Biomarkers of HIV persistence as predictors of HIV rebound off ART

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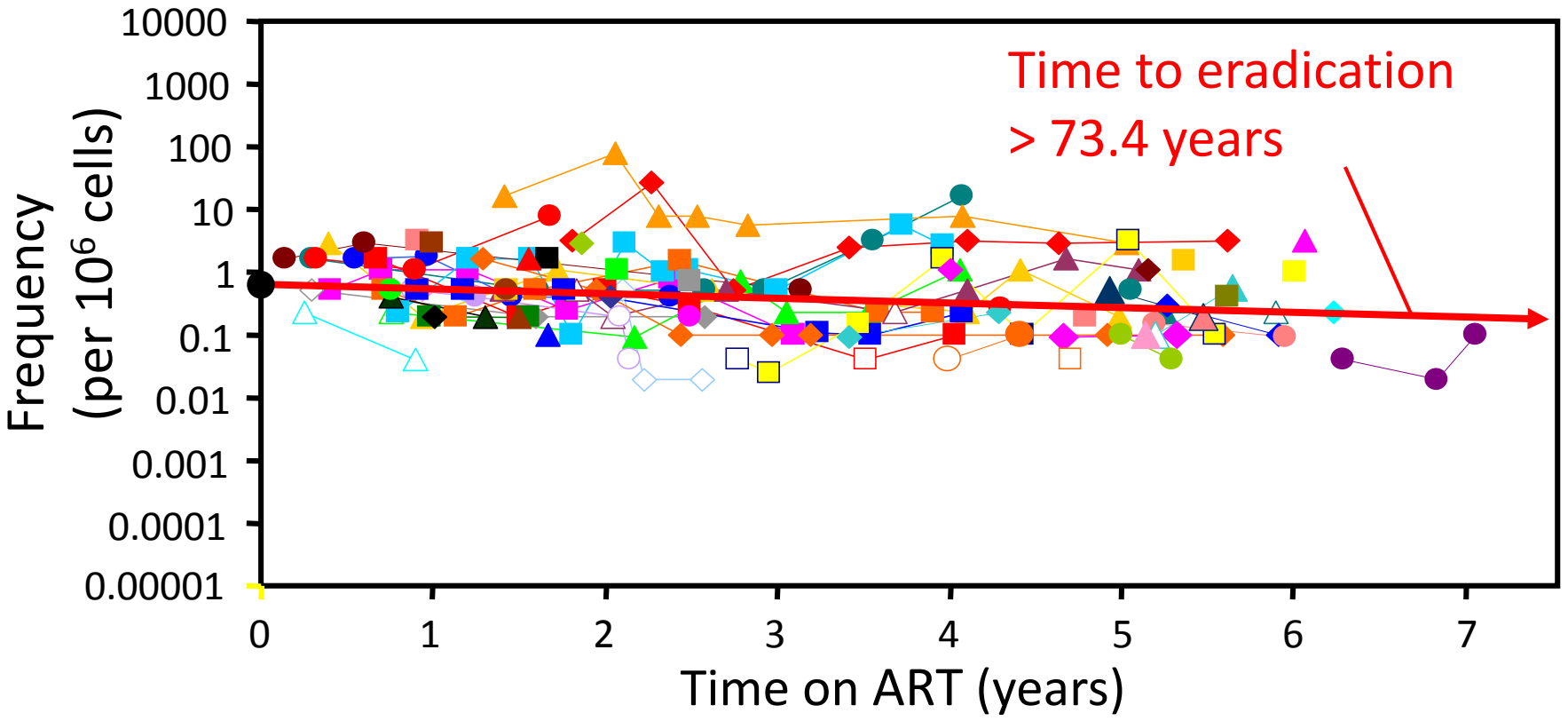
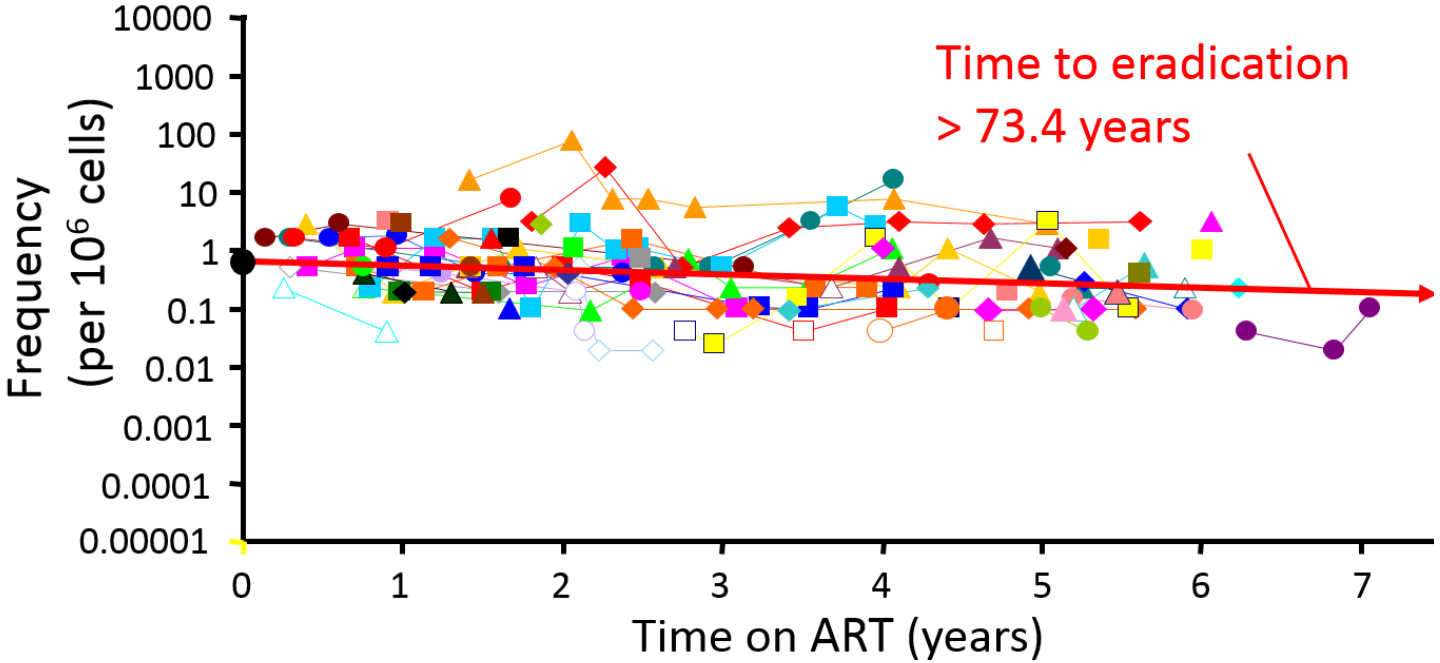
Disclosures: None

The latent reservoir in resting CD4+ T cells is the major barrier to cure



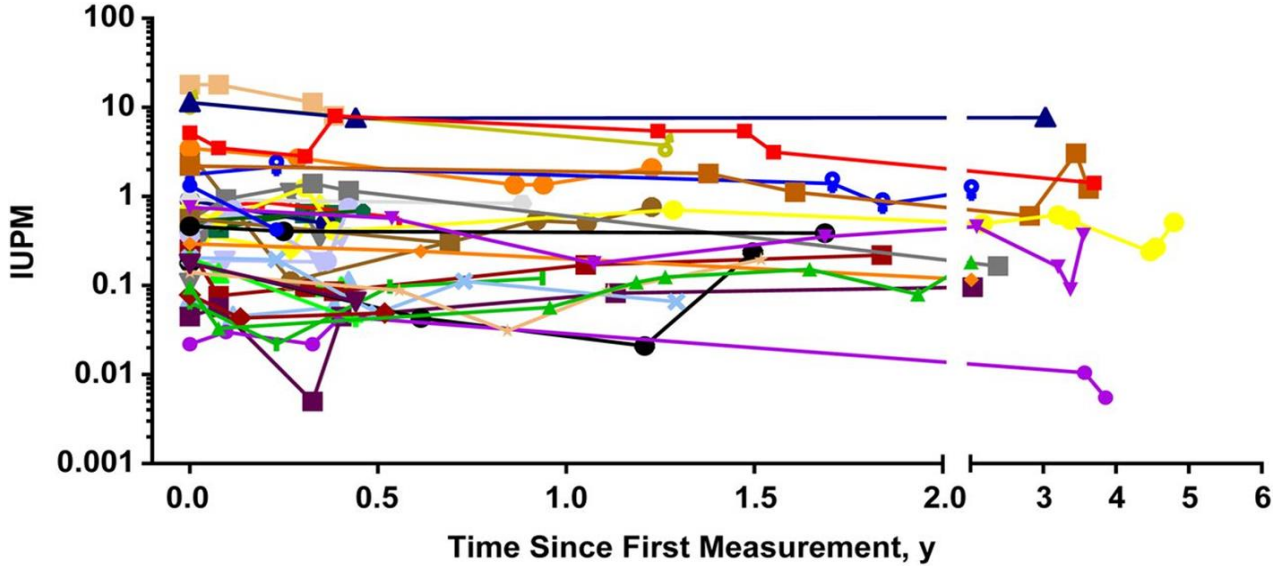
Finzi et al., Nature Med., 1999
Persaud et al., JCI, 2000
Siliciano et al., Nature Med., 2003
Strain et al., PNAS, 20003

