

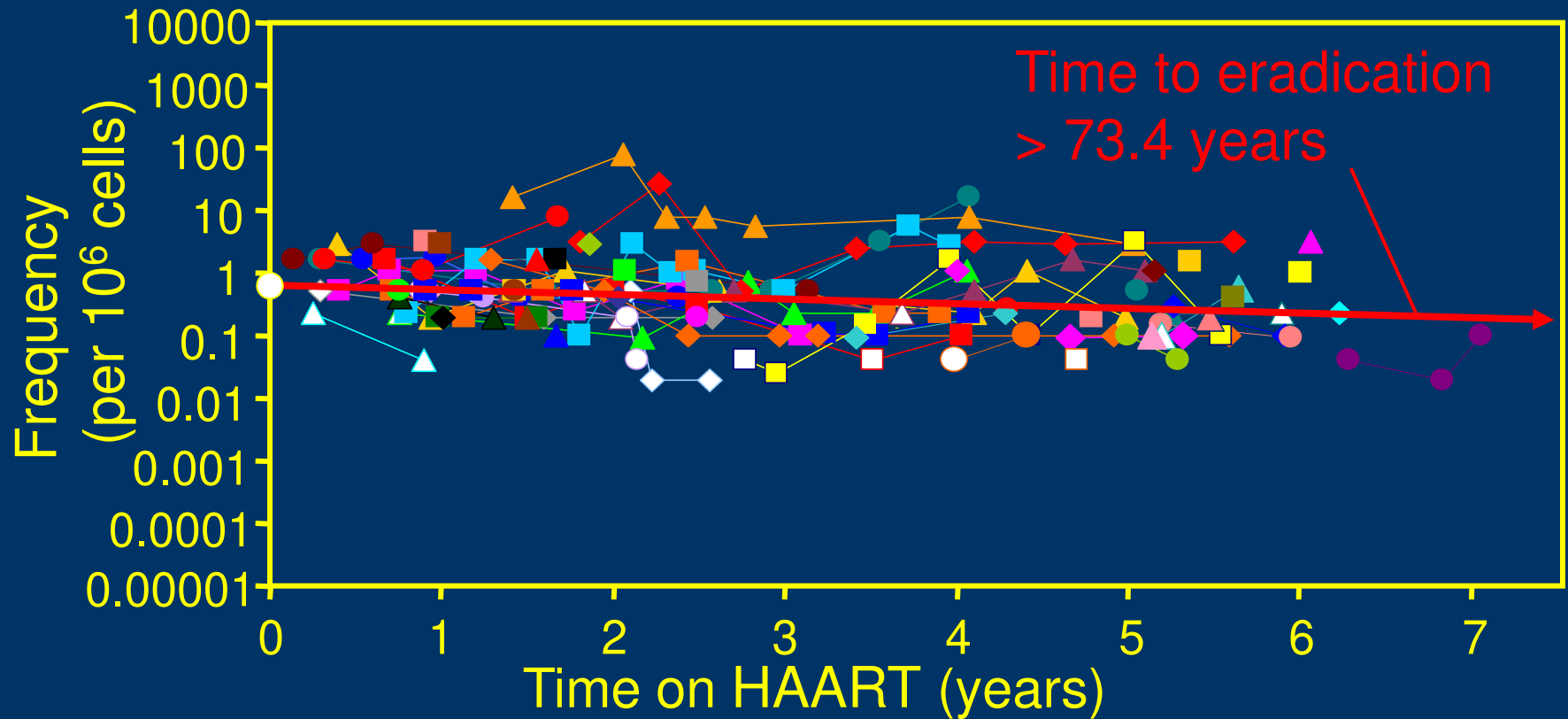
Comparative Analysis of Measures of Viral Reservoirs in HIV-1 Eradication Studies

Janet M. Siliciano PhD
Johns Hopkins University
School of Medicine

Disclosures: None



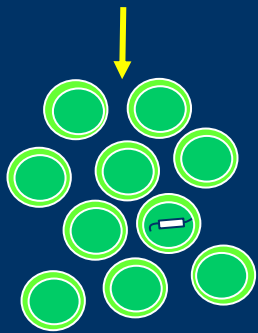
Slow decay of latently infected CD4⁺ T cells



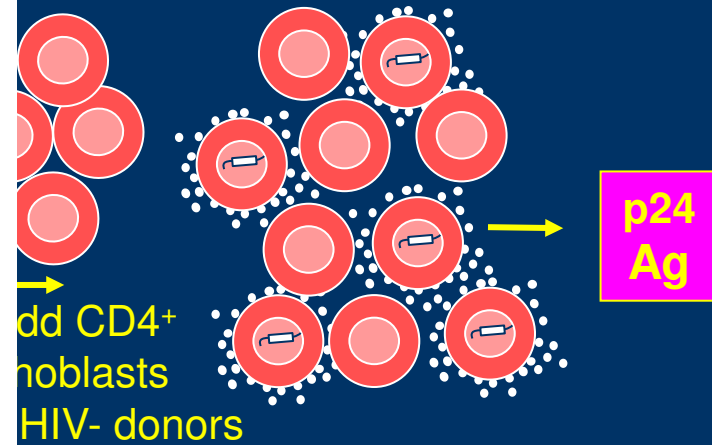
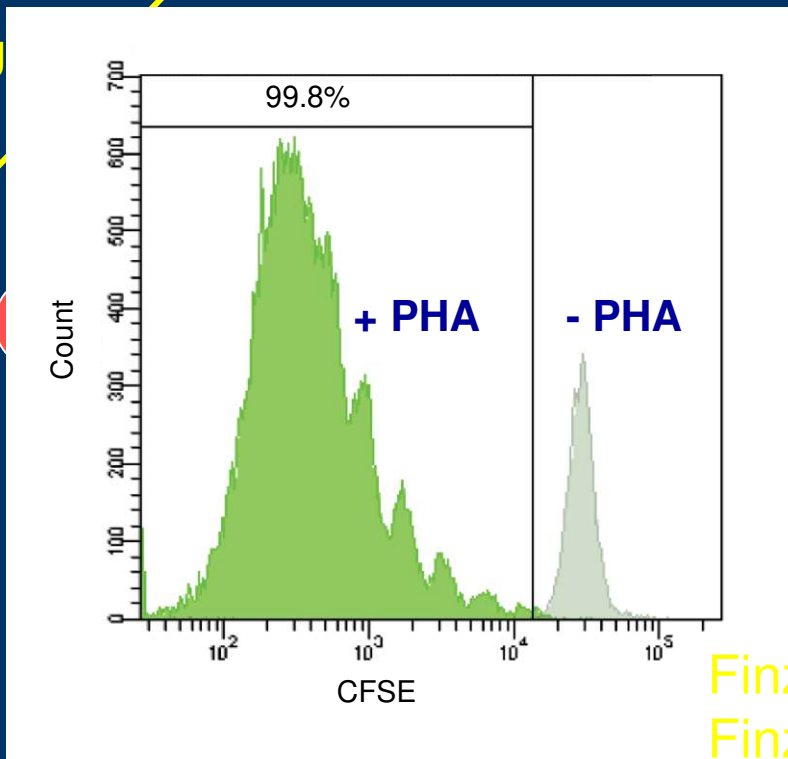
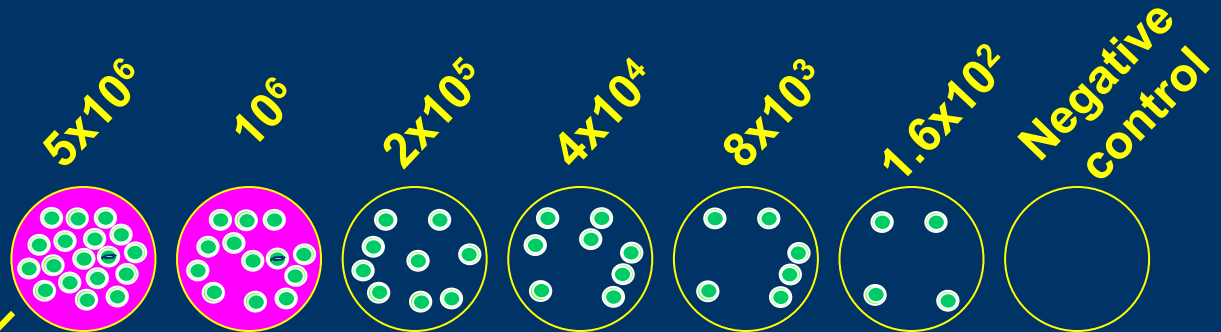
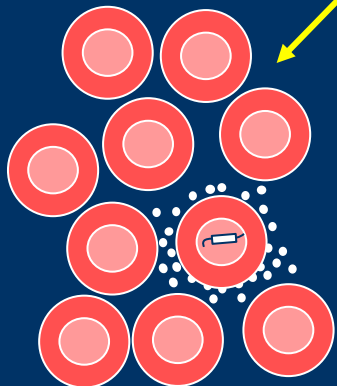
Finzi et al., Nature Med., 1999
Siliciano et al., Nature Med., 2003

