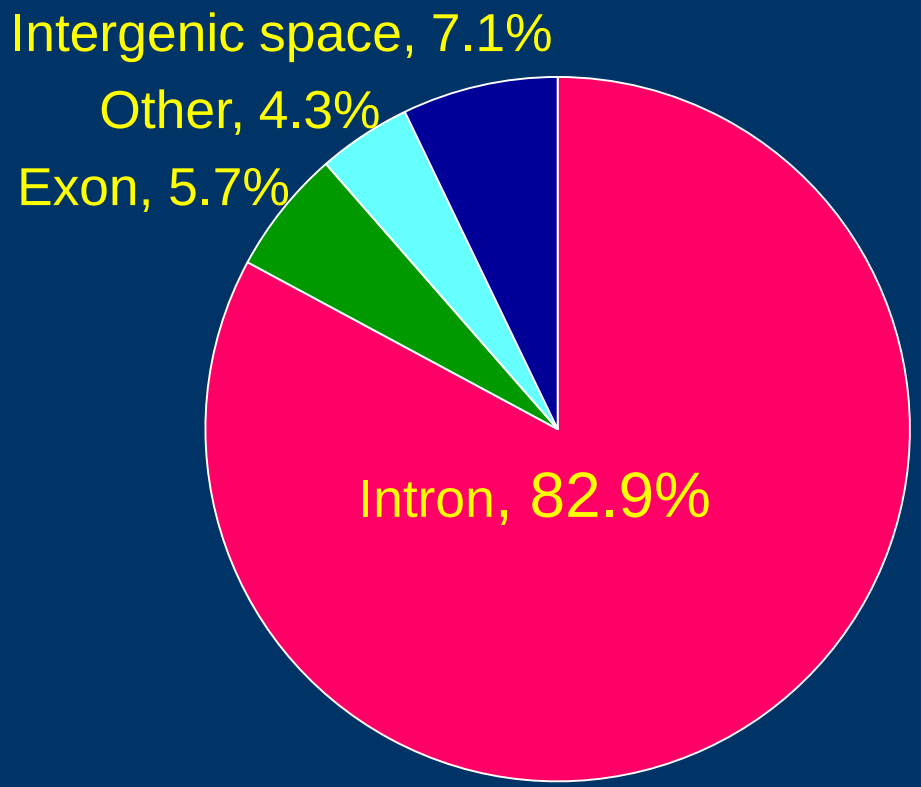
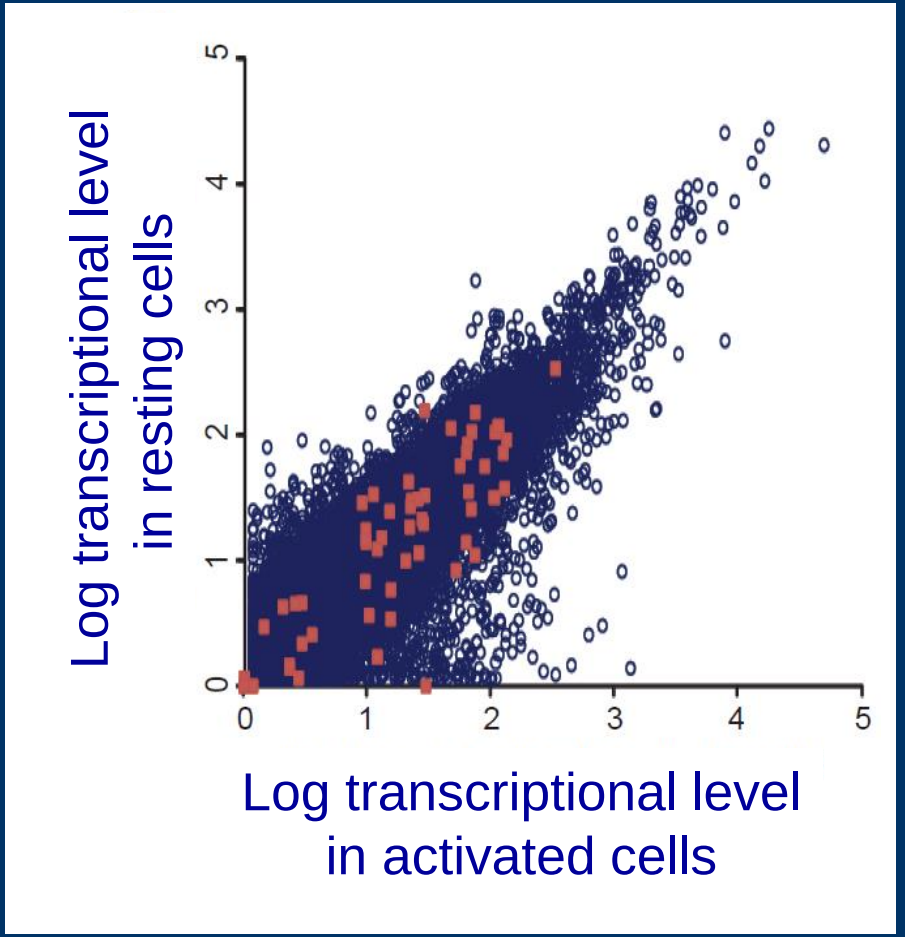
Non-induced proviruses integrate into active transcription units