Slow decay of the reservoir



$t_{1/2}$ = 44 months

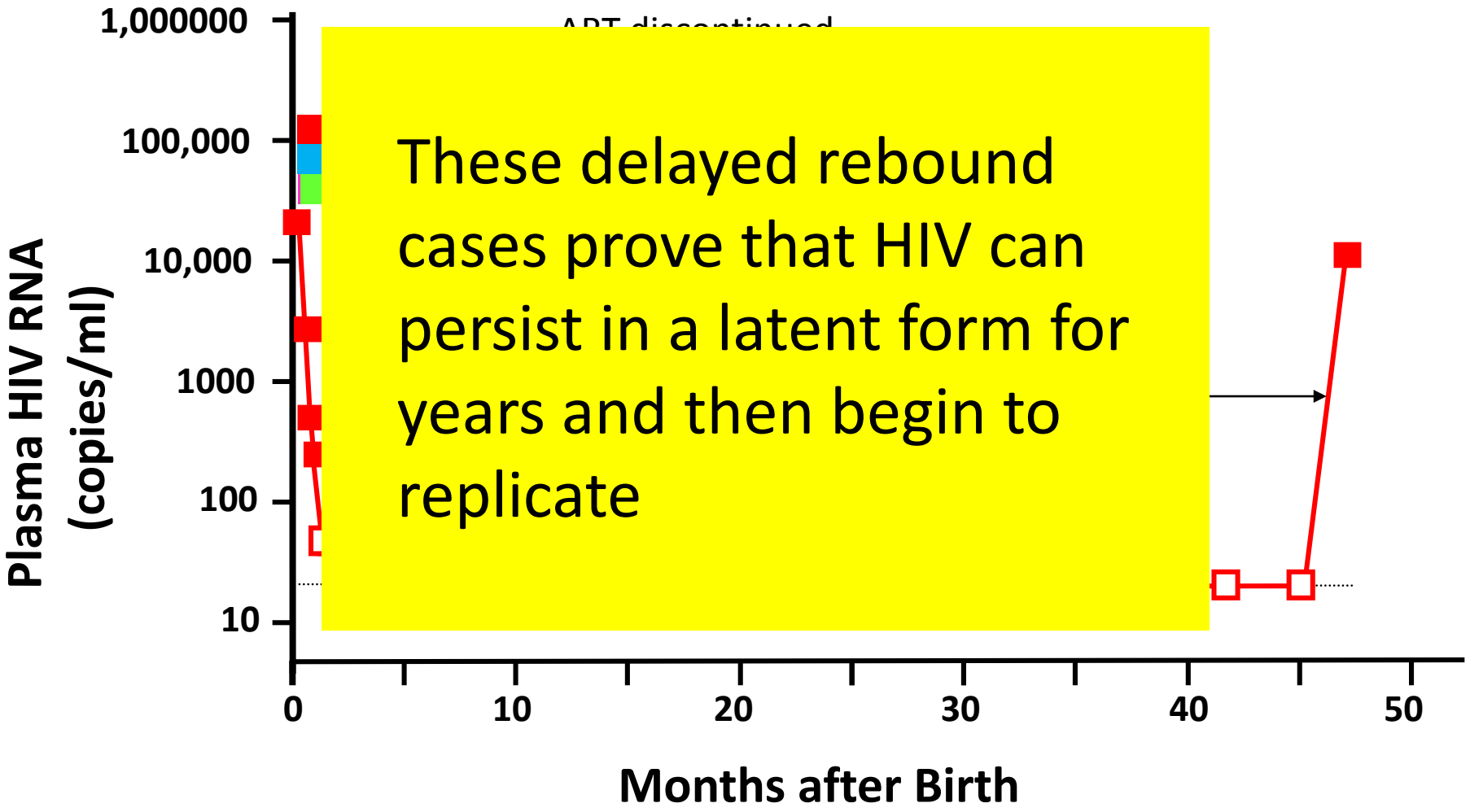
Siliciano et al., Nature Med., 2003



$t_{1/2}$ = 43 months

Crook et al, JID 2015

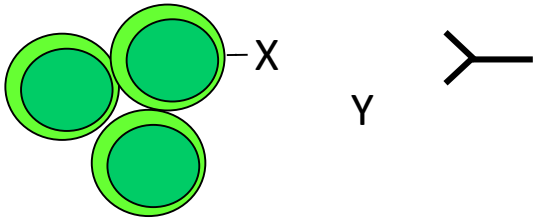
The Mississippi baby



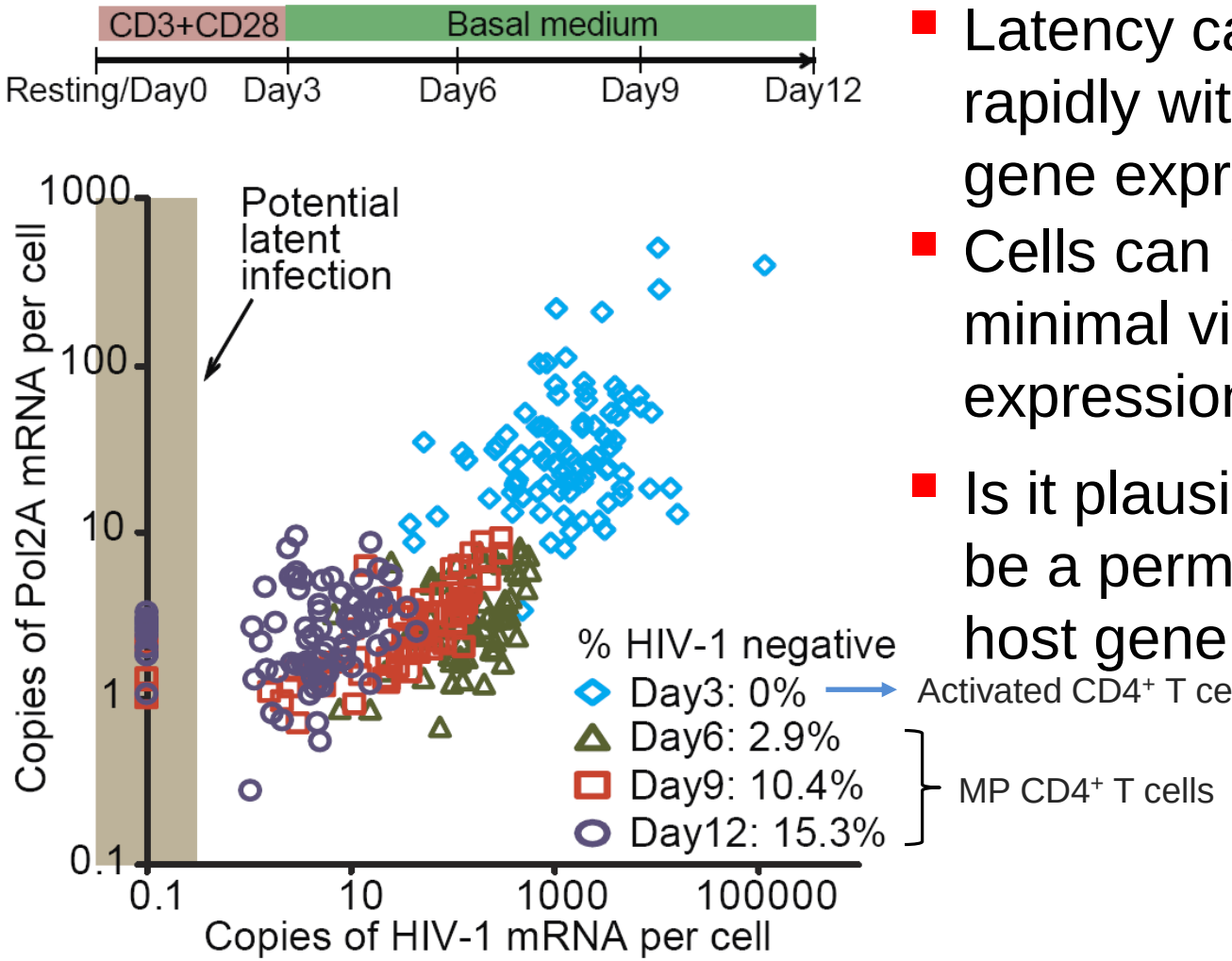
Persaud D et al., NEJM 2013

Biomarkers for HIV persistence

- Non-viral biomarker



Suboptimal environment for HIV-1 transcription facilitates latency

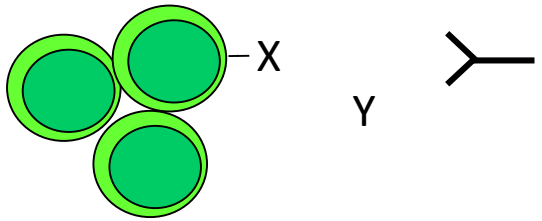


- Latency can be established rapidly with minimal HIV gene expression
- Cells can persist with minimal viral gene expression for years
- Is it plausible that there will be a permanent change in host gene expression?

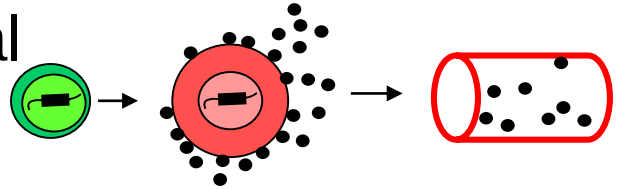
Shan et al., submitted

Biomarkers for HIV persistence

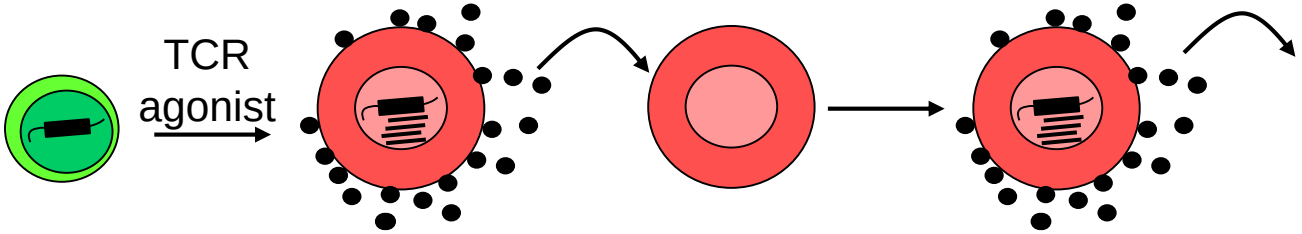
- Non-viral biomarker



- Residual viremia

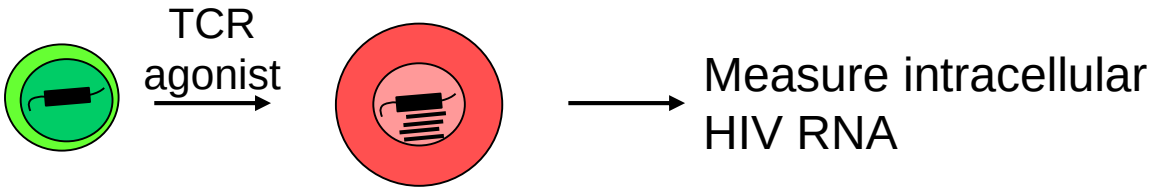


- Viral outgrowth assay (VOA)

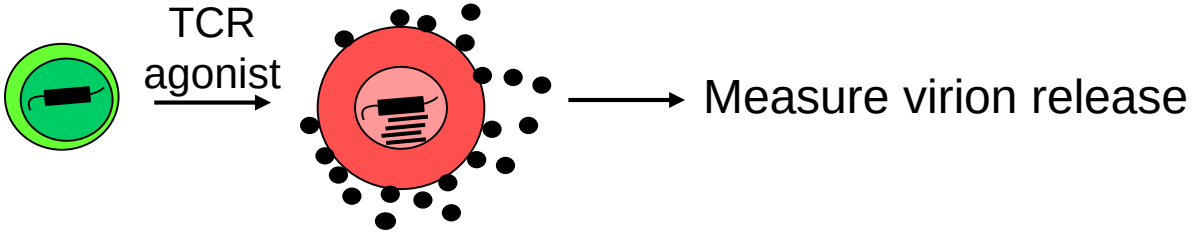


- DNA PCR

- Induction of HIV RNA



- Induction of virion production



An assay for latently infected cells

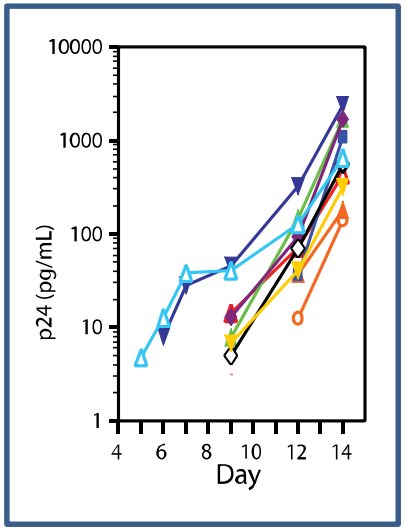
180-200
ml blood

Purified resting
CD4⁺ T cells

PHA + irradiated
allogeneic PBMC

$5x10^6$ 10^6 $2x10^5$ $4x10^4$ $8x10^3$

1/1,000,000



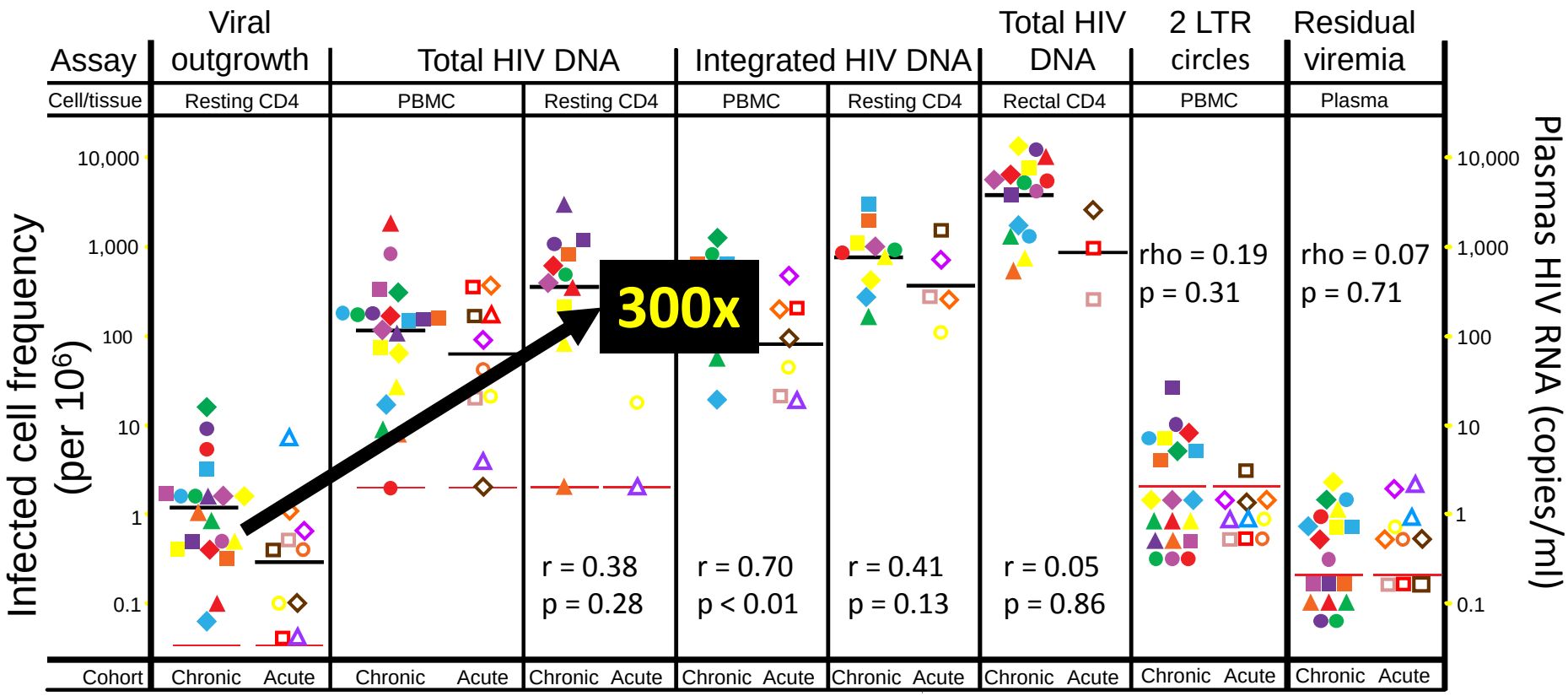
d2: add CD4⁺
lymphoblasts
from HIV-
donors

d7: add CD4⁺
lymphoblasts
from HIV-
donors

p24
Ag

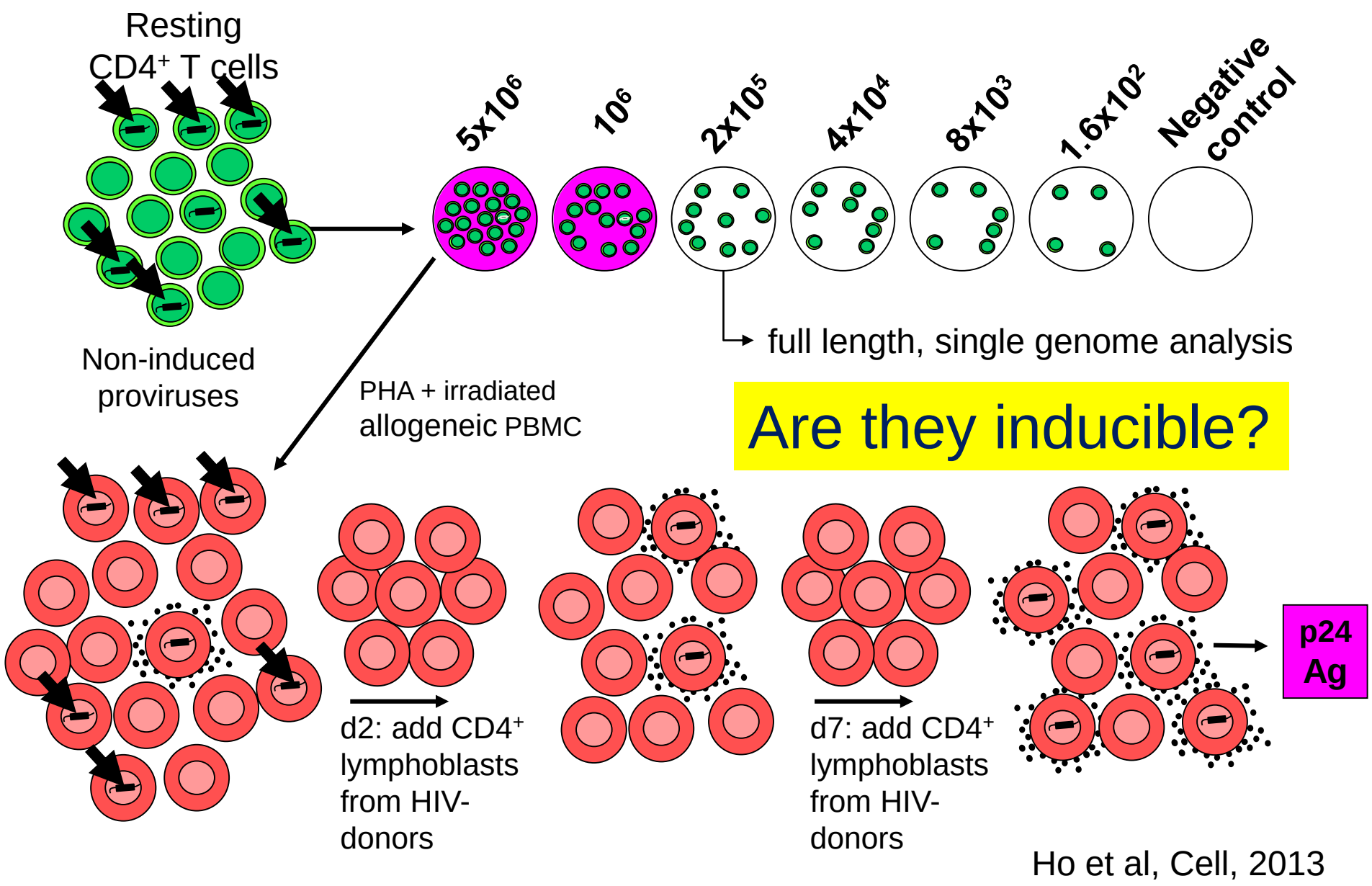
Chun et al., Nature, 1997
Finzi et al., Science, 1997