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

180-200
ml blood



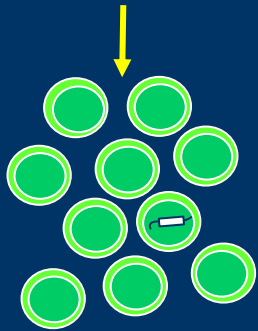
Purified resting
CD4⁺ T cells



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

180-200
ml blood

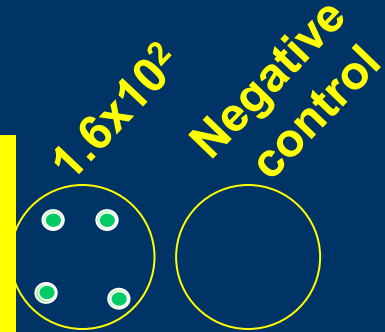


Purified resting
CD4⁺ T cells

- 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

d2: add CD4⁺
lymphoblasts
from HIV- donors

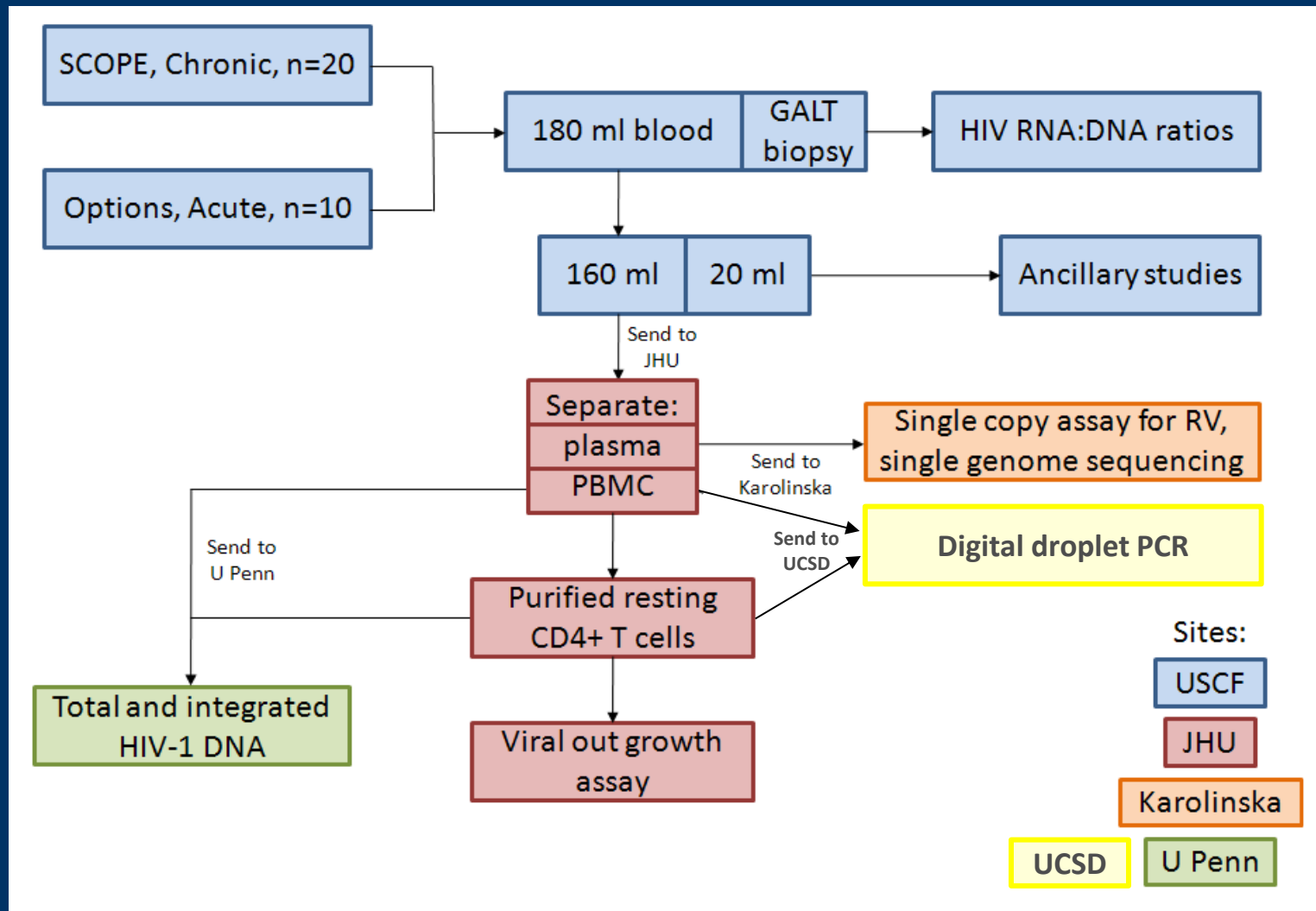
d7: add CD4⁺
lymphoblasts
from HIV- donors