- Location



Transcription units: 92.9% (65/70)

- Activity of genes



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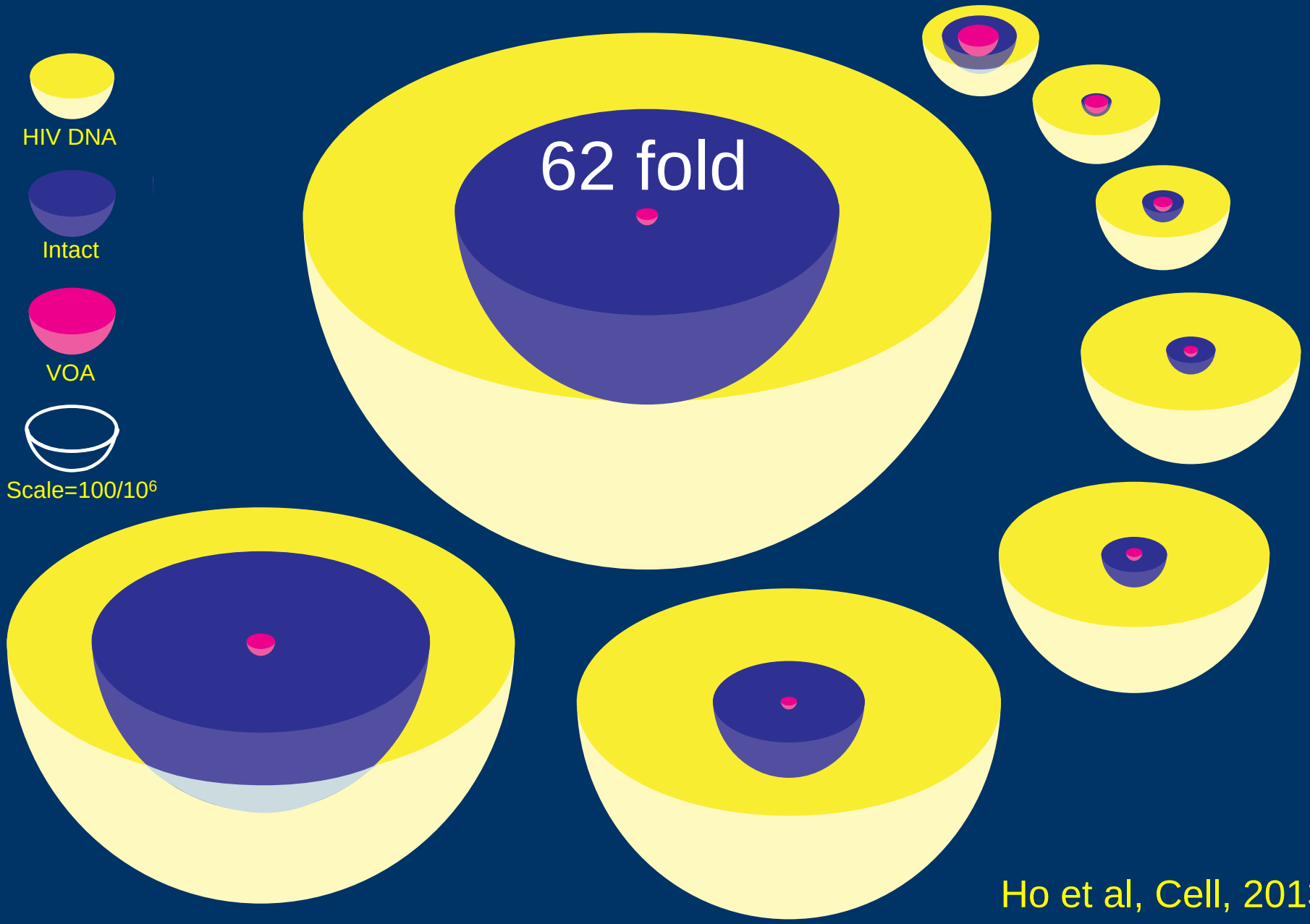
Intact vs induced proviruses