Viral outgrowth vs PCR assays



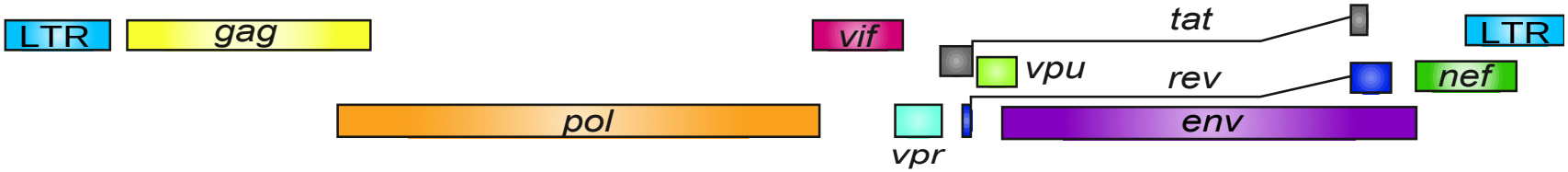
Eriksson et al, PLOS Pathogens, 2013

Non-induced proviruses



Ho et al, Cell, 2013

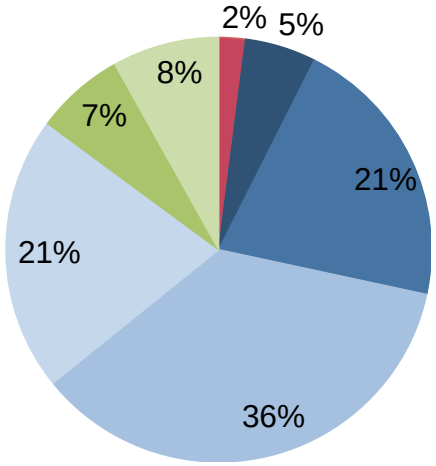
ART initiated in chronic infection



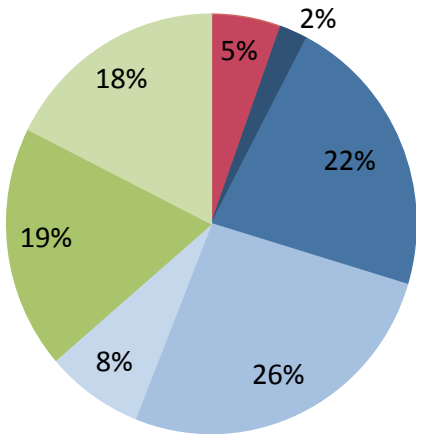
ART initiated during acute infection

Landscape of HIV proviruses

ART during chronic infection



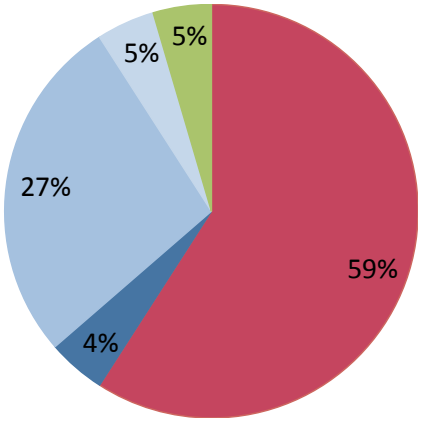
ART during acute infection



Key:

- Intact
- Hypermutated
- Hypermutated and deleted
- Packaging signal deletion
- Very large internal deletion
- Deletion at 3’ end of genome
- Deletion at 5’ end of genome

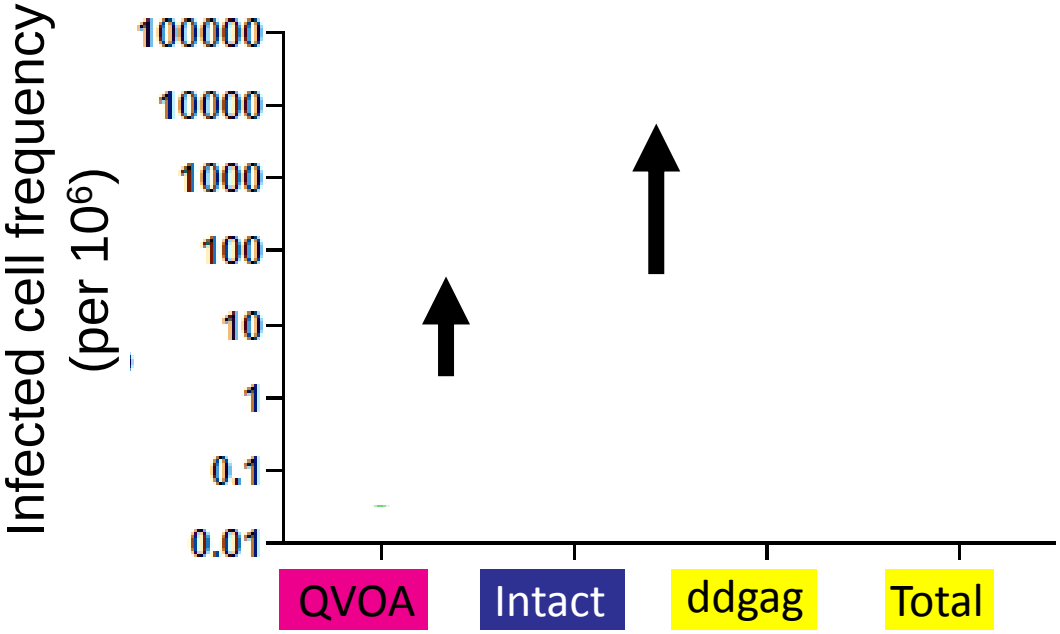
Single round of infection

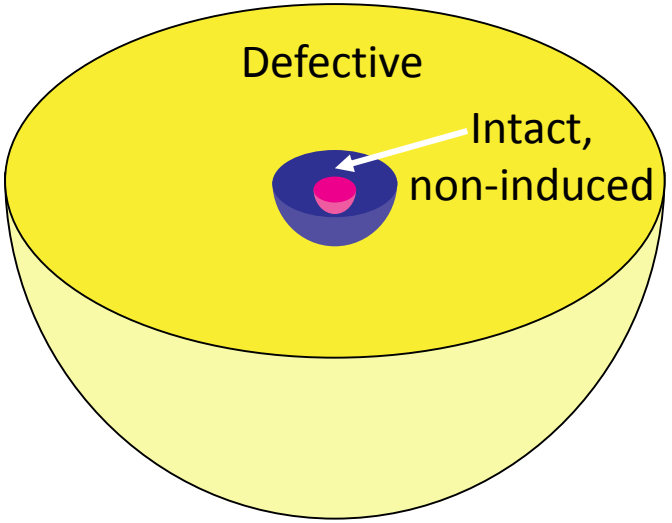


- Arise during (-) strand synthesis
- Not in plasma virus
- Missed by subgenomic PCR

Bruner et al, Nature Med 2016

QVOA, intact, and total proviruses

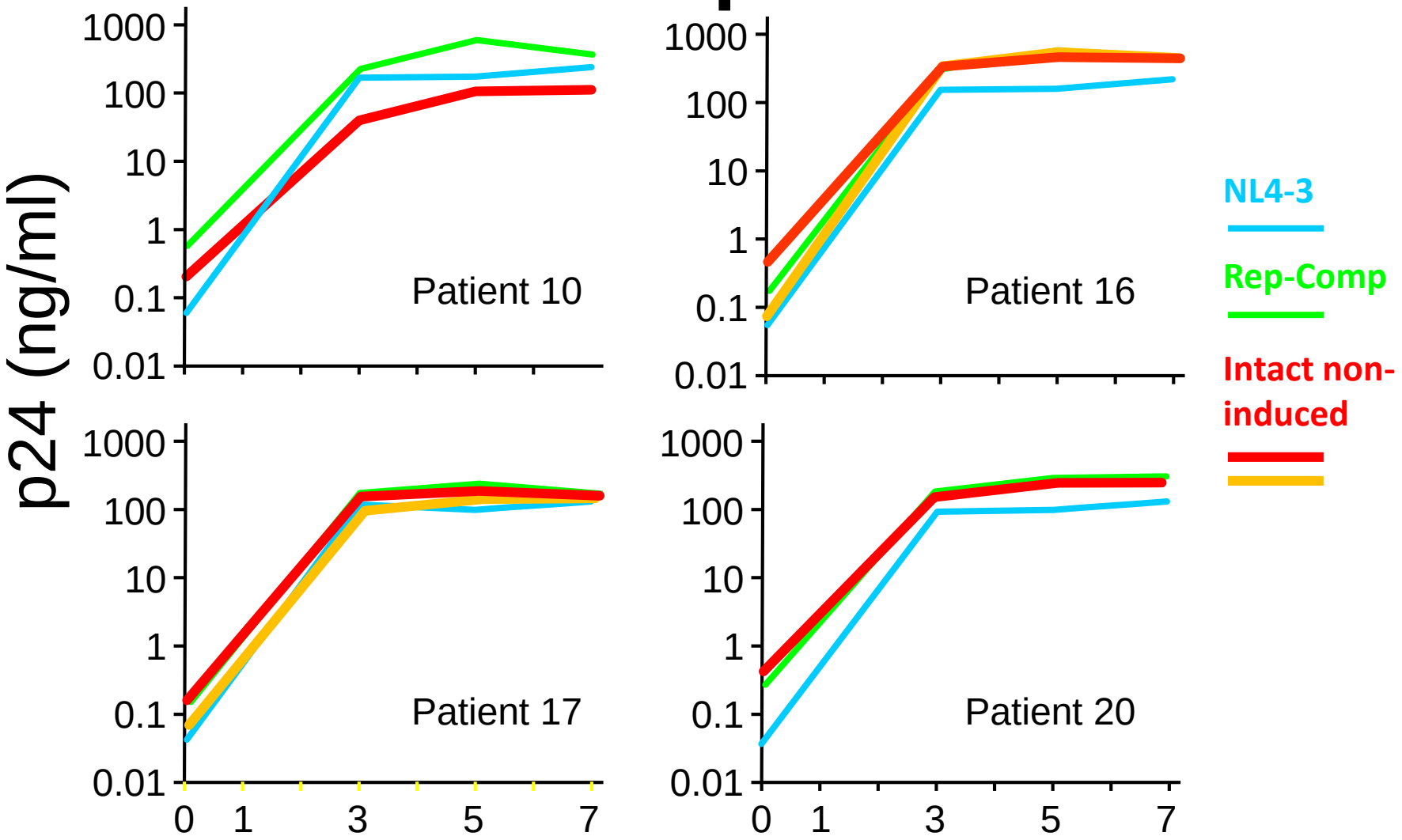




- Are they replication-competent?
- Can they be induced *in vivo*?

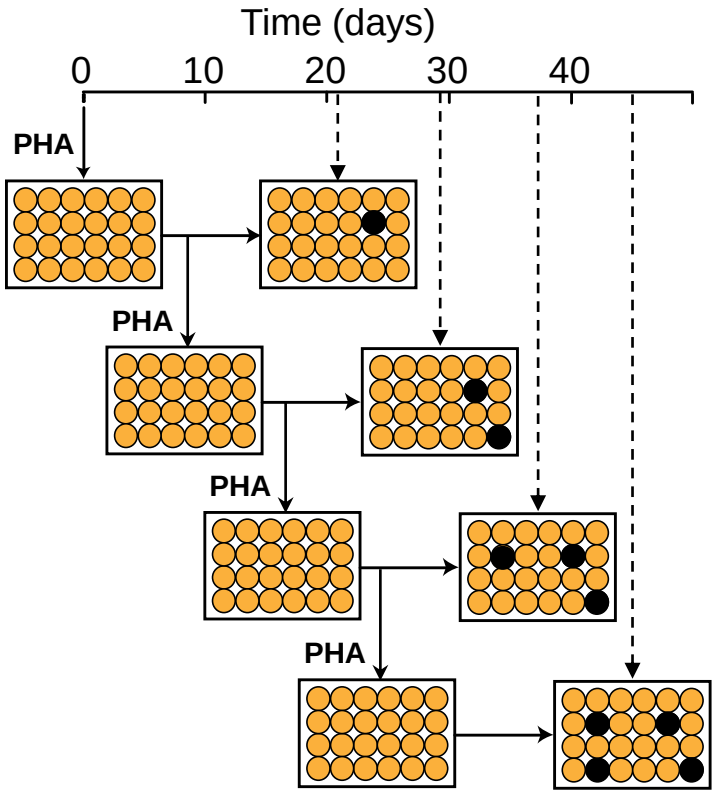
Ho et al Cell, 2013
Bruner et al, Nature Med 2016

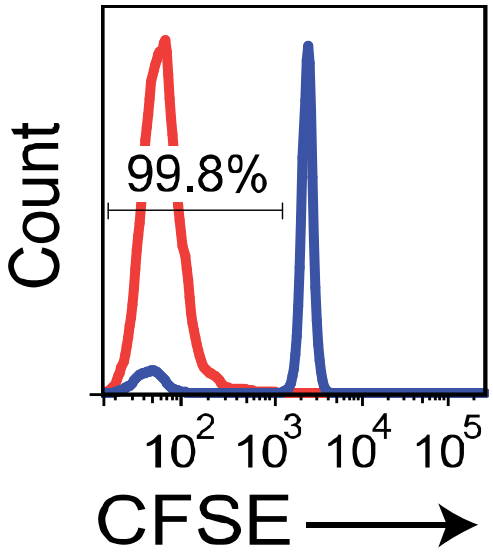
Replication capacity of intact non-induced proviruses



Ho et al, Cell, 2013

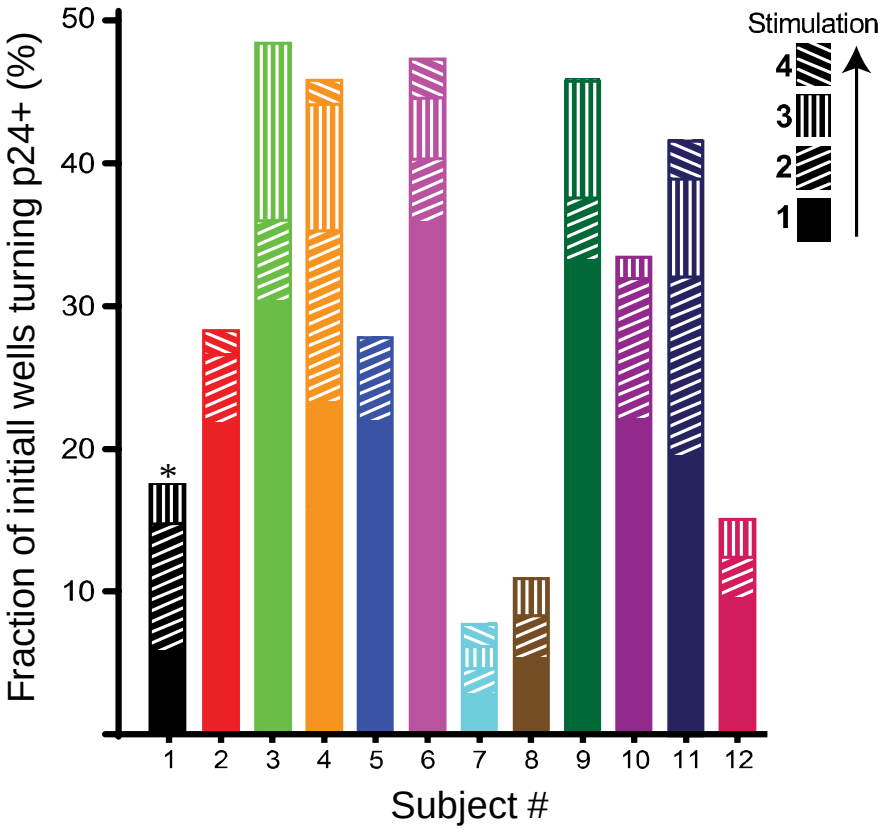
Can intact non-induced proviruses be induced?