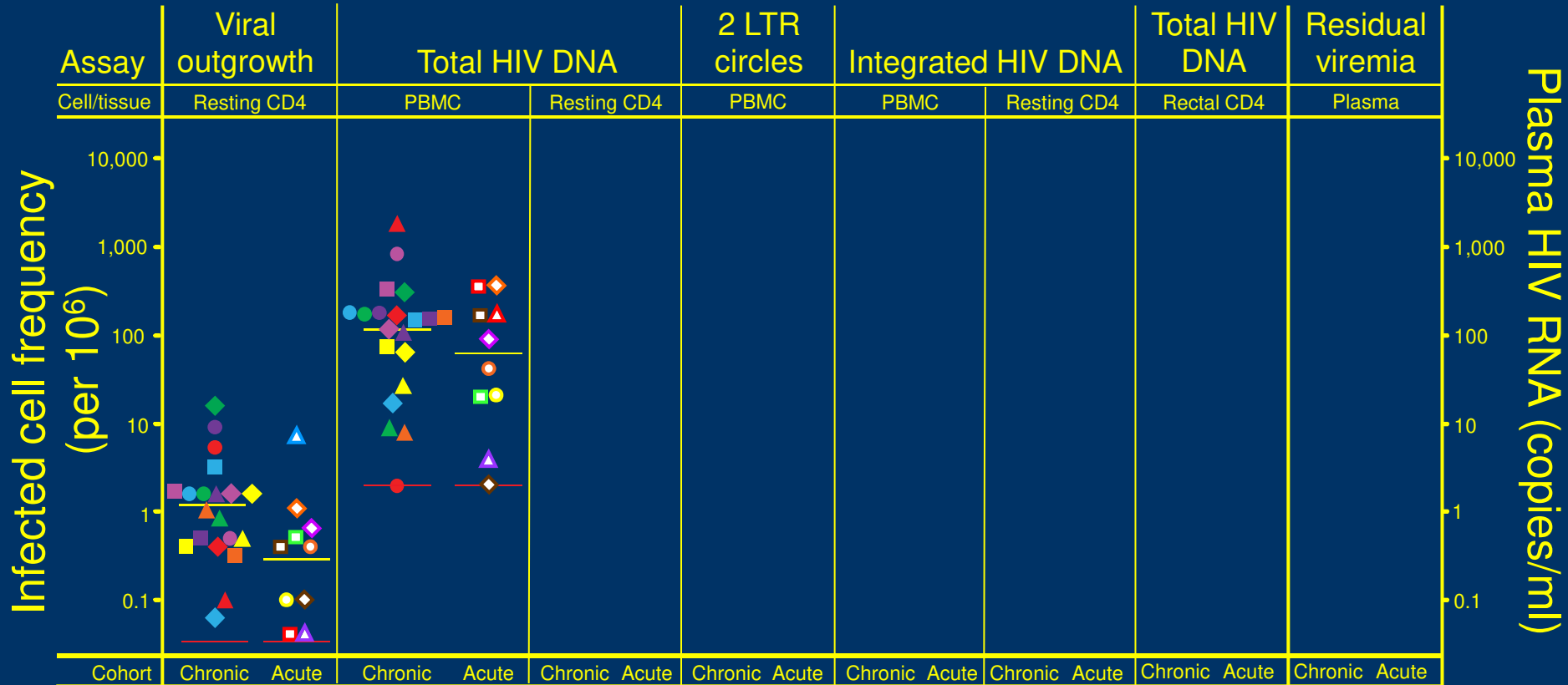
p24
Ag

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

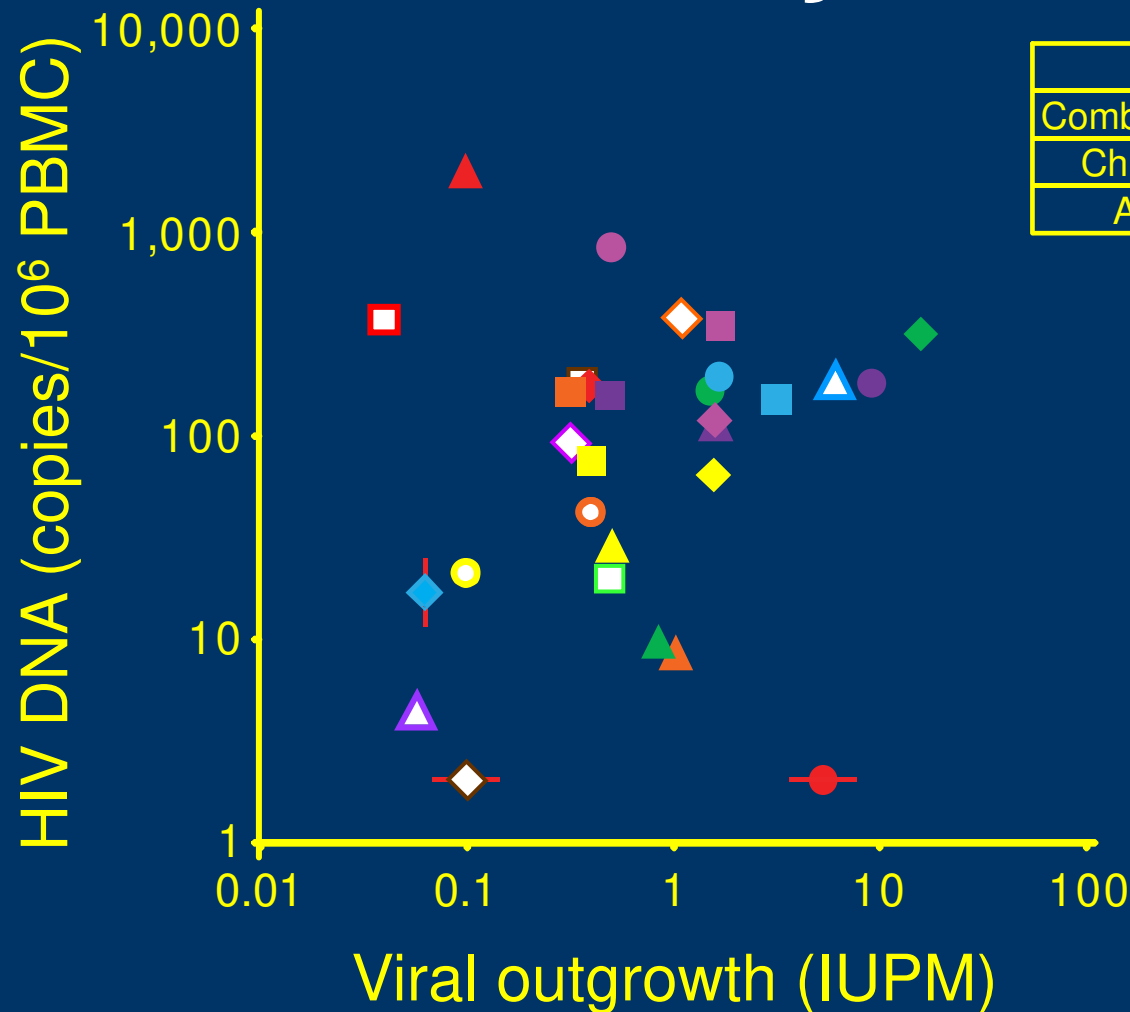
Comparison of reservoir assays



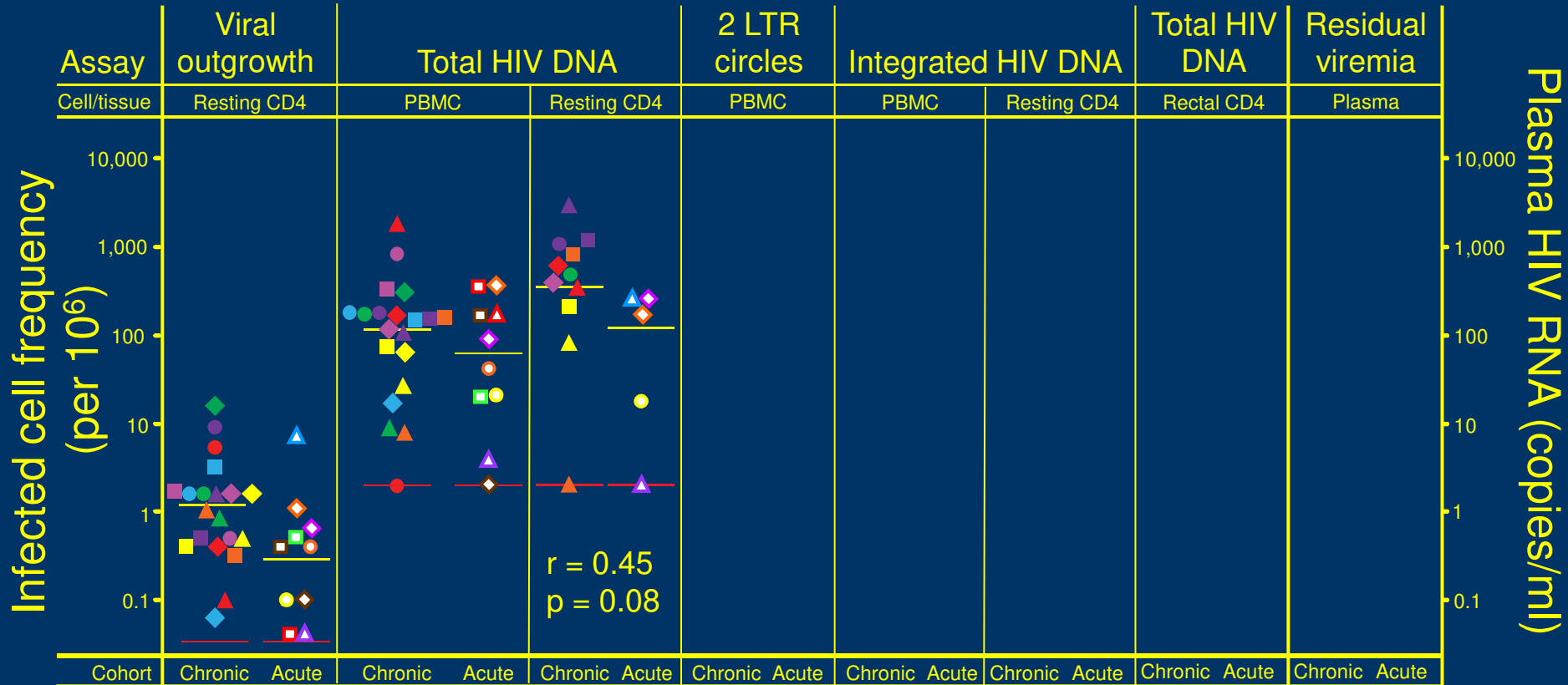
Assay for persistent HIV in patients on HAART



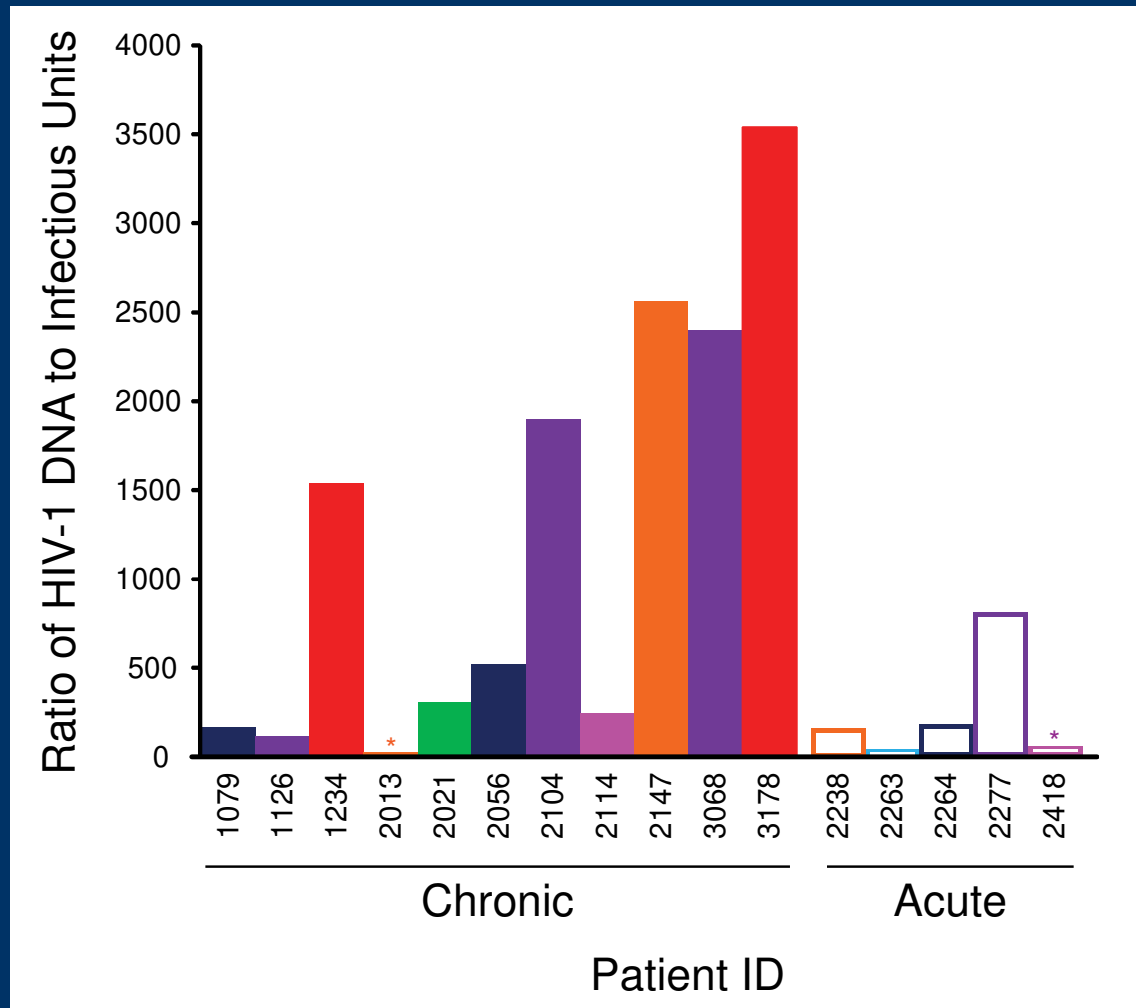
Correlation between culture and PCR assays



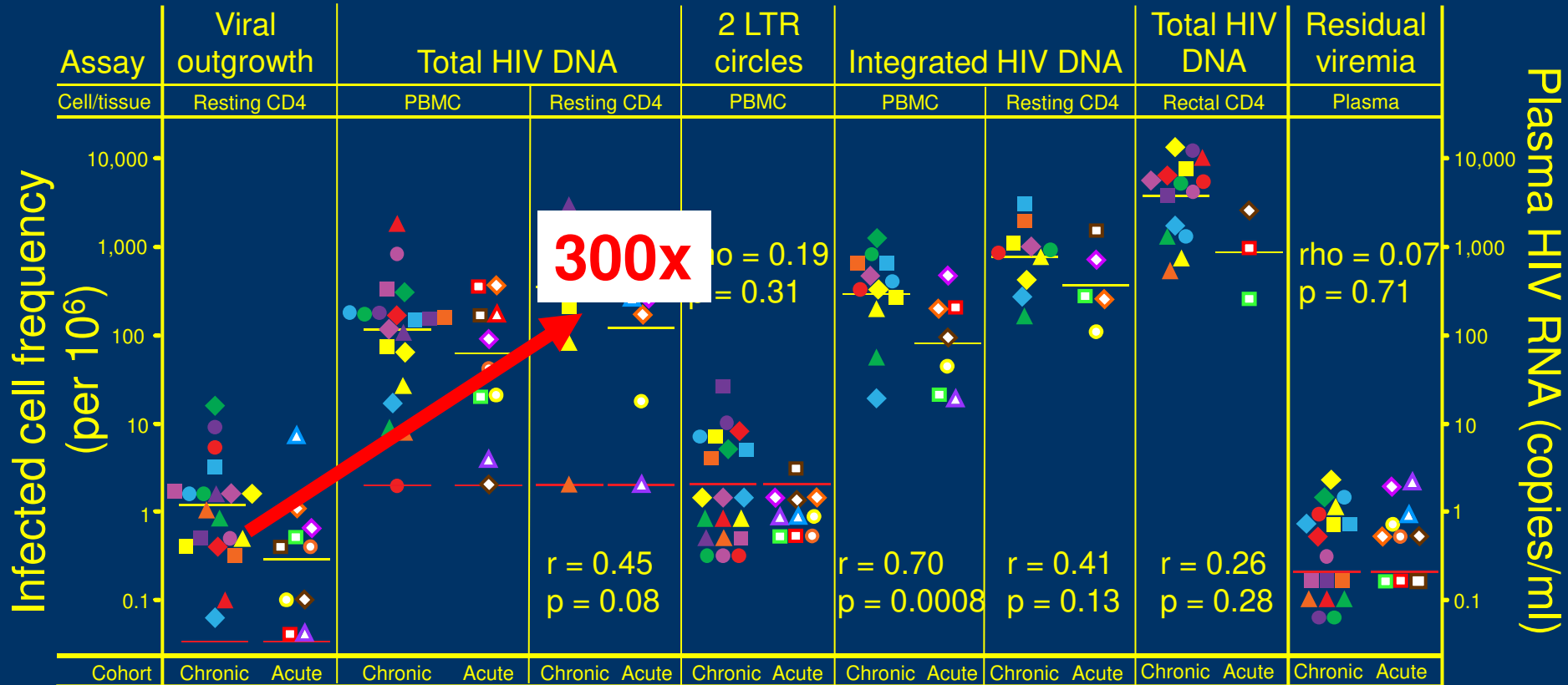
Assay for persistent HIV in patients on HAART