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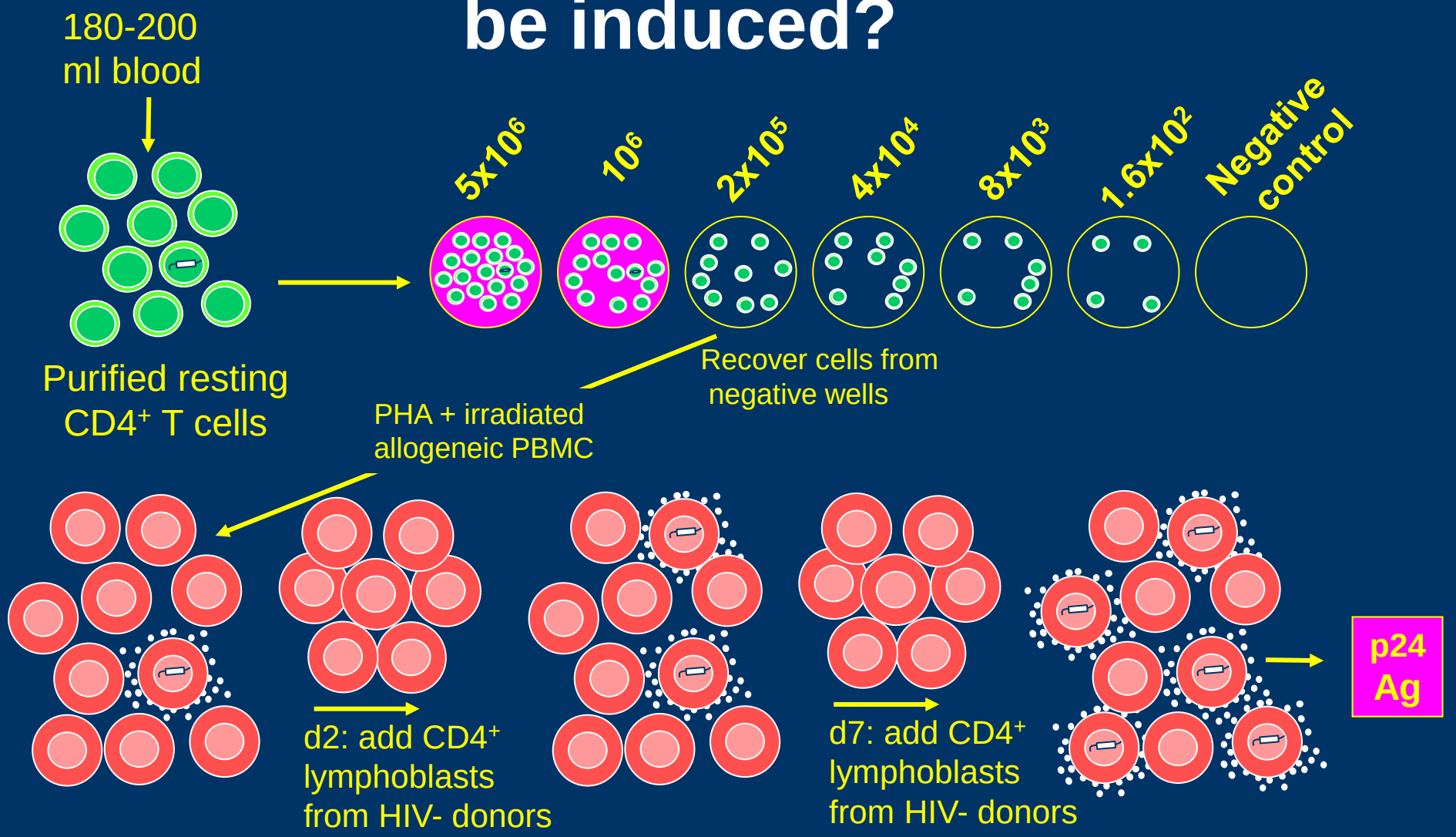
Can intact non-induced proviruses be induced?