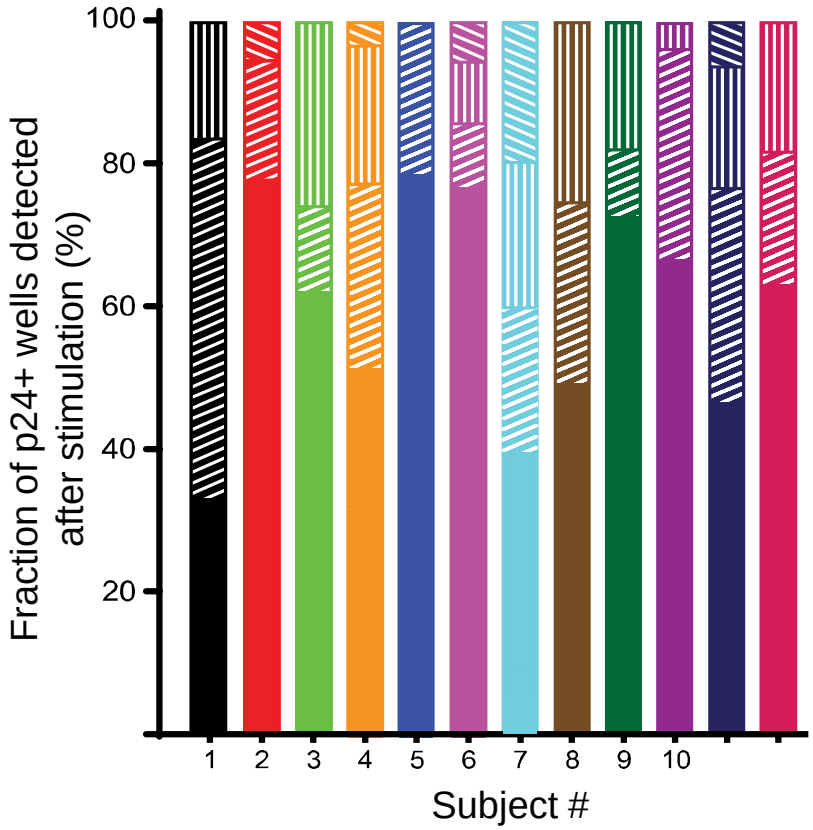




Ho et al Cell, 2013
Hosmane et al, JEM in press

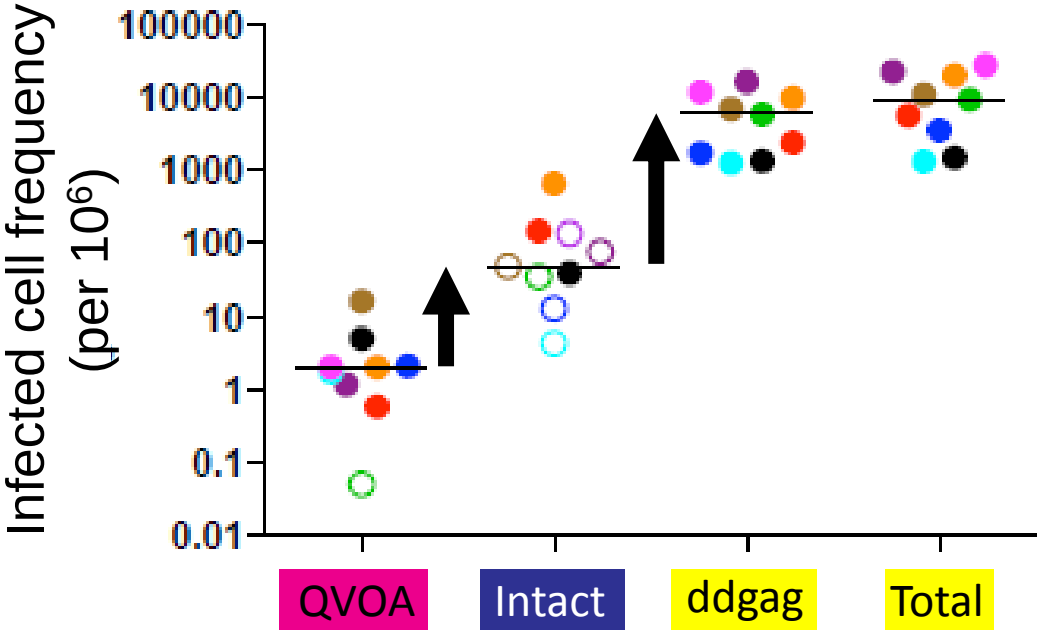
Repetitive stimulation induces additional proviruses

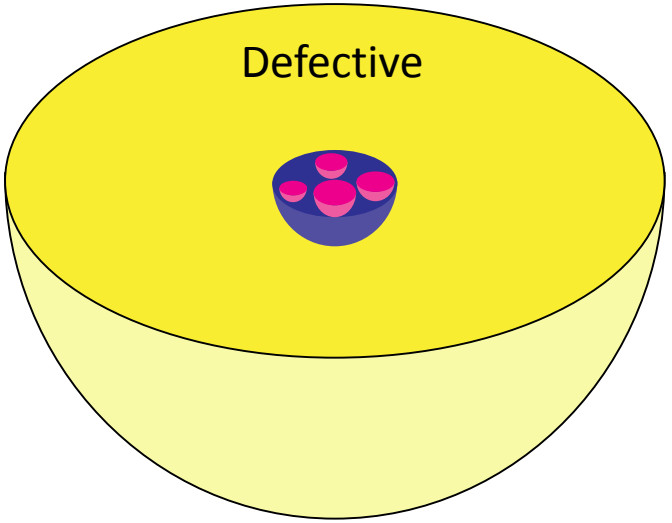




Ho et al Cell, 2013
Hosmane et al, JEM in press

QVOA, intact, and total proviruses

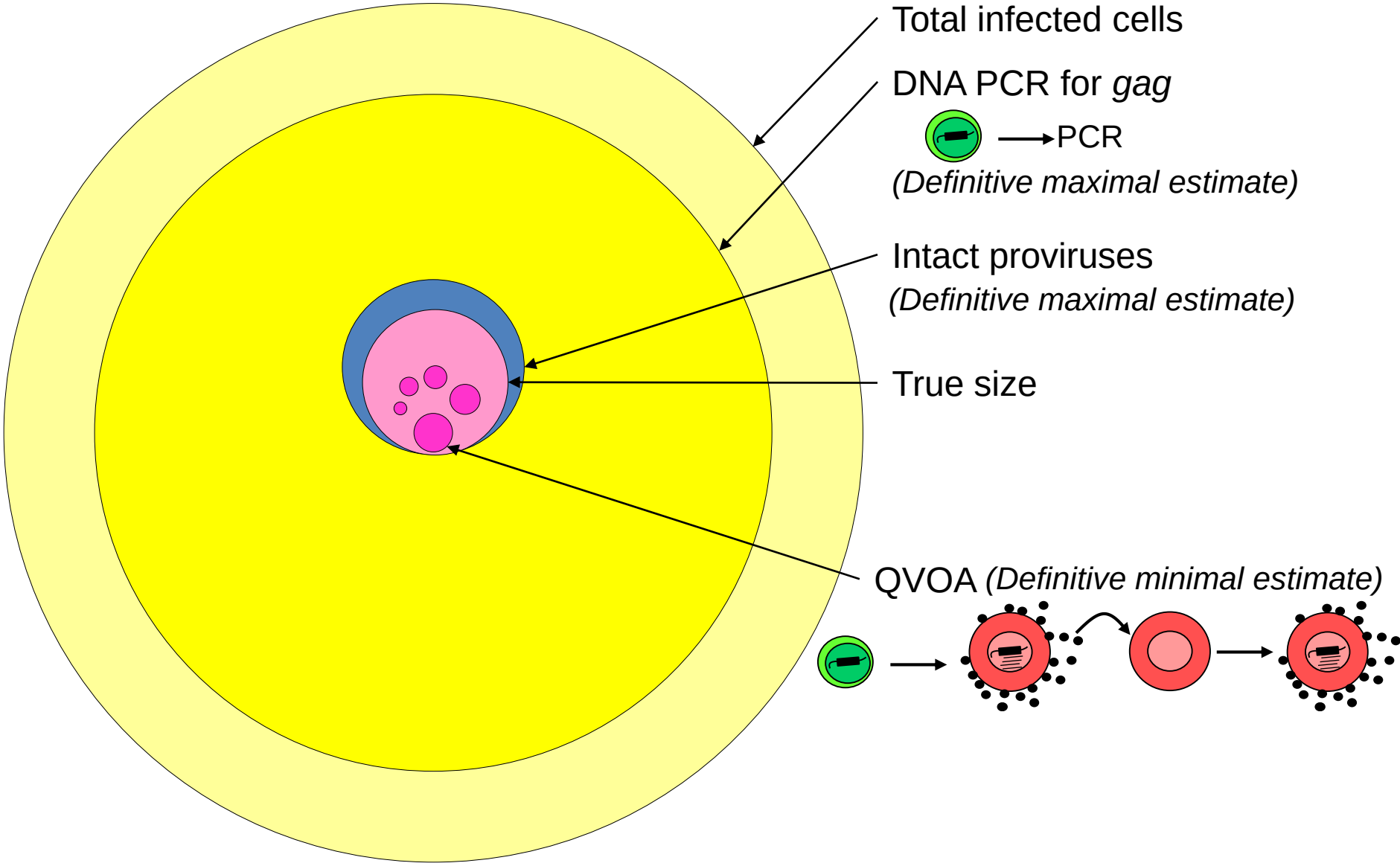




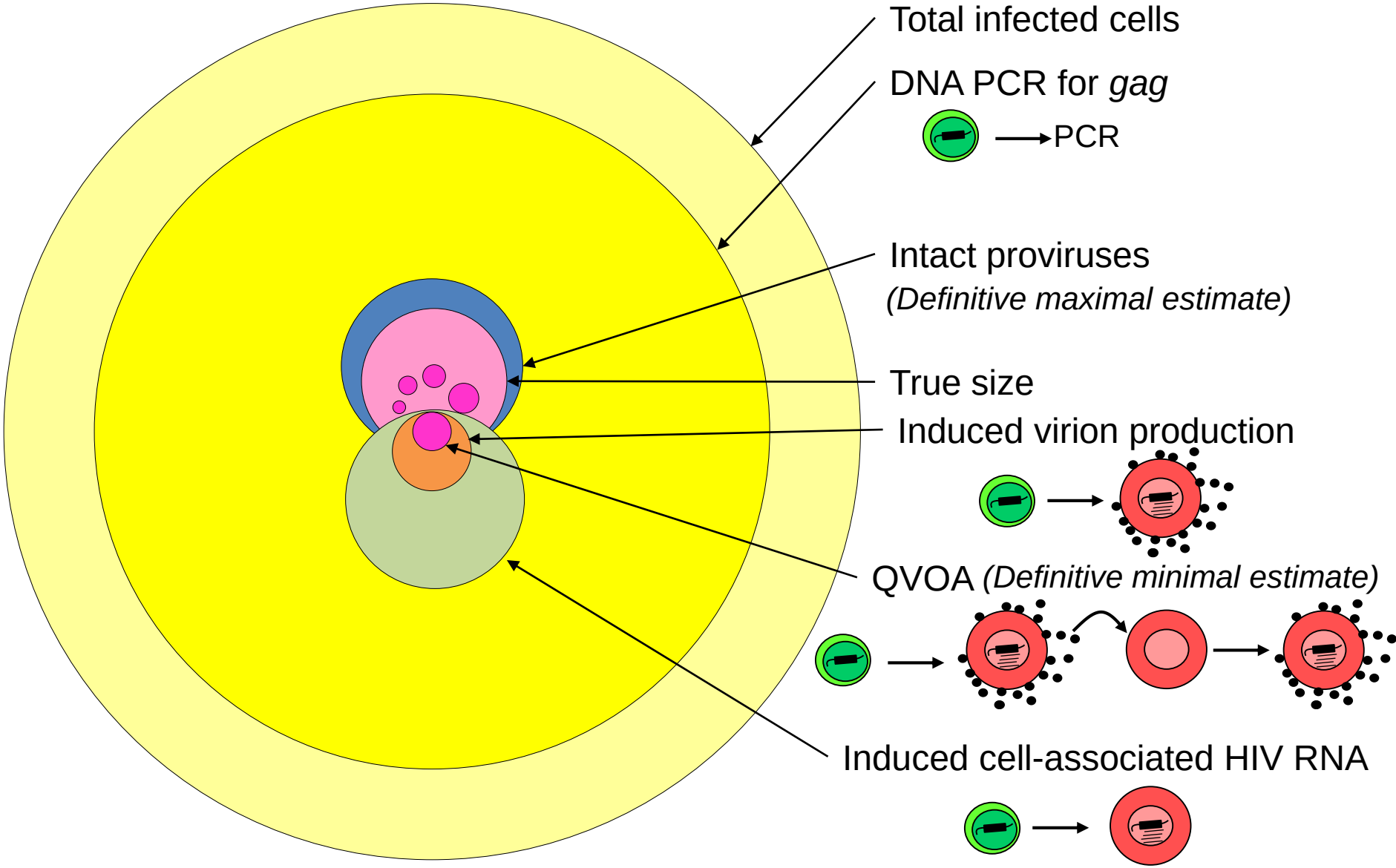
- Each round of stimulation induces additional proviruses
- A single round of maximal T cell activation does not induce all latent proviruses
- The number of intact proviruses provides a much more accurate upper limit on reservoir size than standard DNA PCR assays
- We need a scalable assay for intact proviruses to guide clinical trials of cure strategies

Ho et al Cell, 2013
Bruner et al, Nat Med, 2016
Hosmane et al, JEM in press

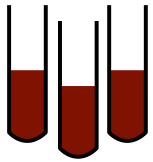
Best assay for latent reservoir?



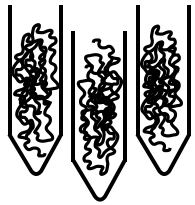
Best assay for latent reservoir?