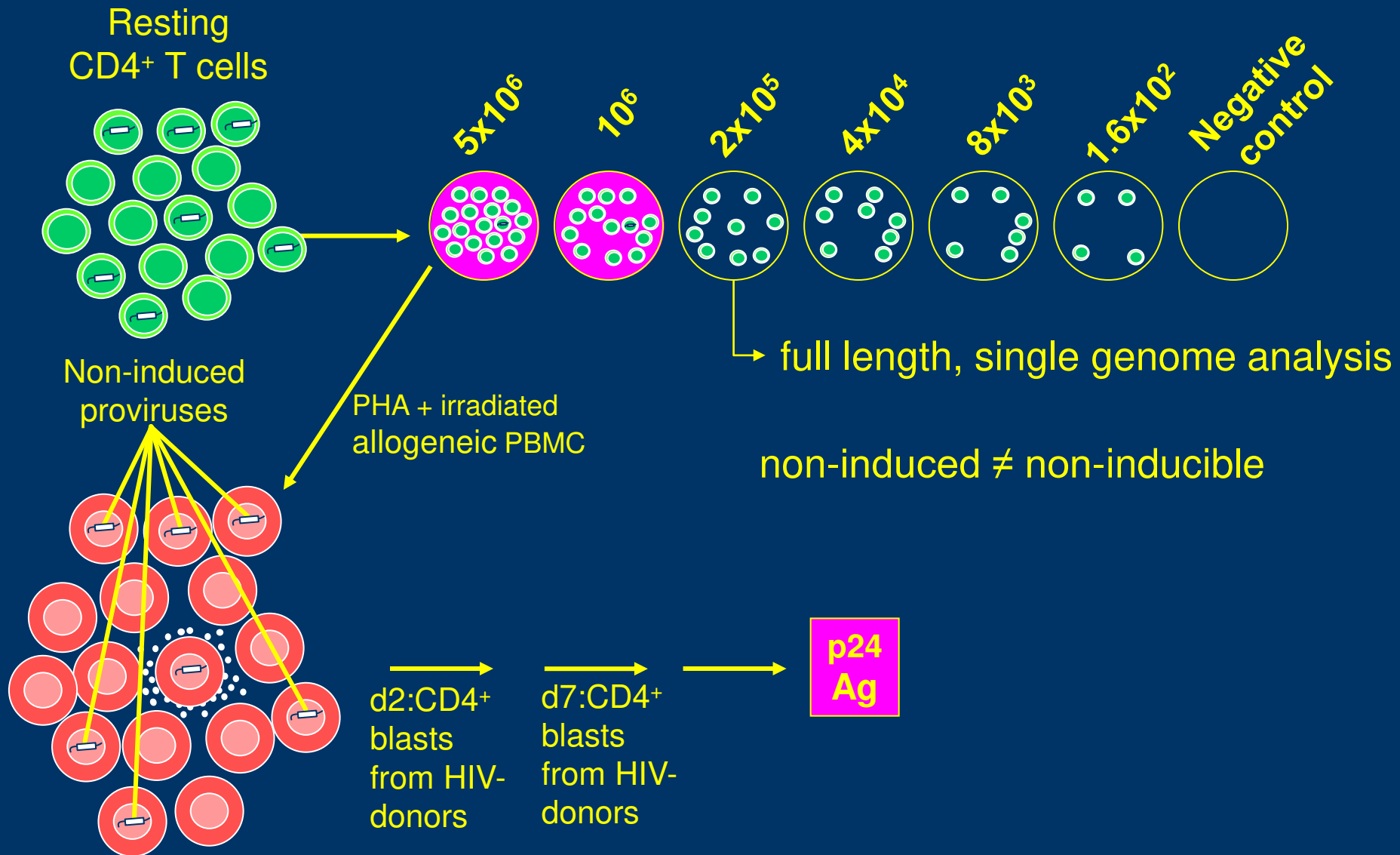
Ratio of infected cell frequencies by PCR and culture assays



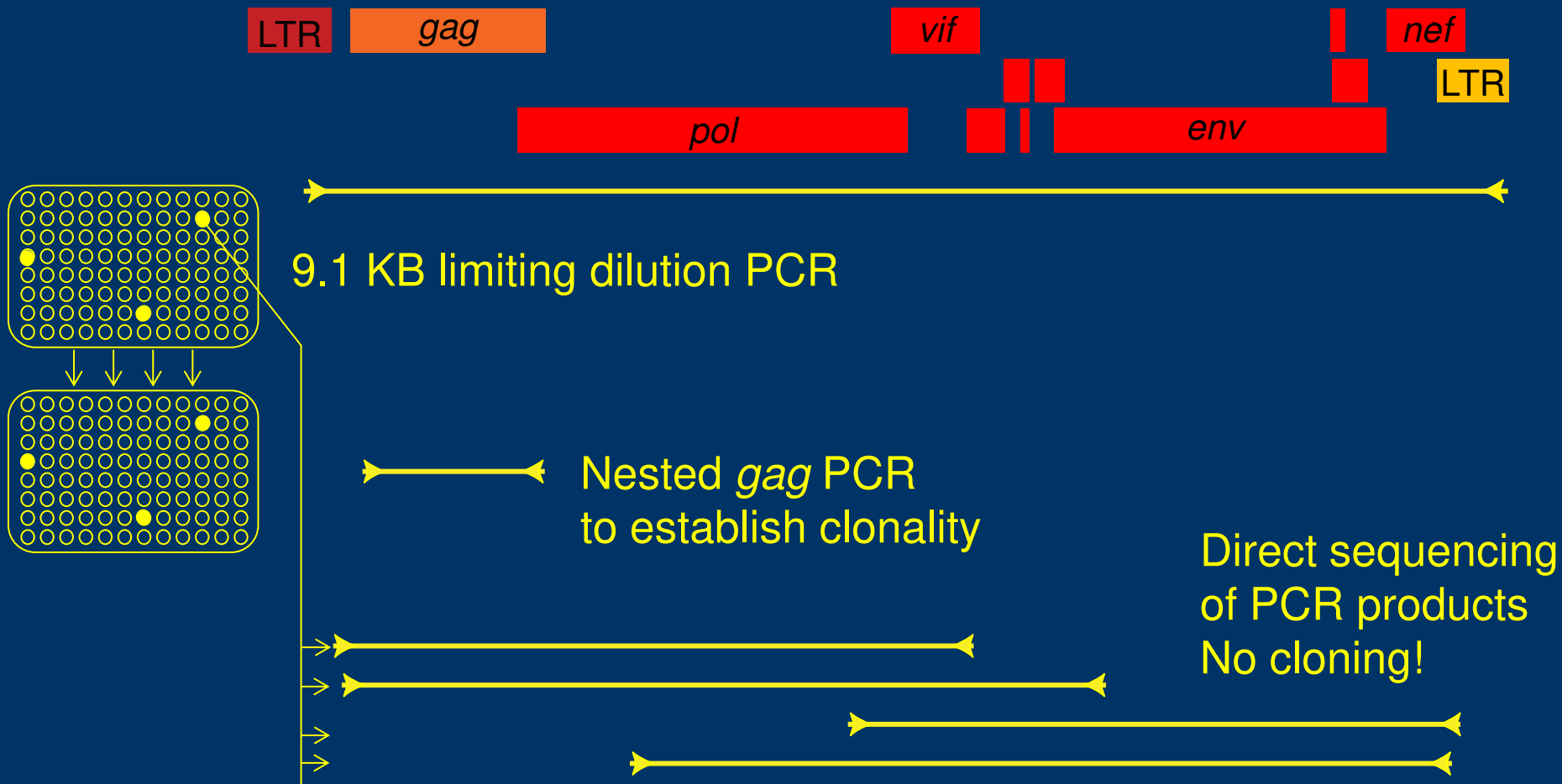
Which assay should be used?