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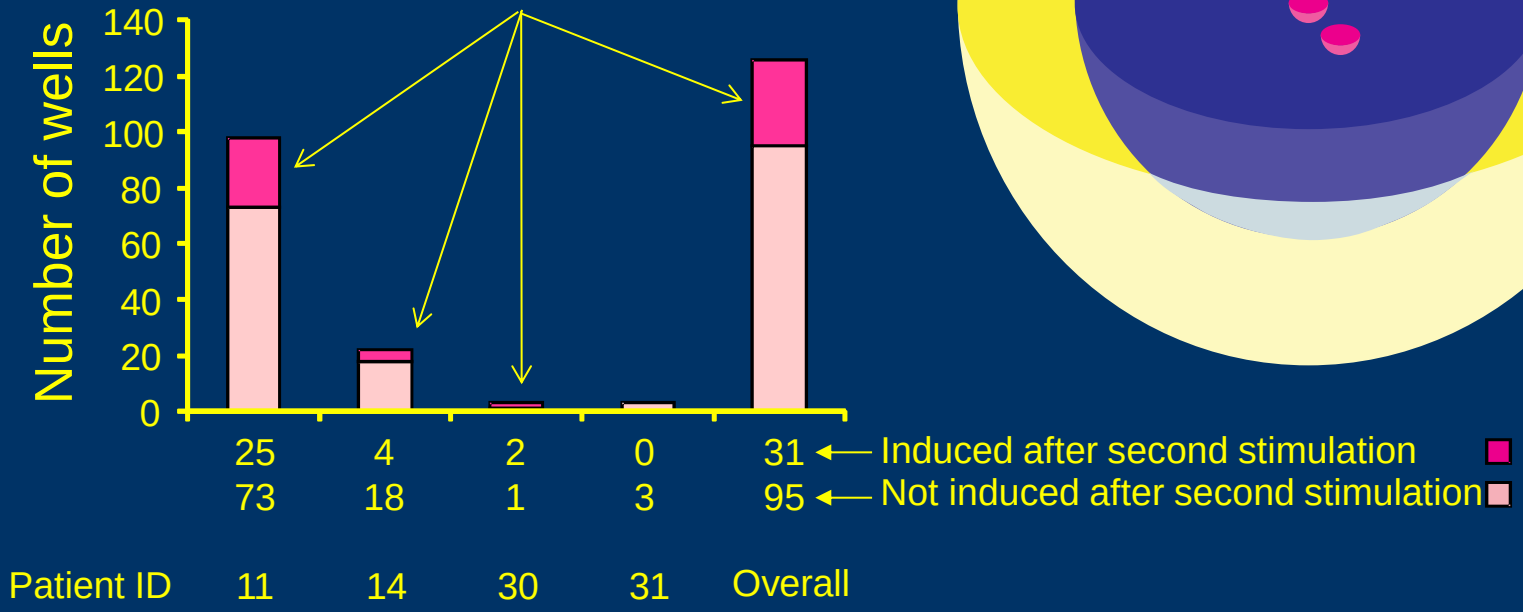
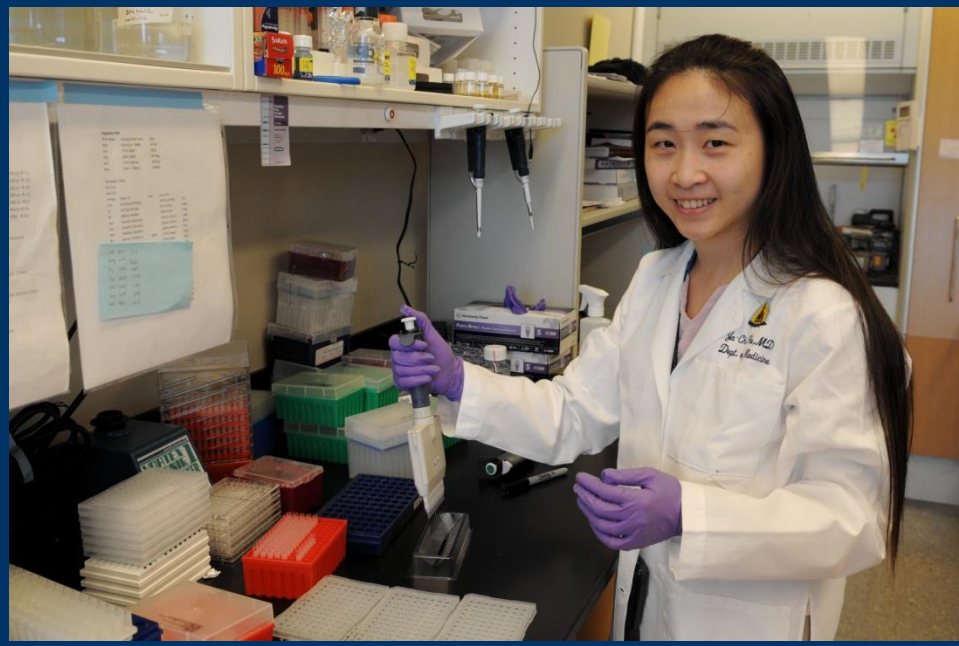


Ho et al, Cell, 2013

Thanks

Collaborators

Robert Siliciano
Steve Deeks
Dave Margolis
Doug Richman
Jon Karn
Martin Nowak
Matt Strain
Sarah Palmer
Una O'Doherty
Joe Wong
Steve Yukl



Funding

Foundation for AIDS Research (amFAR): ARCHE
NIH: Martin Delaney Collaboratories CARE and DARE
Johns Hopkins Center for AIDS Research
Howard Hughes Medical Institute