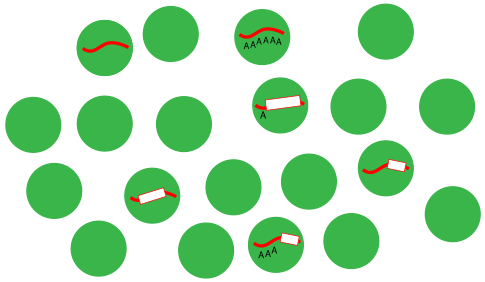
ddPCR assay for intact proviruses



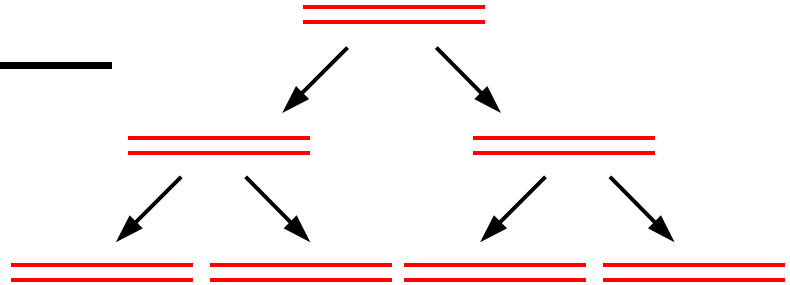
Take blood from HIV+ patients



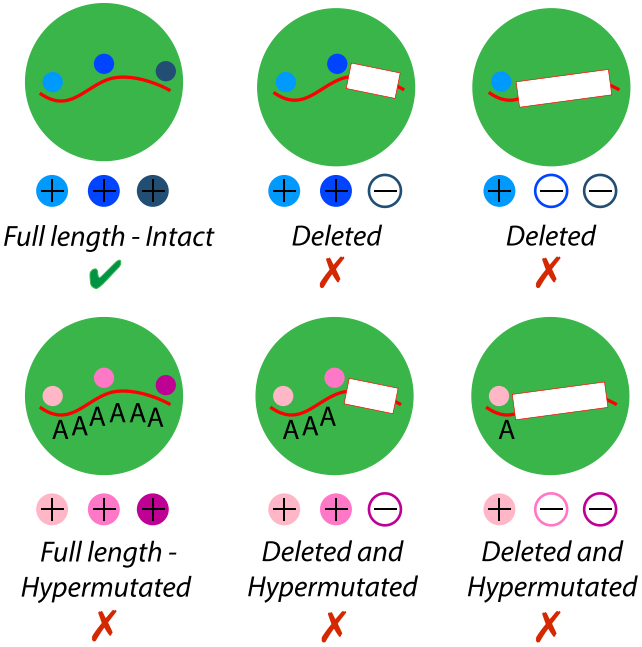
Extract DNA from resting CD4+ T Cells



Droplet Formation



PCR



Detection and Analysis

Analysis

6 total proviruses

- 1 intact

-2 deleted

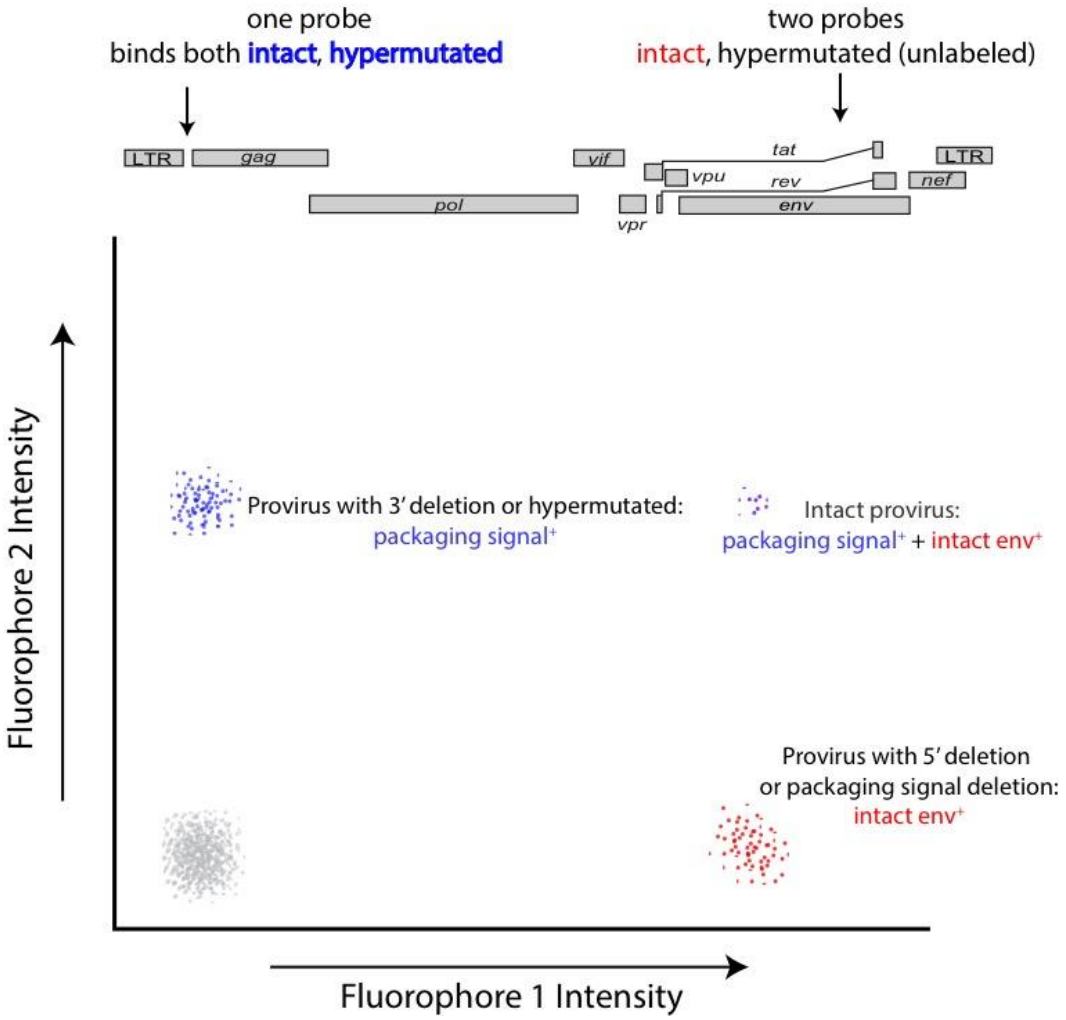
-1 full length hypermutated

-2 hypermutated and deleted

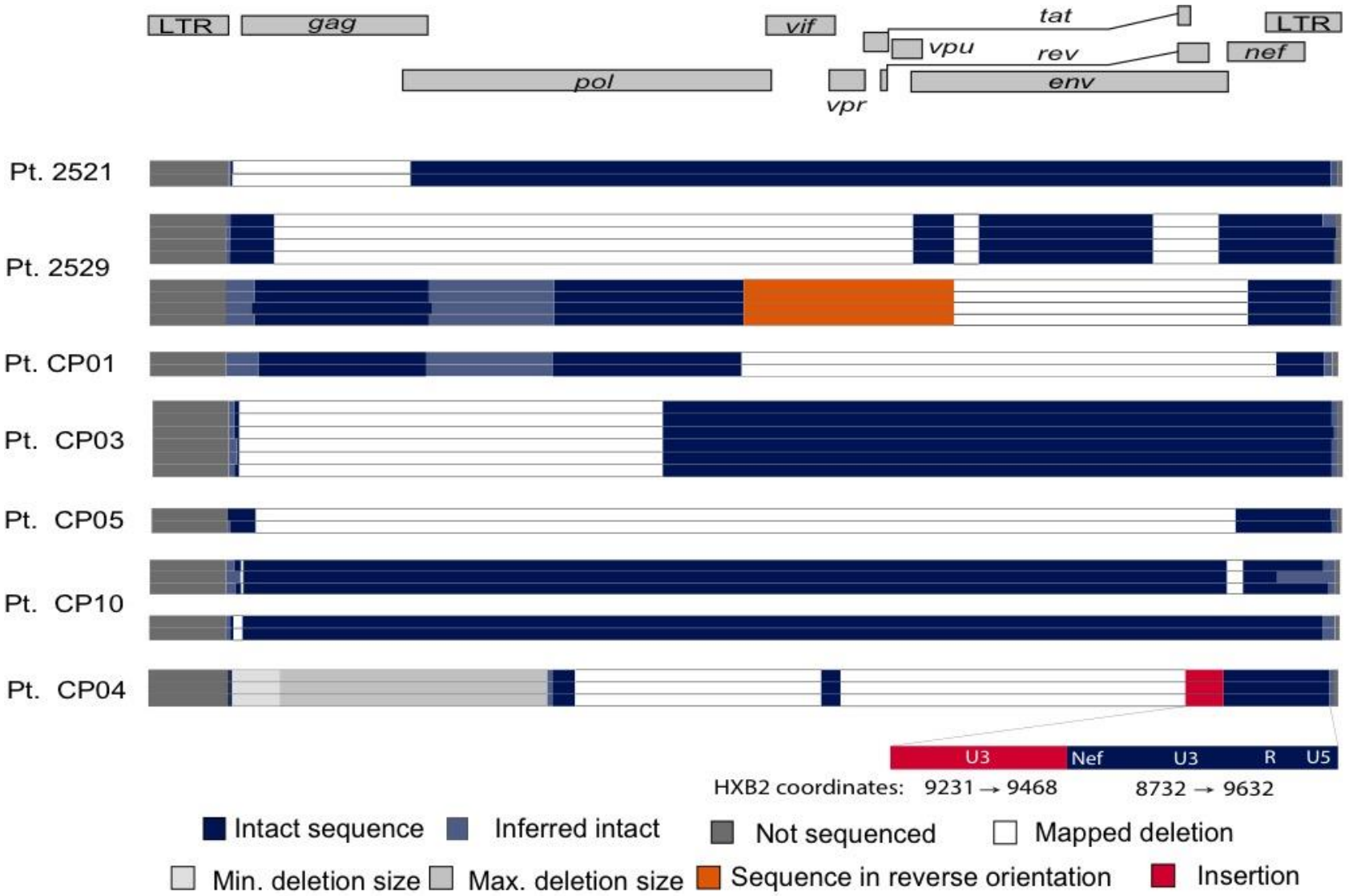
✔ = *intact*

✗ = *defective*

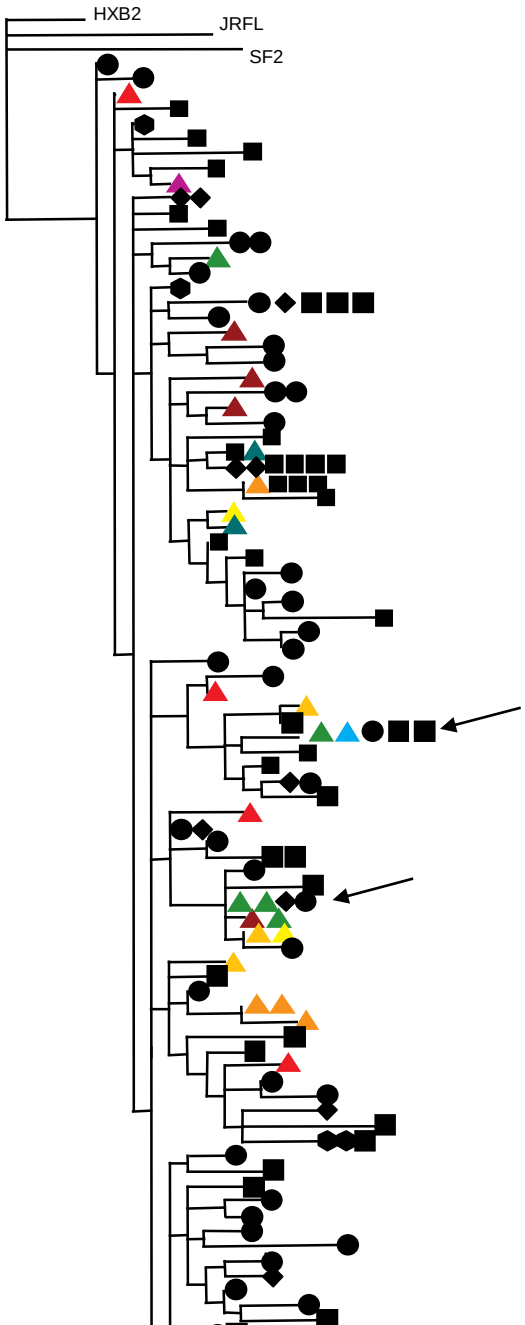
Sample results on patient samples

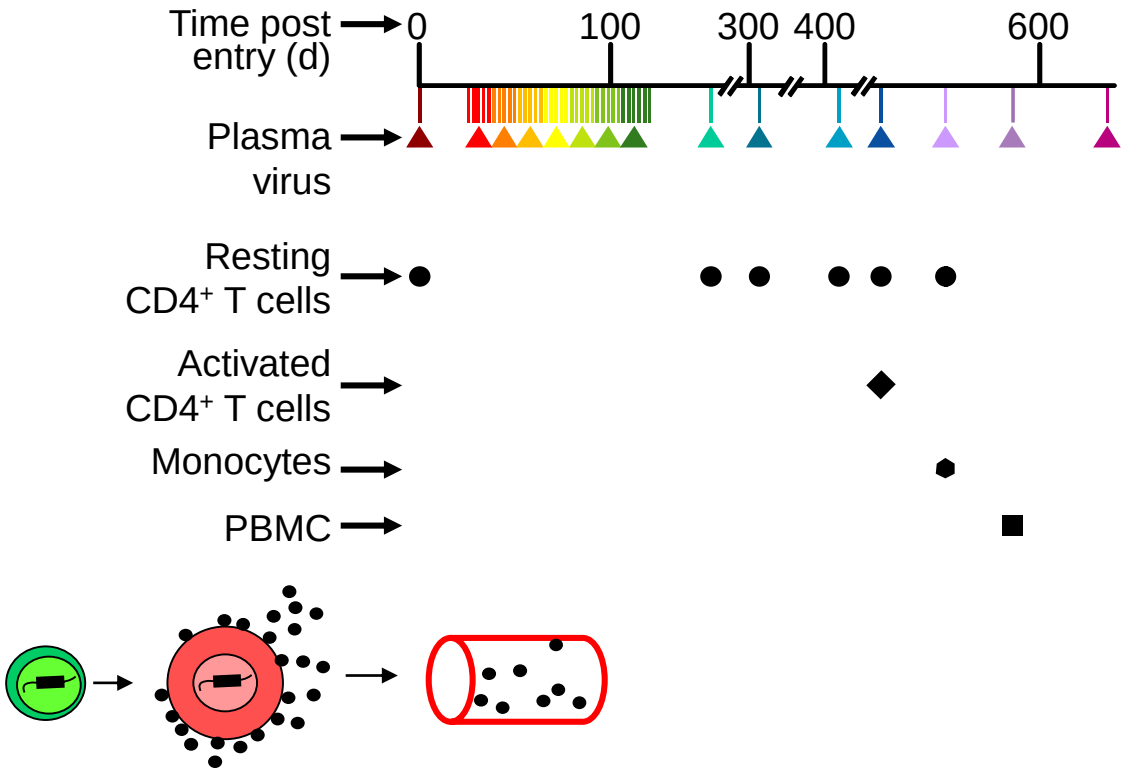


Expanded clones with major defects

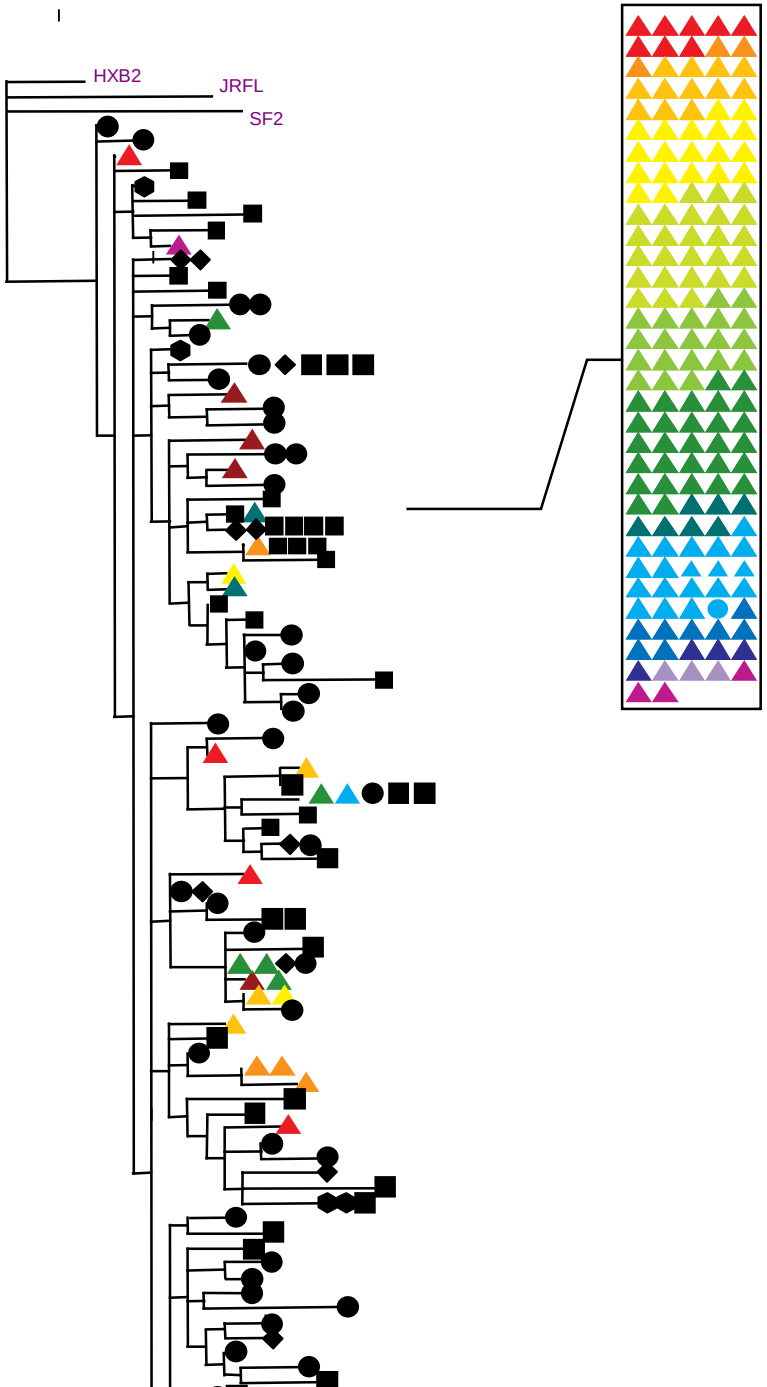


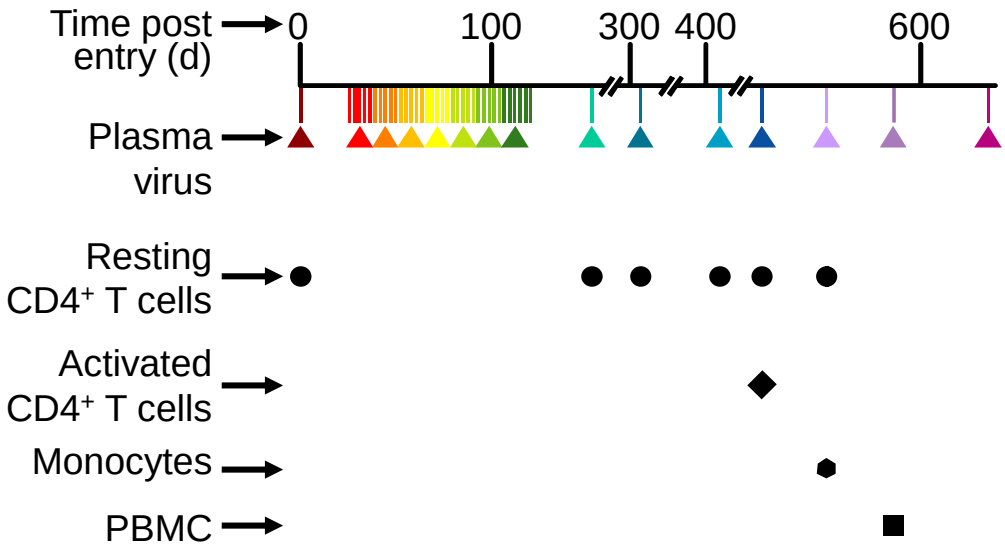
Bruner et al, Nat Med 2016





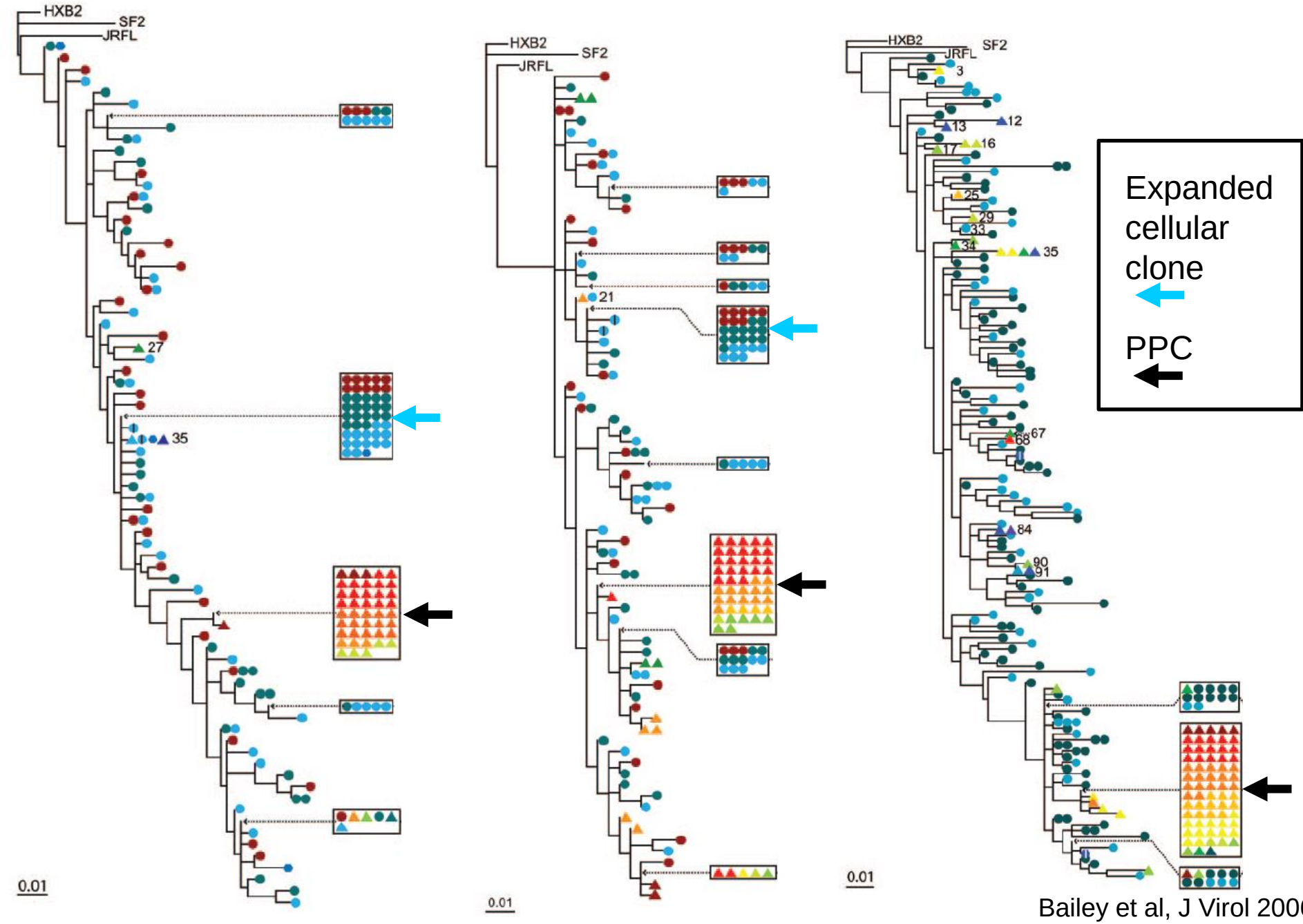
Bailey et al, J. Virol., 2006





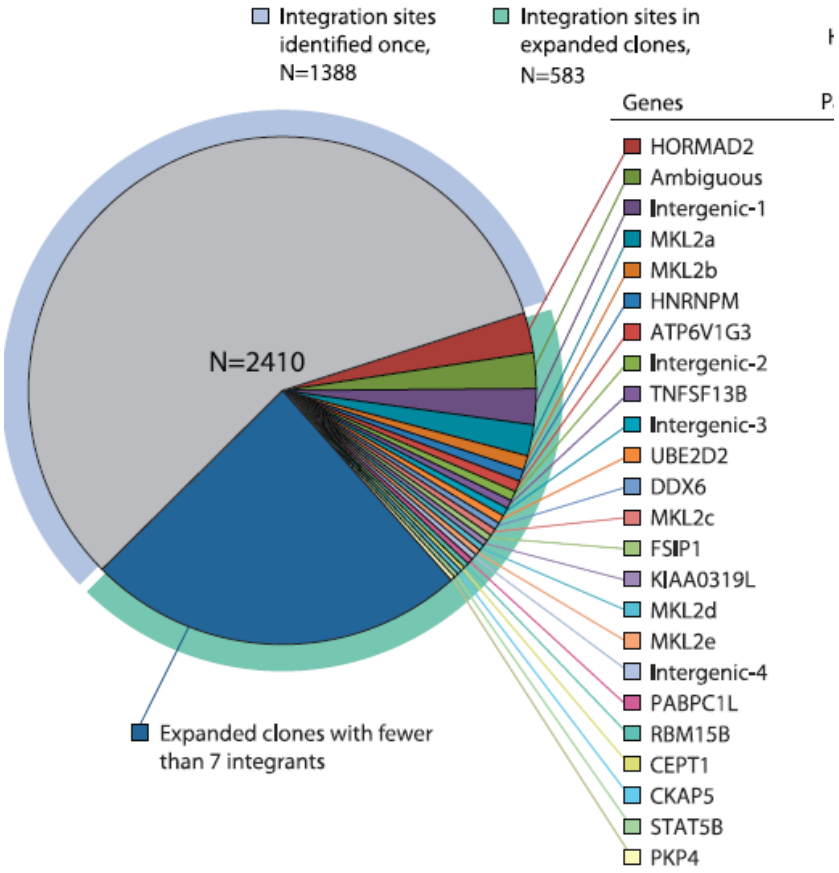
- In half of patients studied, residual viremia is dominated by a small number of clones