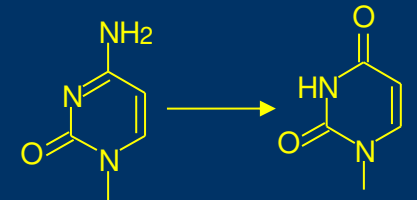
Non-induced proviruses



Clonal analysis of non-induced proviruses



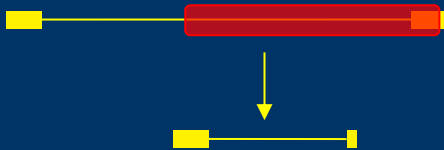
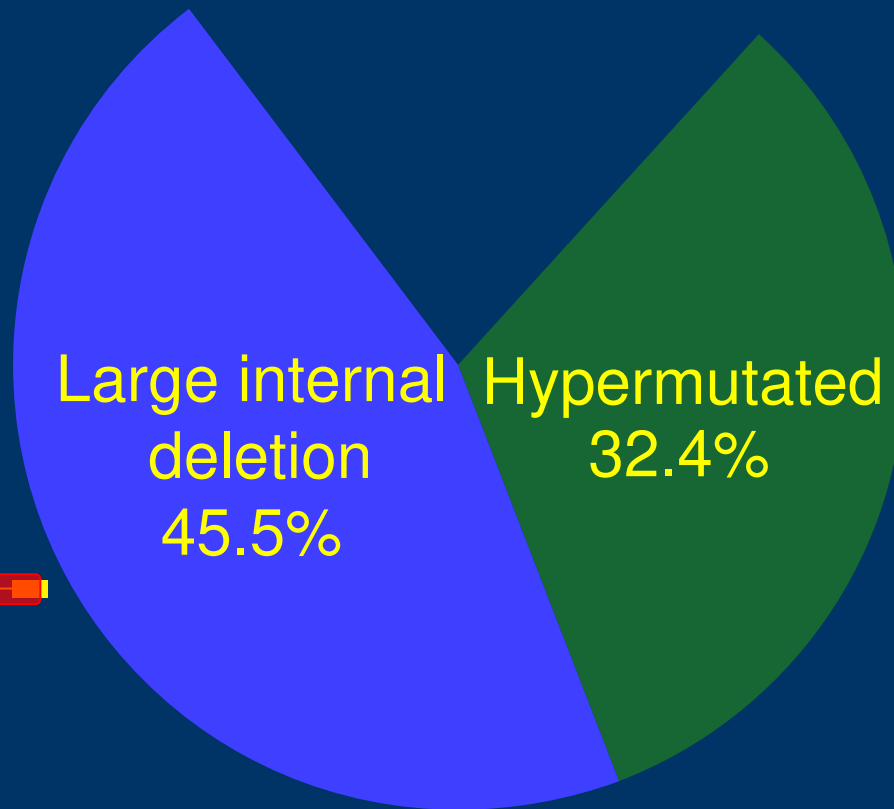
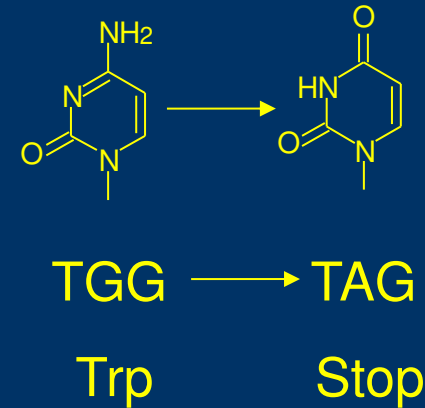
Non-induced proviral clones (n=213)



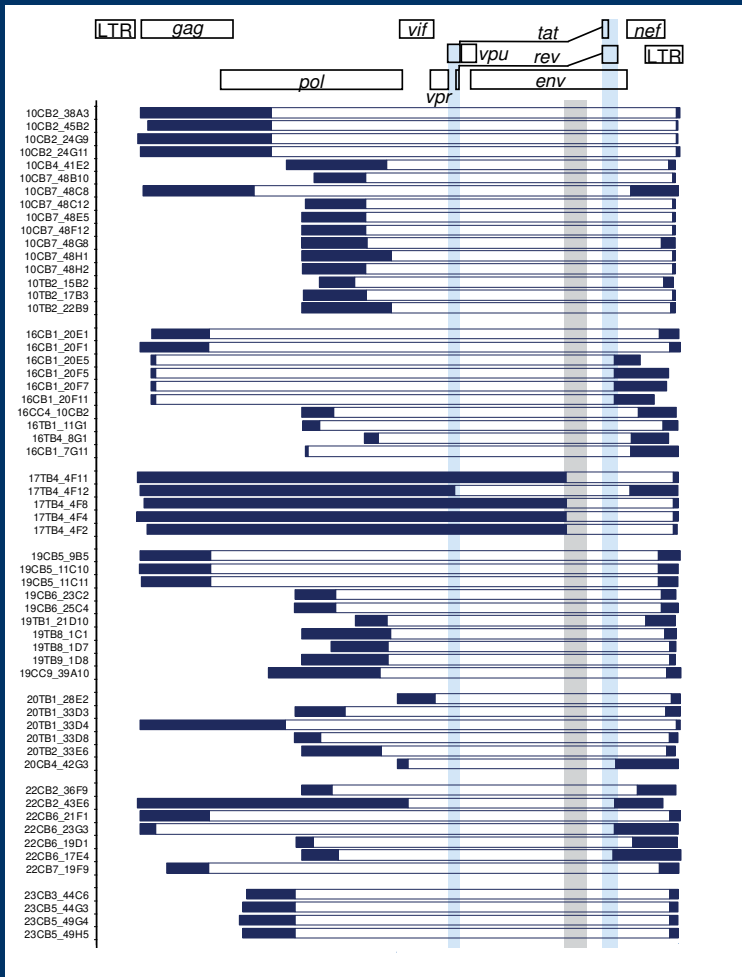
TGG → TAG
Trp Stop

Hypermutated
32.4%

Non-induced proviral clones (n=213)



45.5% of non-induced proviruses have large internal deletions



Non-induced proviral clones (n=213)