- These sequences do not show evidence of sequence evolution.
- These sequences appear to represent clonal expansion of individual infected cells

Bailey et al, J. Virol., 2006

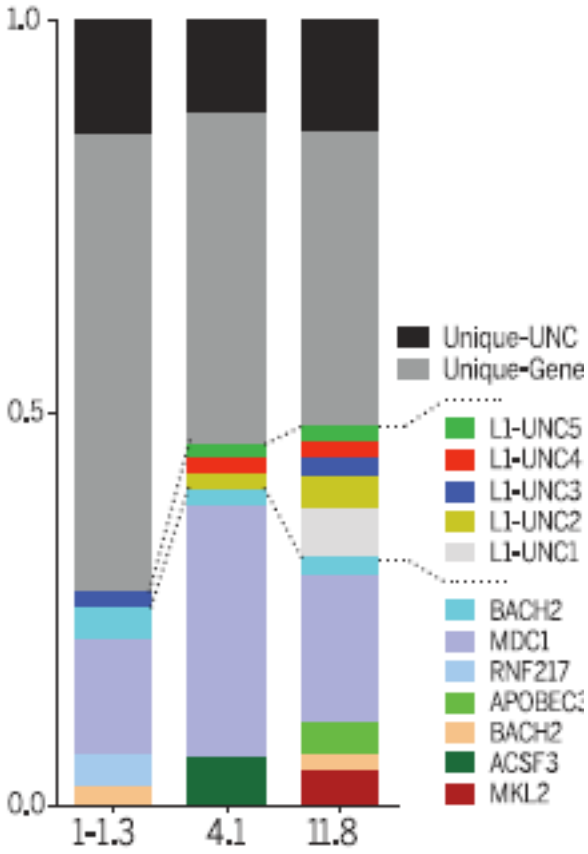


Bailey et al, J Virol 2006

Clonal expansion detected by integration site analysis



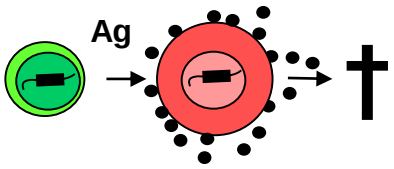
Maldarelli et al, Science, 2014



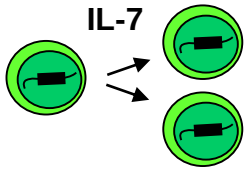
Wagner et al, Science, 2014

Proliferation of infected cells

- Antigen drives T cell proliferation but also induces viral gene expression. Productively infected cells die quickly.

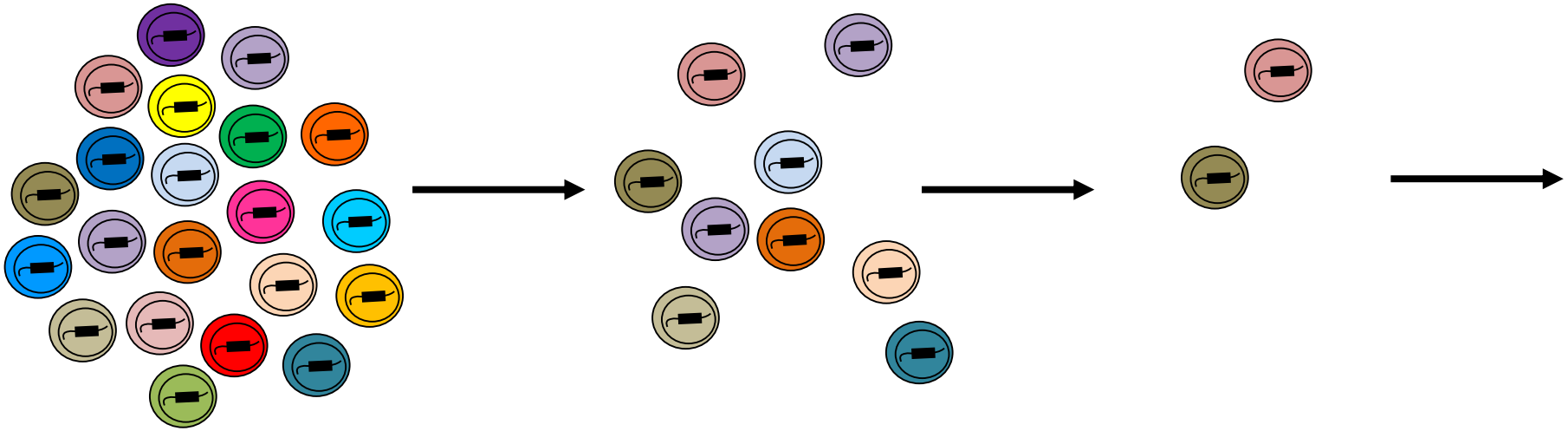


- Cytokines like IL-7 can drive homeostatic proliferation of memory T cells, possible expanding the reservoir, but may also reverse latency.

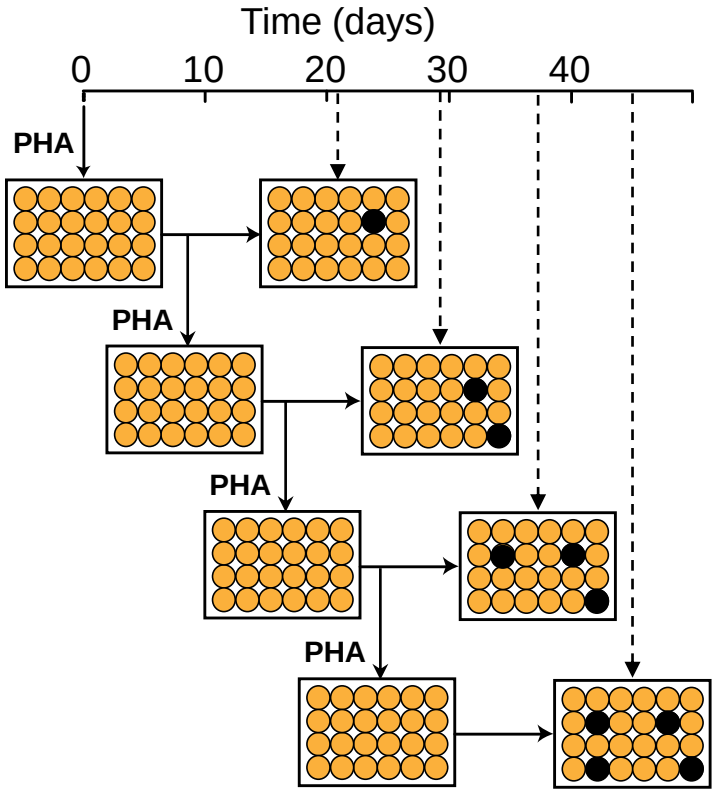


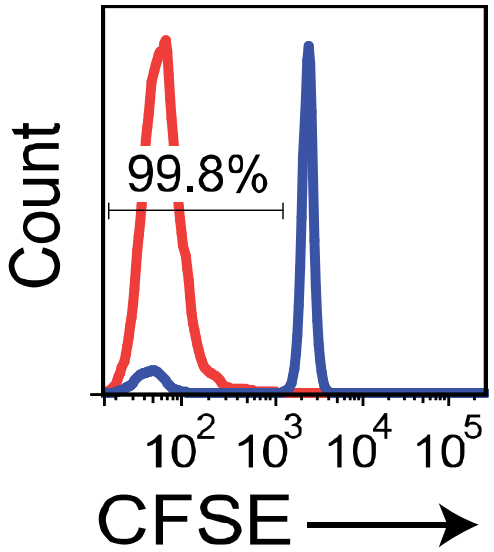
Fundamental assumption of cure strategies

- Generation of new latently infected cells is completely stopped by ART
- Therefore, reductions induced by curative strategies are stable
- Repeat cycles may lead to cure

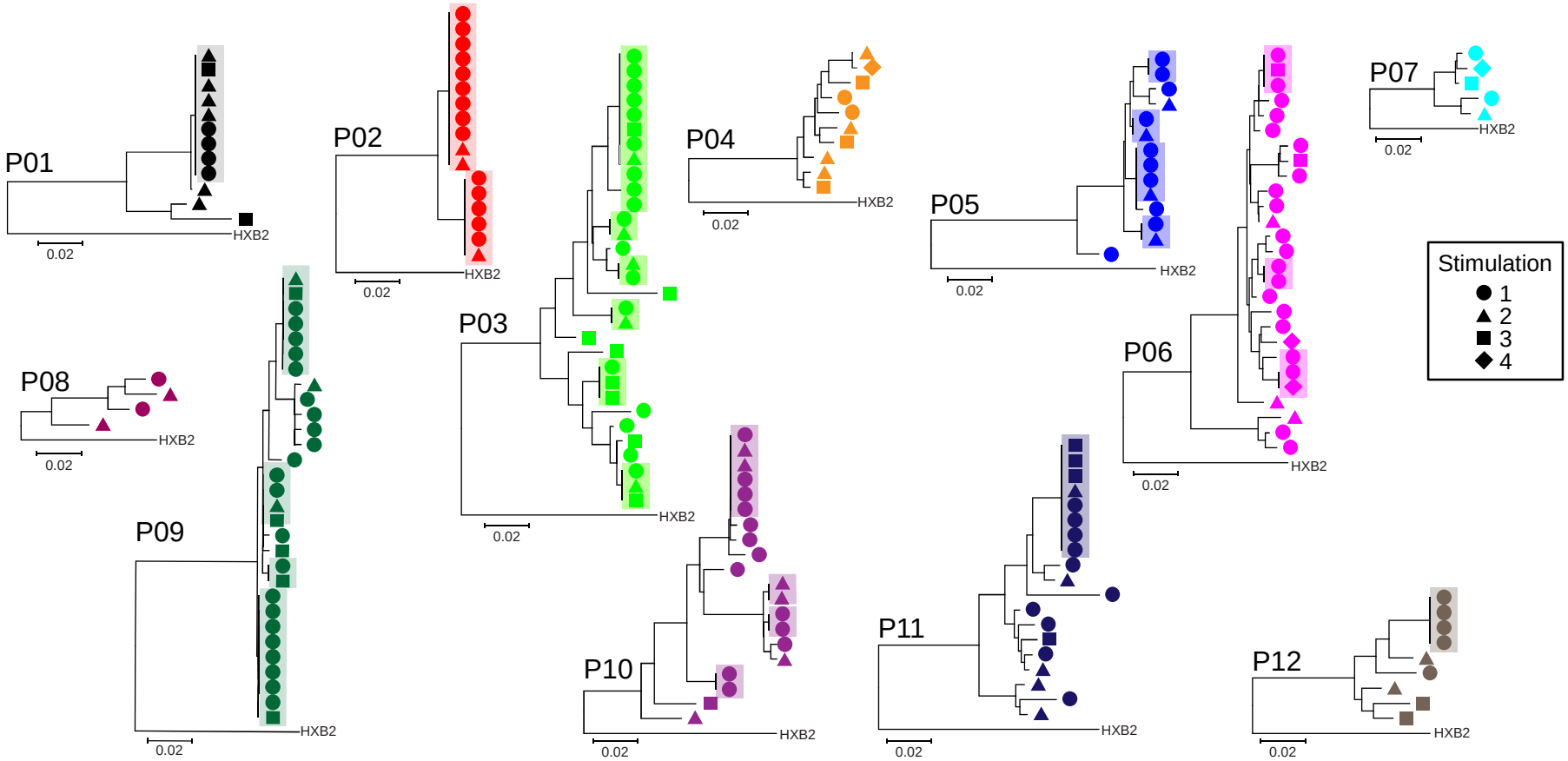


In vitro proliferation of latently infected cells

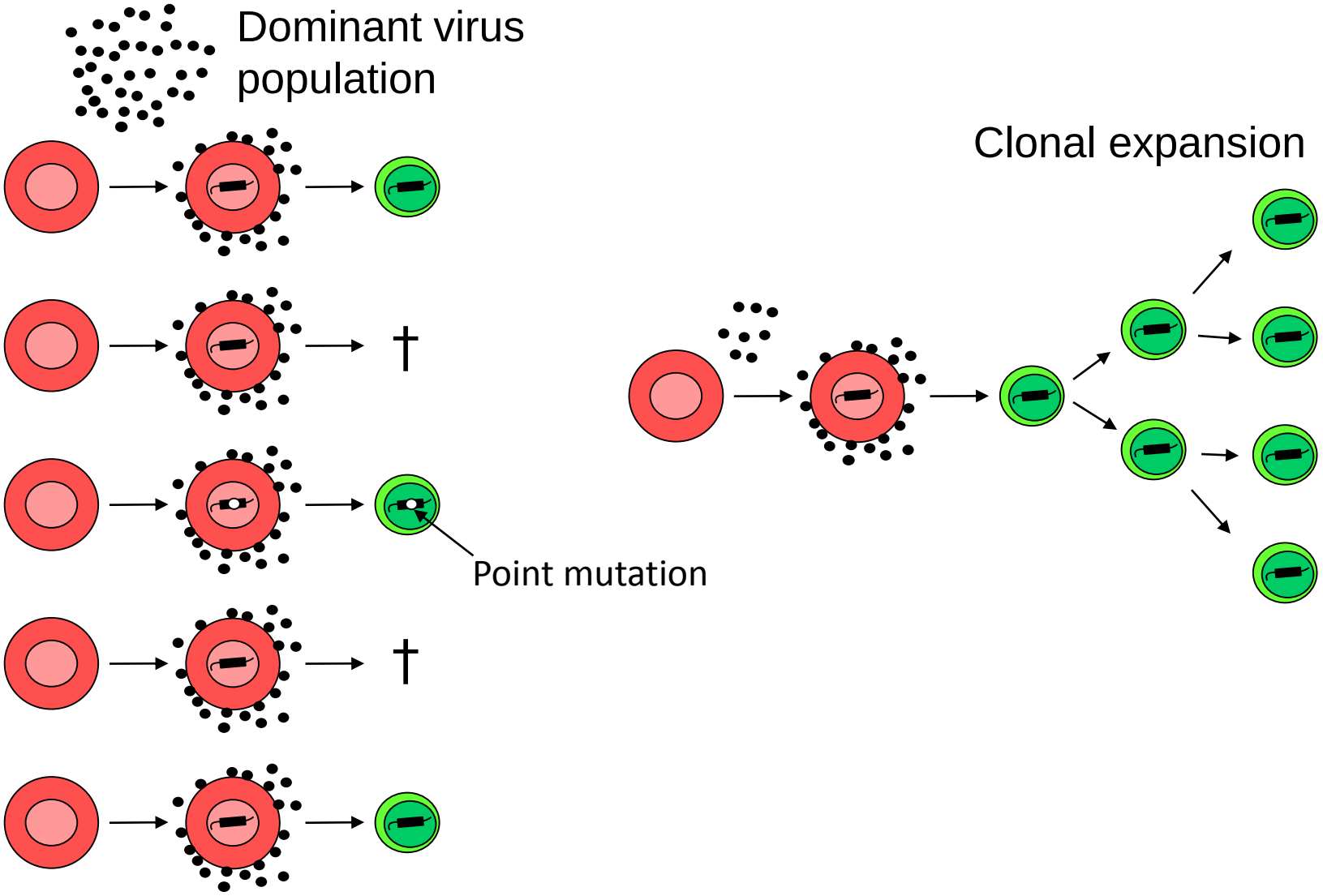




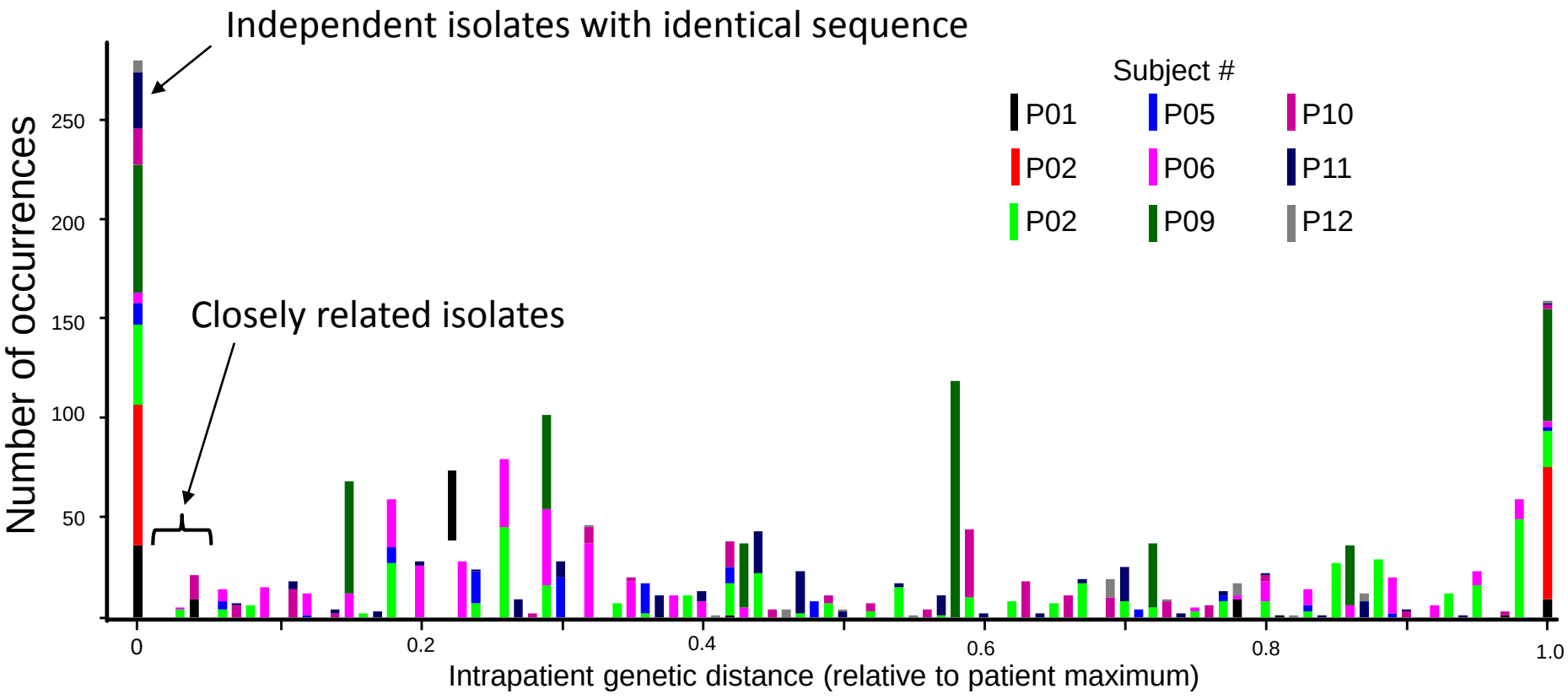
Independent isolates of replication-competent HIV with identical sequence



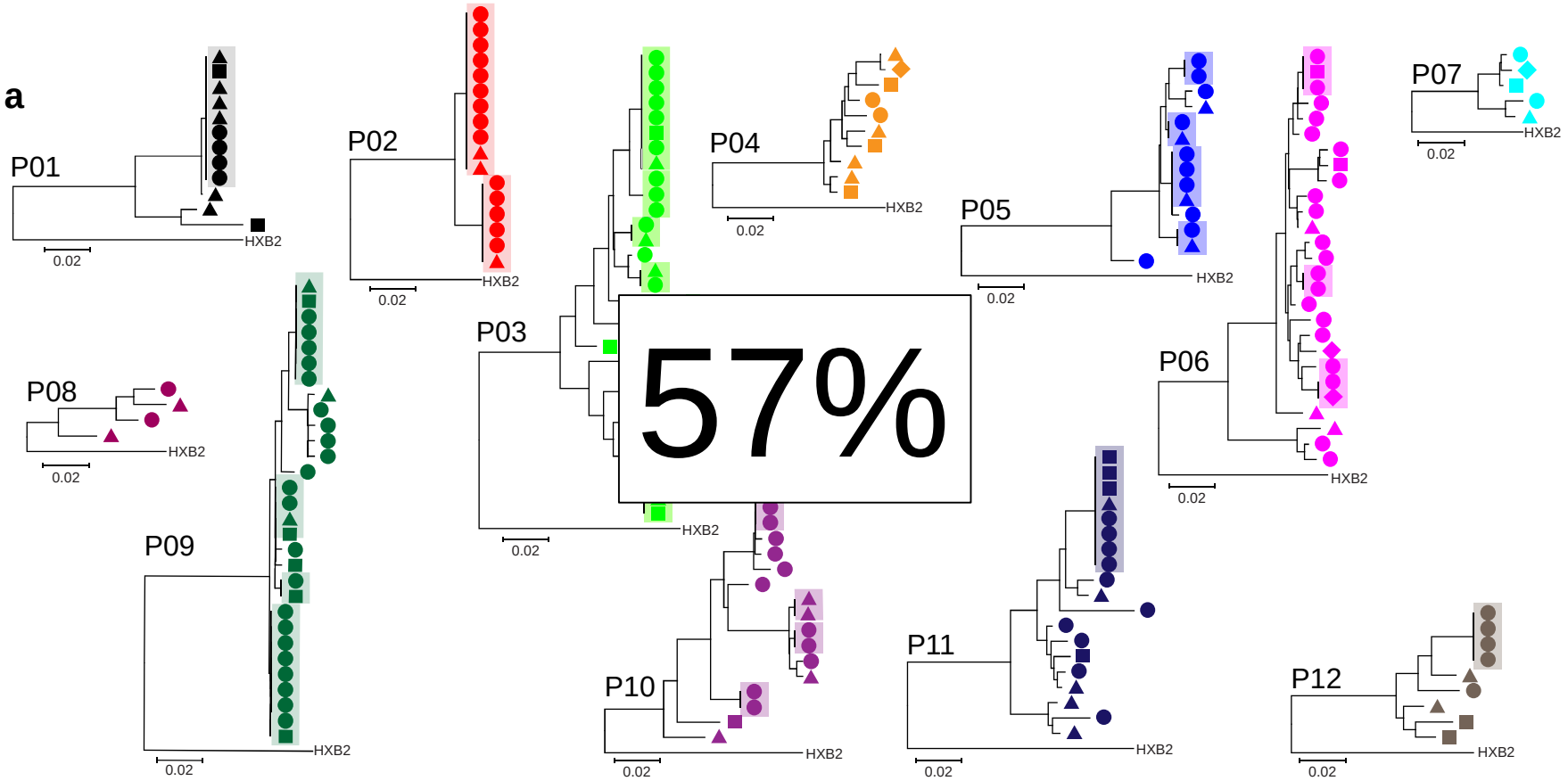
Hypotheses to explain identical isolates