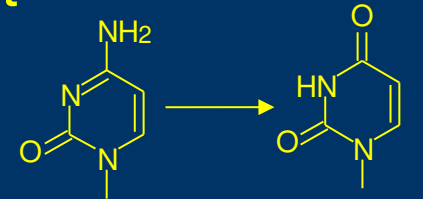
Nonsense mutations/
INDELS 3.8%

Deletion in ψ /
MSD site 6.5%

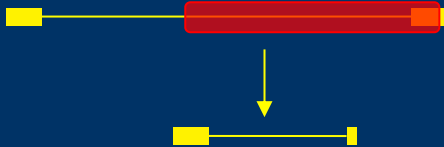
11.7% Intact
genome

Large internal
deletion
45.0%

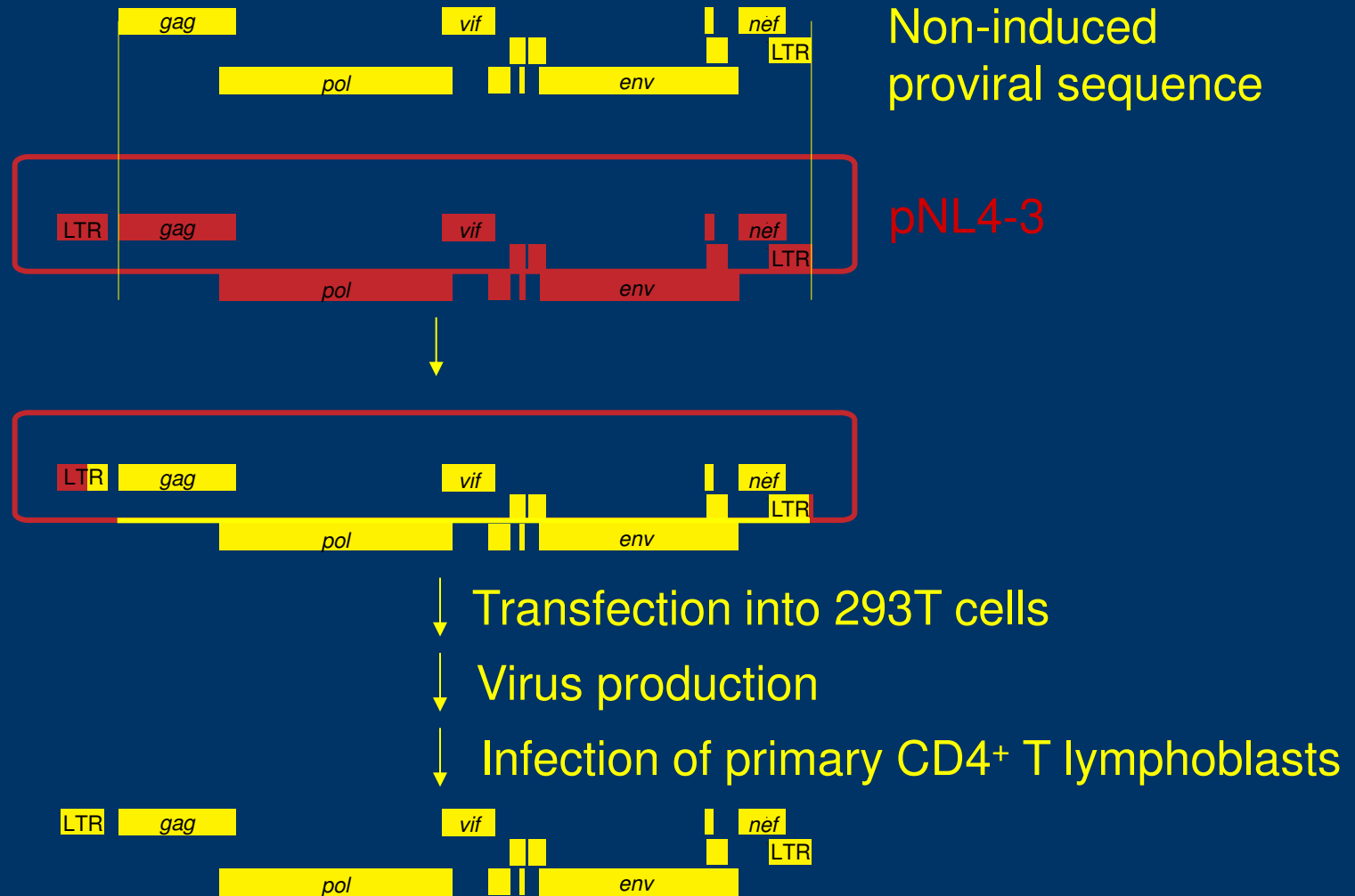
Hypermuted
32.4%



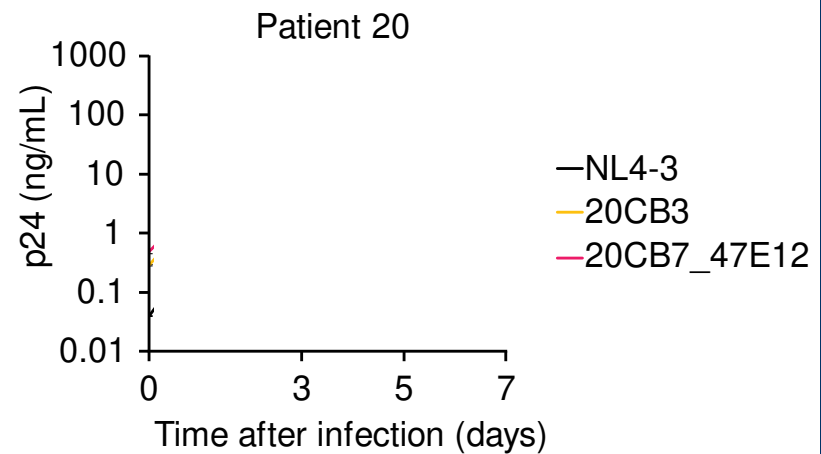
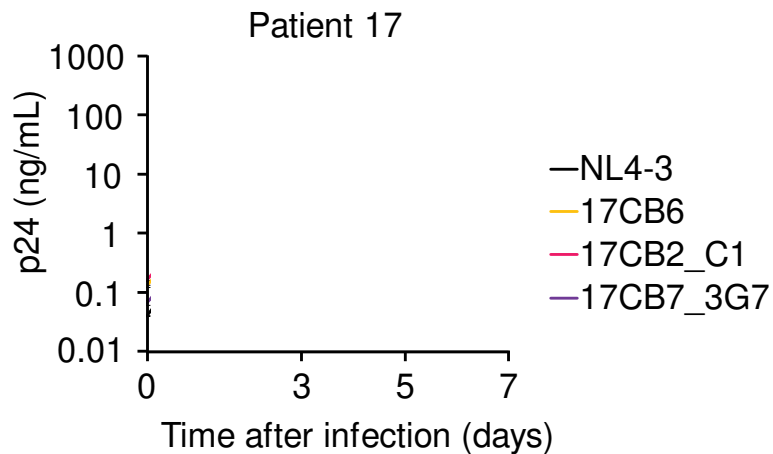
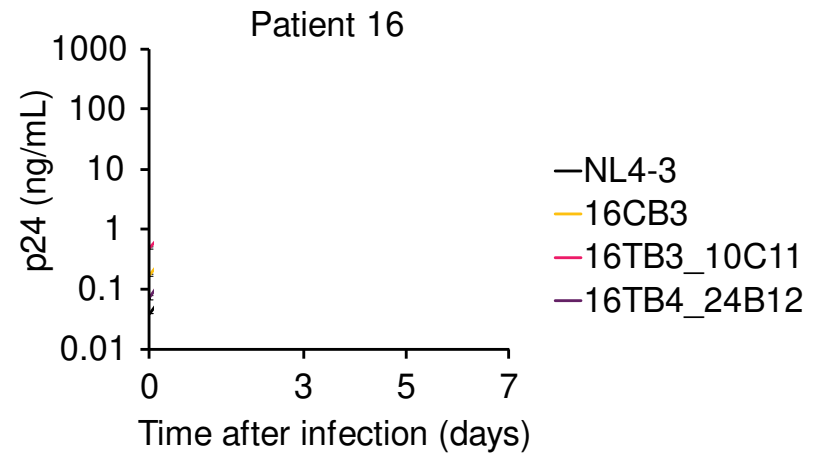
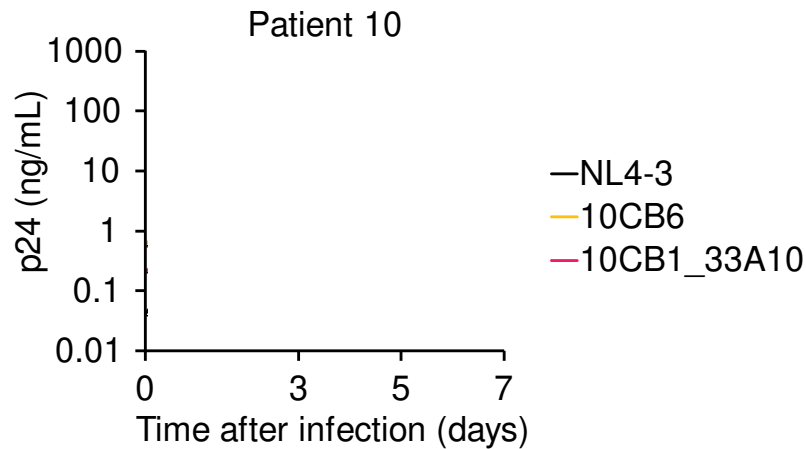
TGG → TAG
Trp Stop



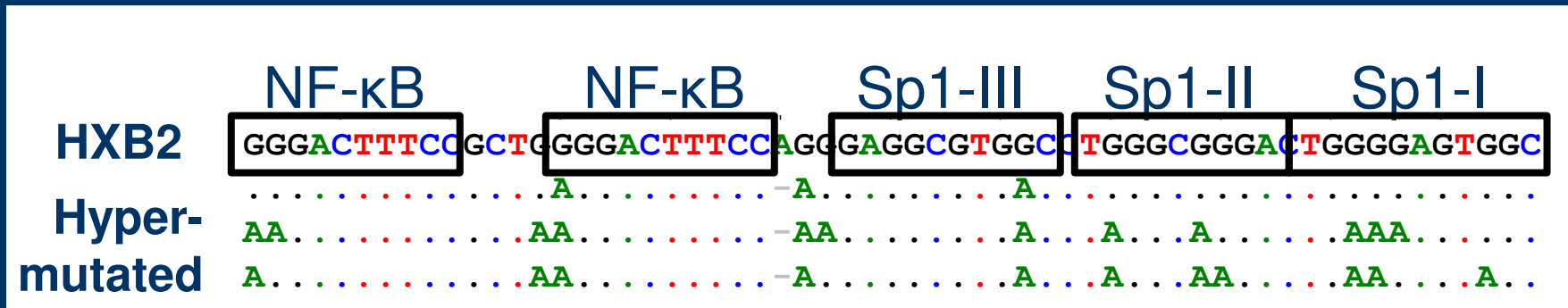
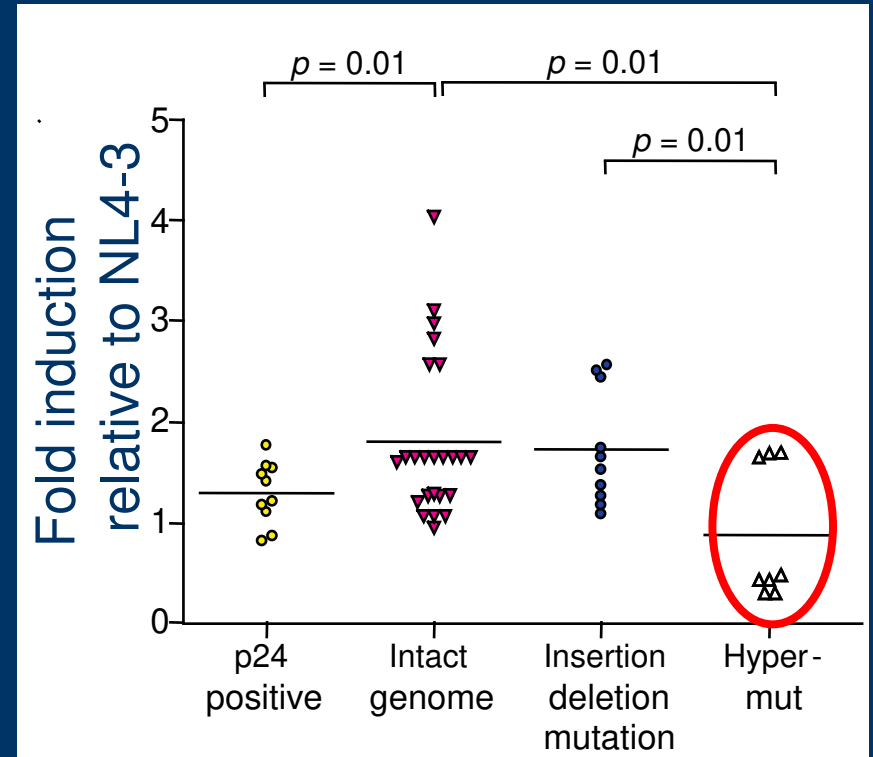
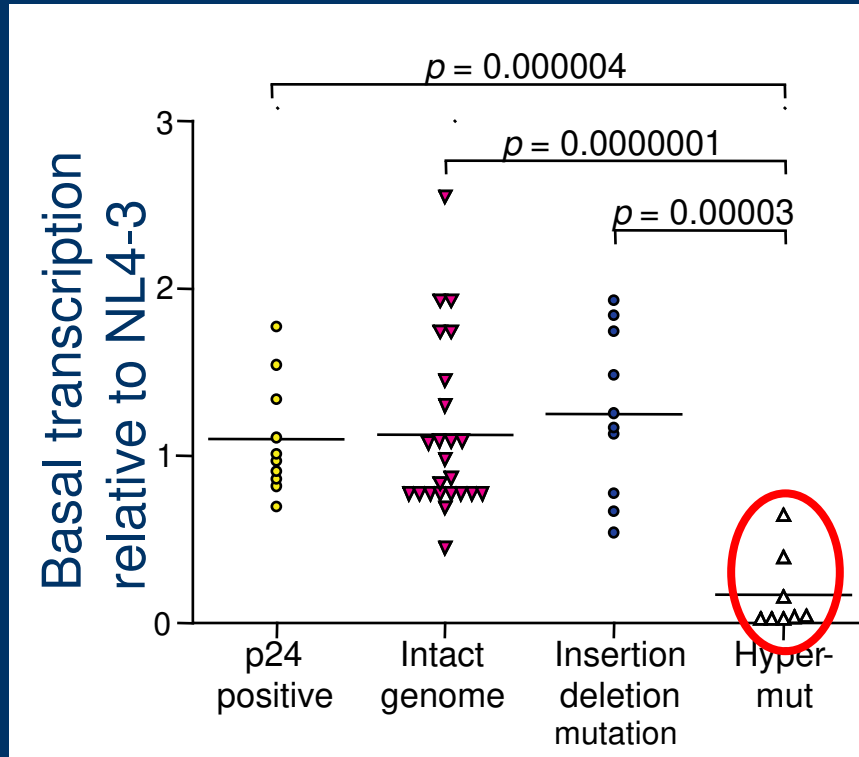
Replication-competence of non-induced proviruses



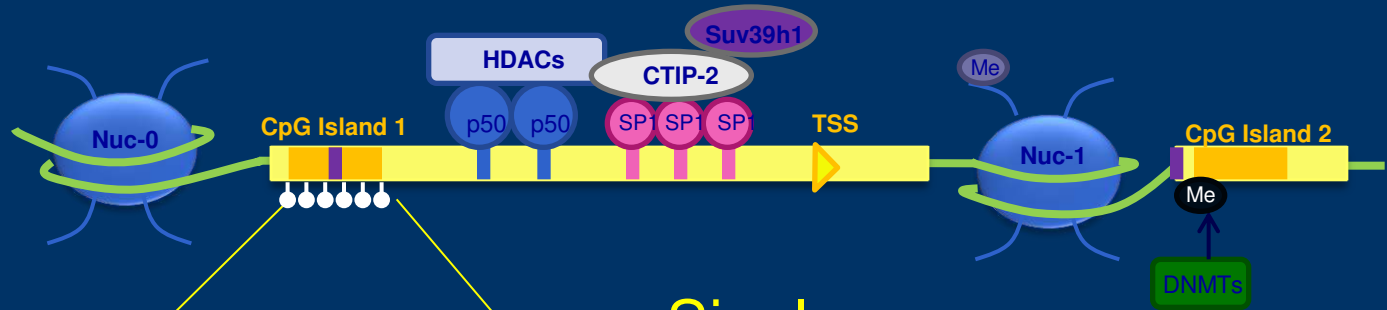
Replication of non-induced proviruses clones



Non-induced proviruses have functional LTRs except for hypermutated clones



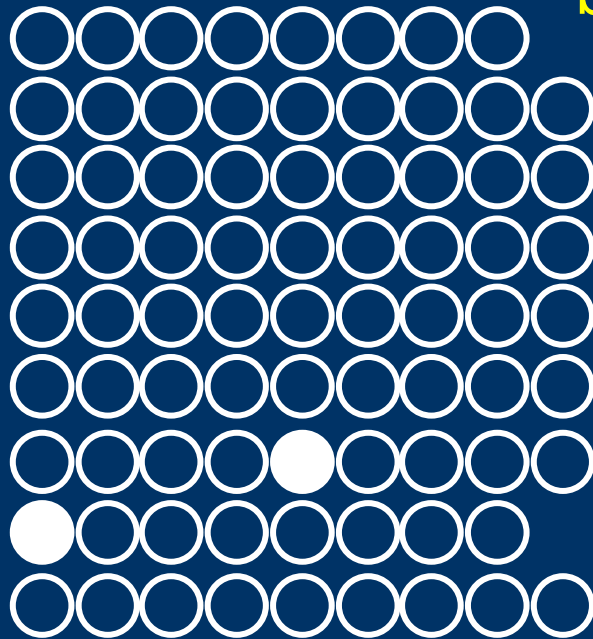
Clonal analysis of DNA methylation



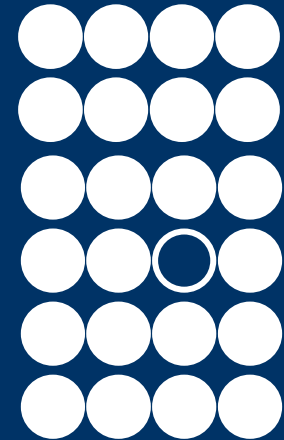
Single genome
bisulfite sequencing

Patient 20

Cells from
p24 negative
co-culture
well



env



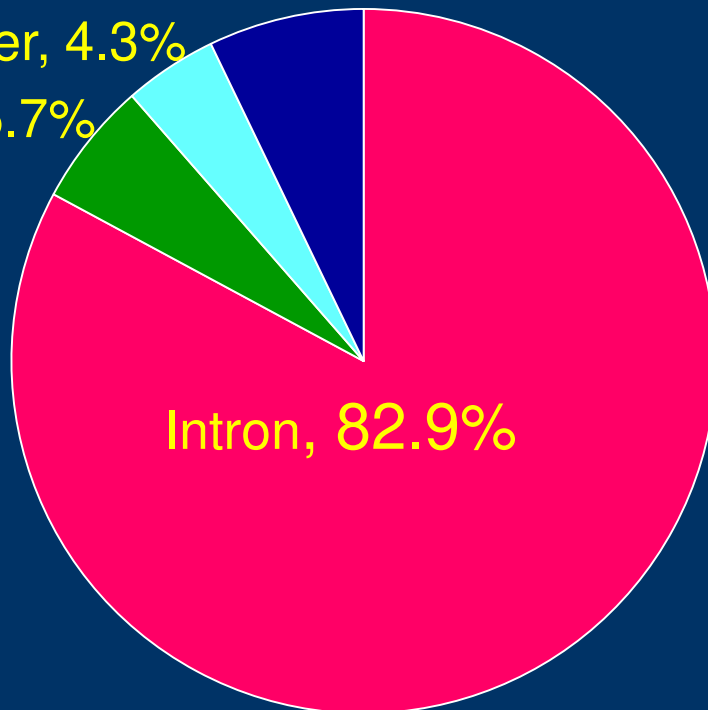
Non-induced proviruses integrate into active transcription units

- Location

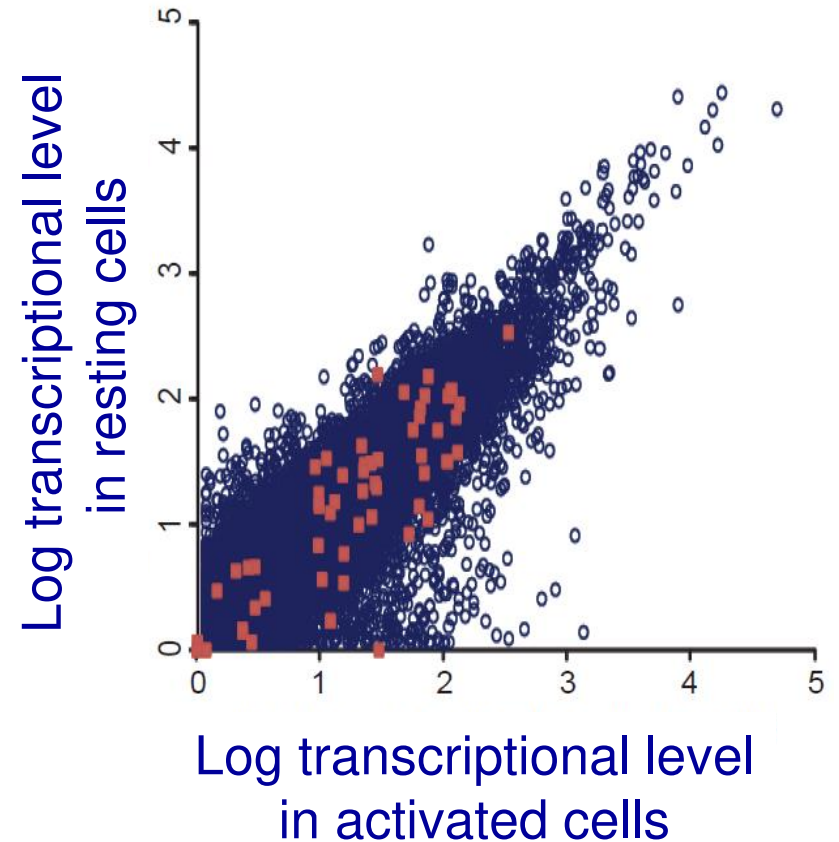
Intergenic space, 7.1%

Other, 4.3%

Exon, 5.7%

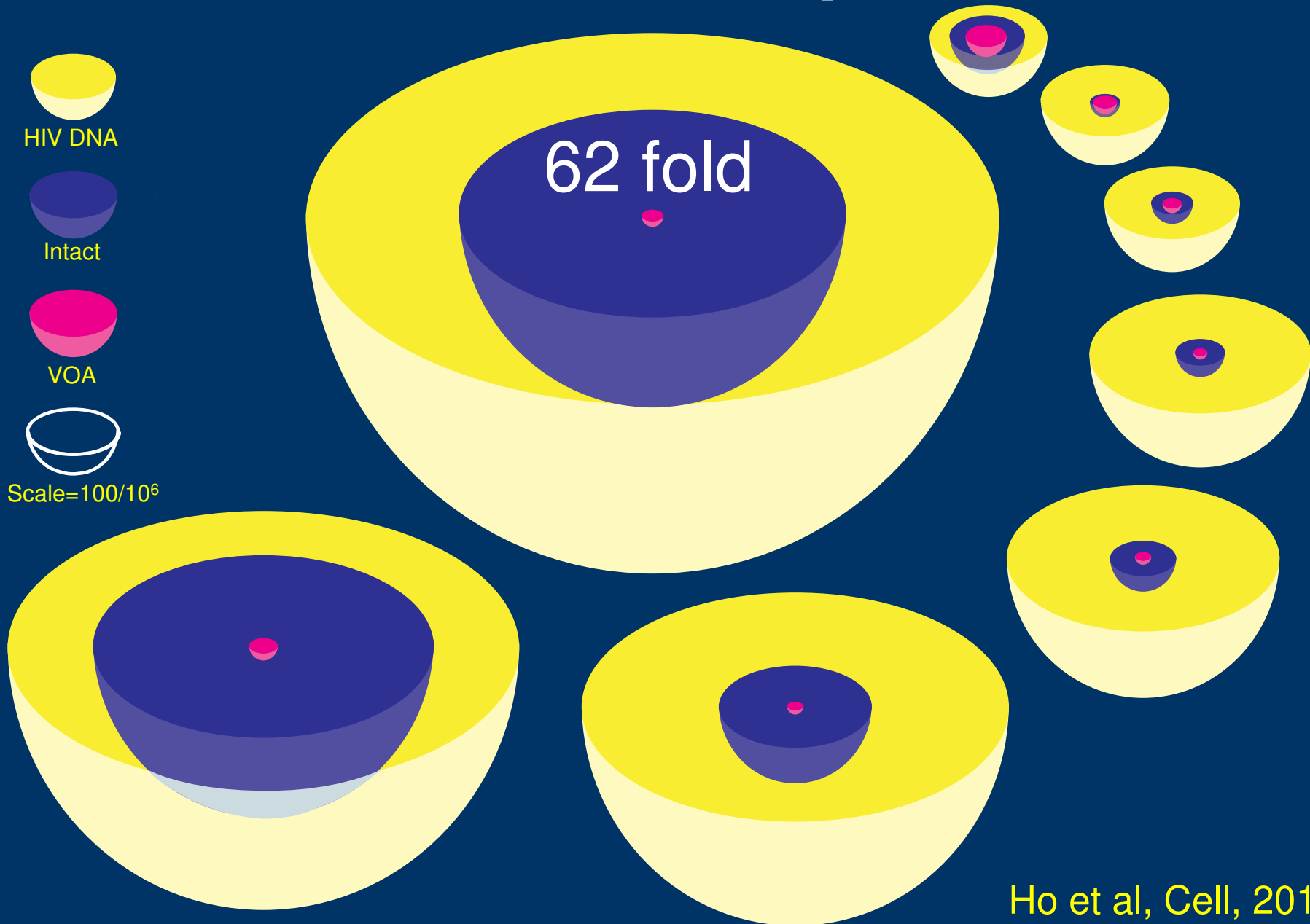


- Activity of genes



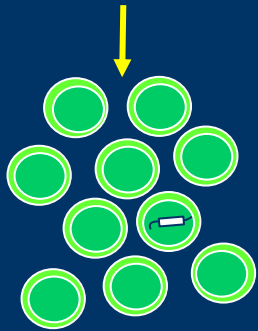
Transcription units: 92.9% (65/70)

Intact vs induced proviruses



Can intact non-induced proviruses be induced?

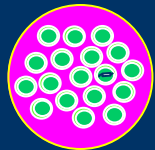
180-200 ml blood



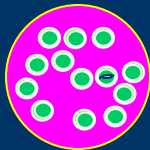
Purified resting CD4⁺ T cells

PHA + irradiated allogeneic PBMC

5×10^6



10^6



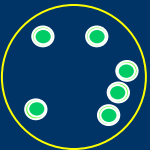
2×10^5



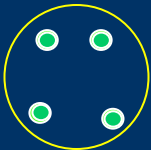
4×10^4



8×10^3



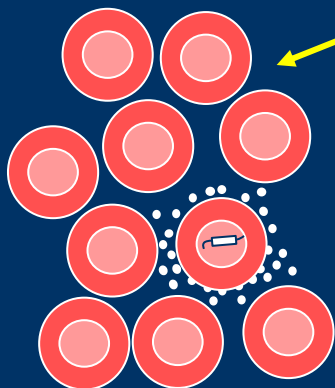
1.6×10^2



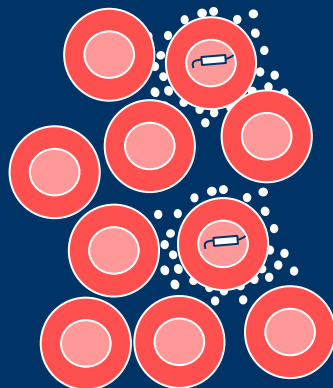
Negative control



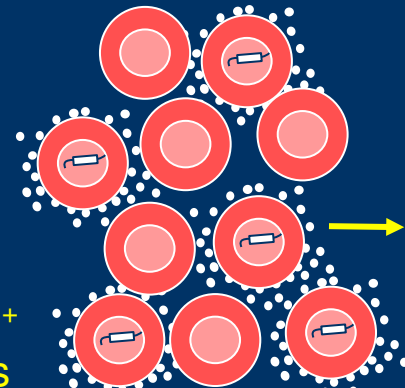
Recover cells from negative wells



d2: add CD4⁺ lymphoblasts from HIV- donors

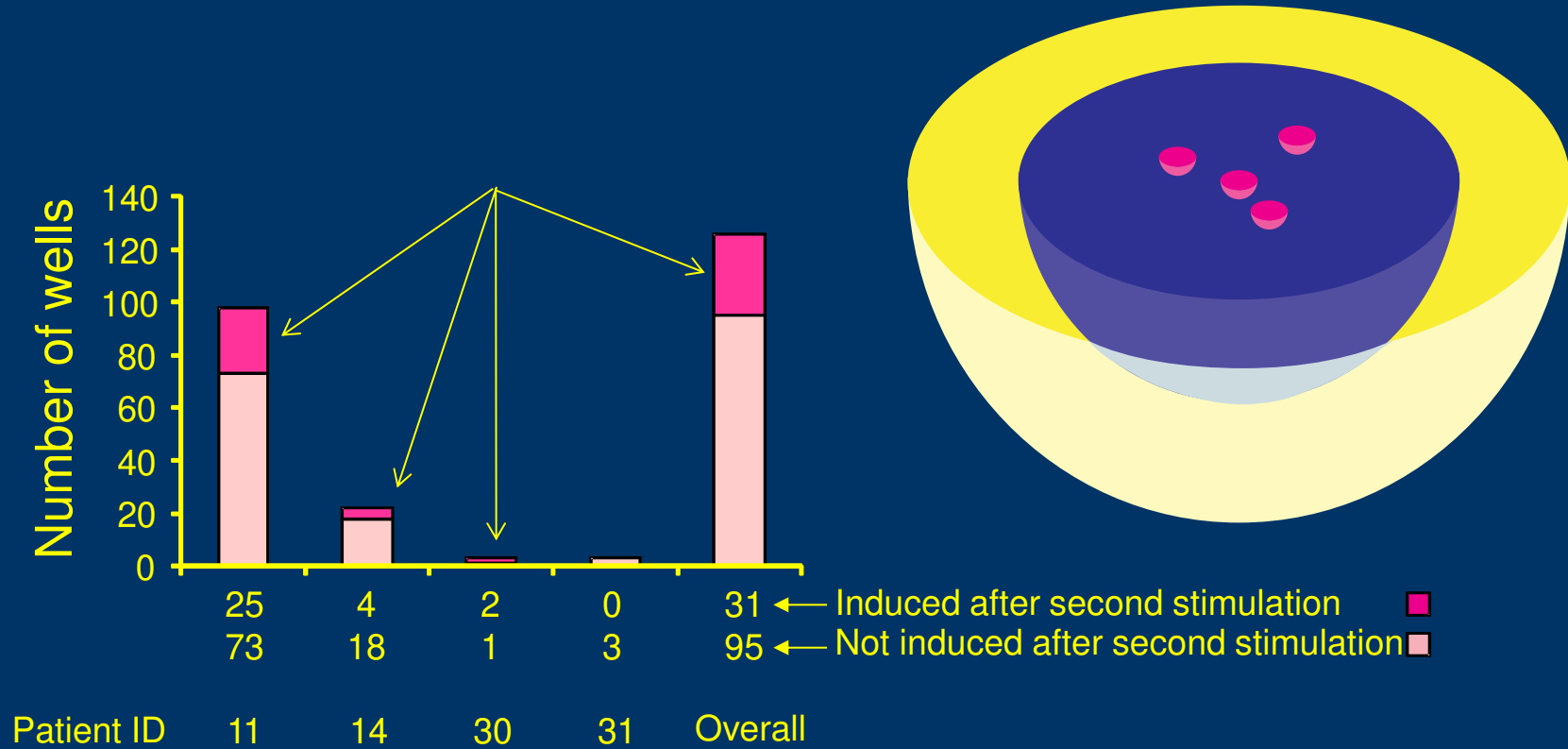


d7: add CD4⁺ lymphoblasts from HIV- donors



p24 Ag

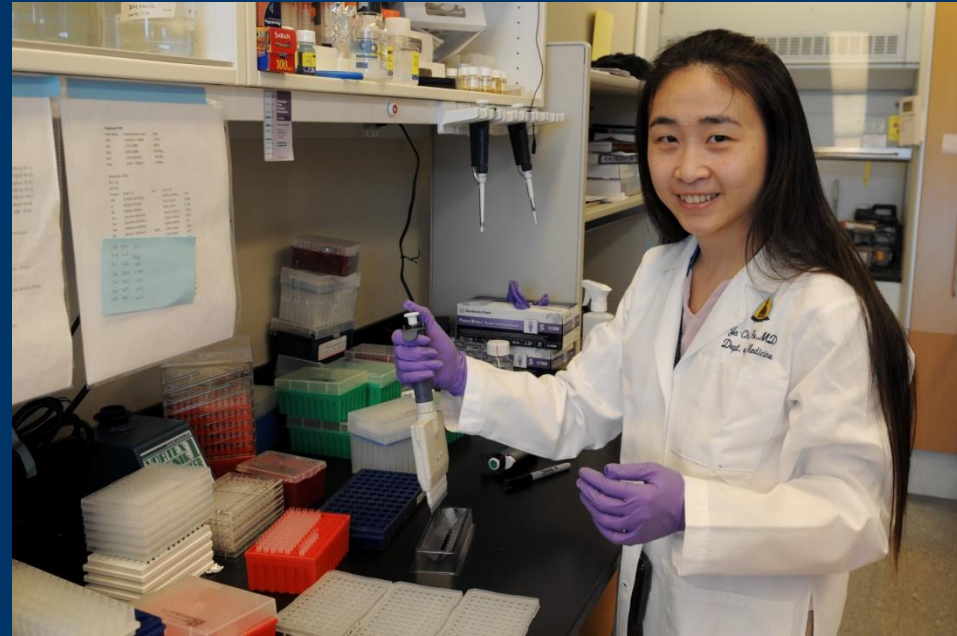
Can intact non-induced proviruses be induced?



Thanks

Collaborators

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



Funding

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