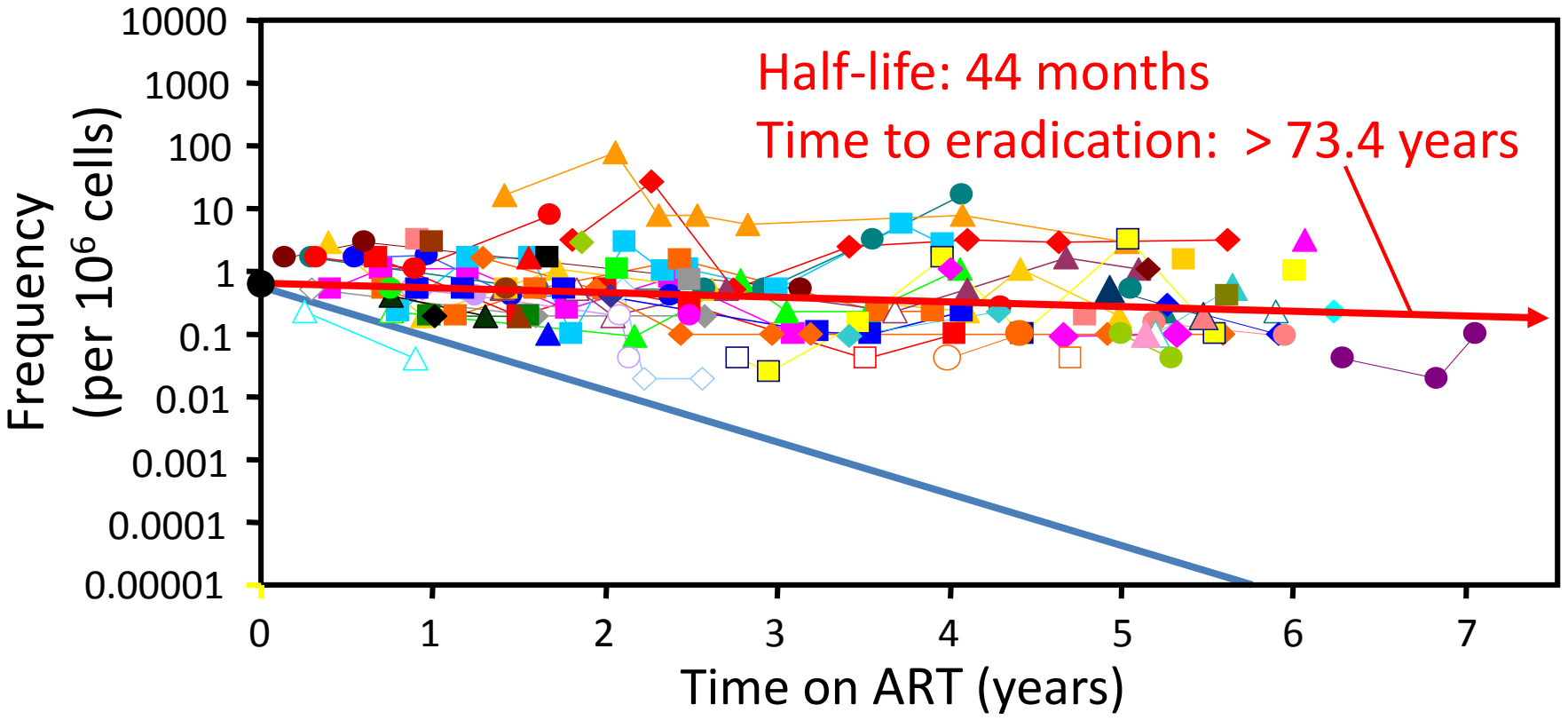
Intrapatient genetic distances between isolates



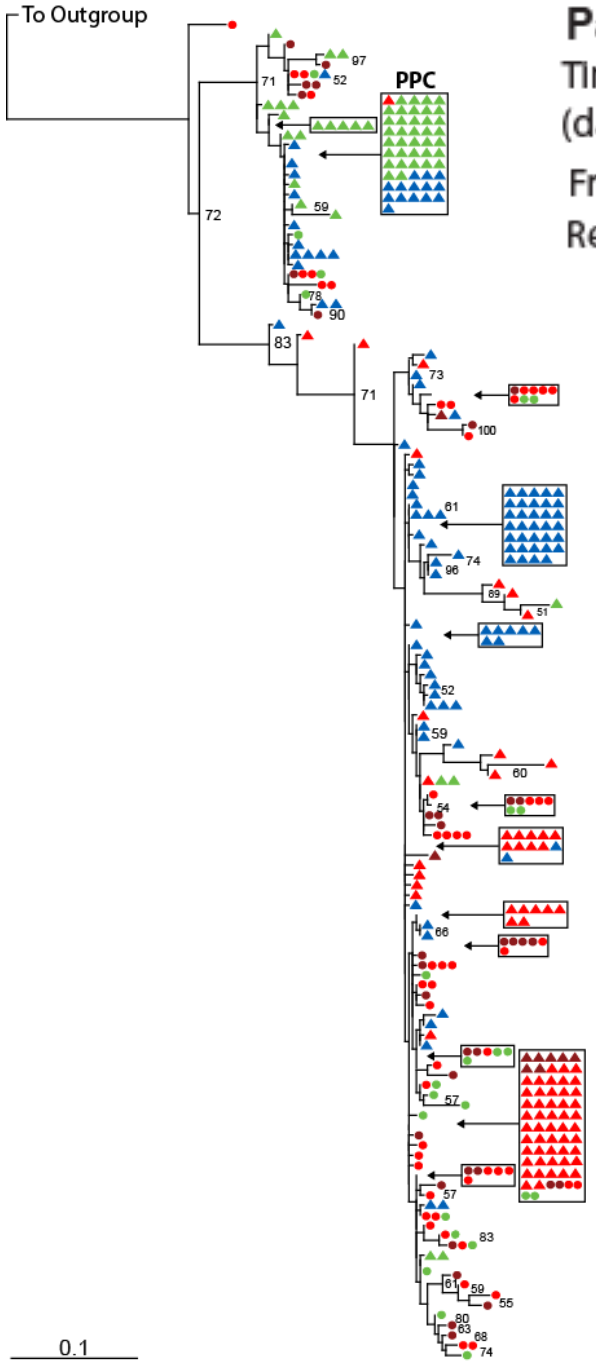
Expanded cellular clones account for the majority of the reservoir

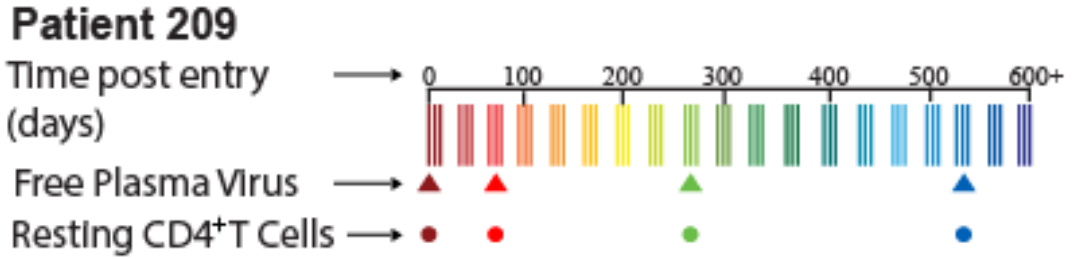


Slow decay may reflect more rapid decay balanced by proliferation



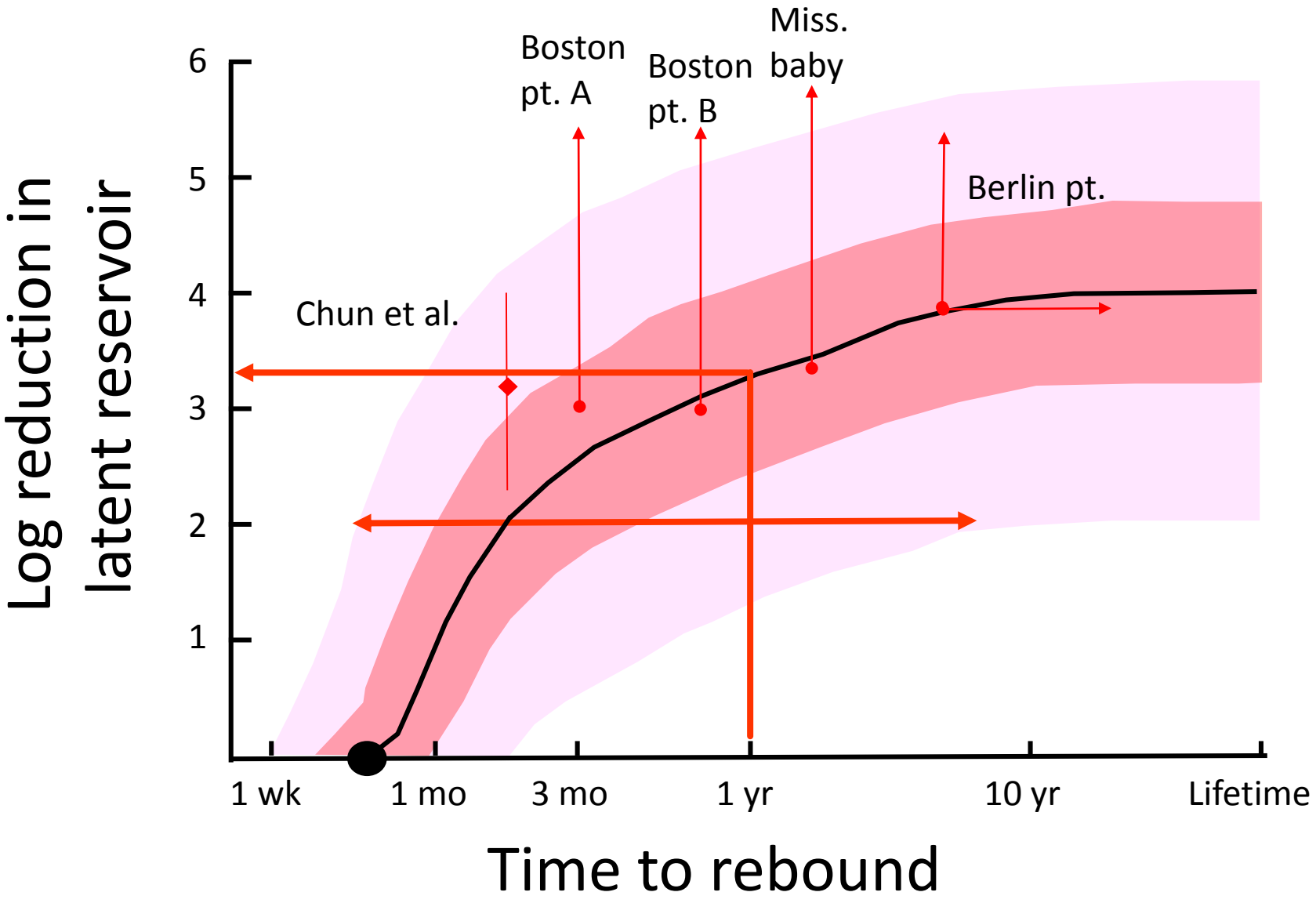
Finzi et al., Nature Med., 1999
Siliciano et al., Nature Med., 2003





- Predominant plasma clones wax and wane over time
- Consistent with antigen driven proliferation rather than a general homeostatic process or a cell autonomous proliferative stimulus based on integration site

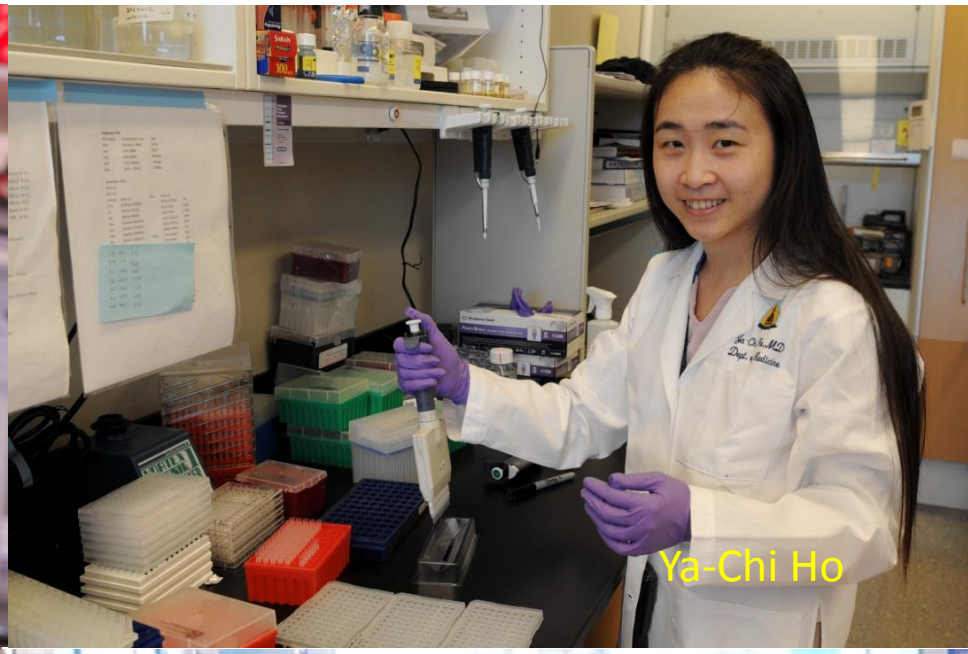
Time to rebound



Hill et al, PNAS 2014

Thanks


Janet Siliciano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Thanks

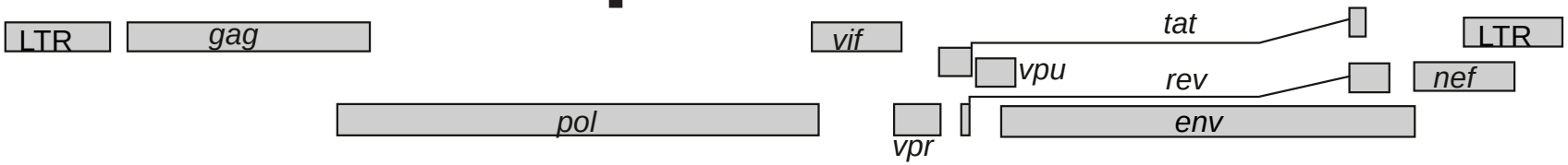
Collaborators

Steve Deeks
Doug Richman
Brad Jones
Richard Flavell
Dave Margolis
Joel Gallant
Joe Cofrancesco
Jon Karn
Martin Nowak
Matt Strain
Sarah Palmer
Una O'Doherty
Joe Wong
Steve Yukl
John Mellors

Funding

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

Strategy for unbiased analysis of proviruses



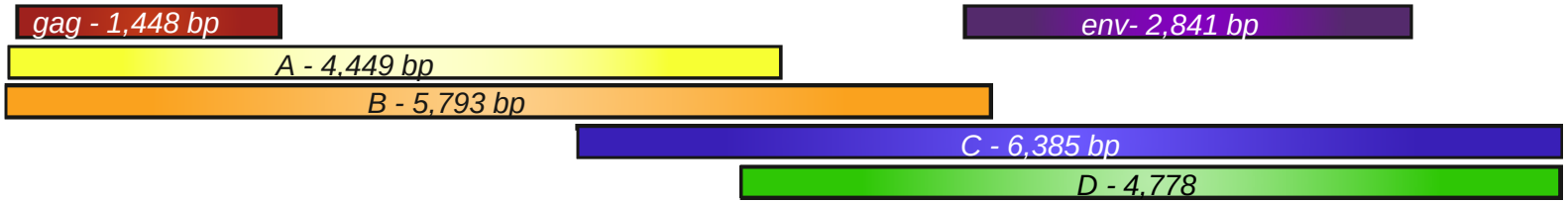
Plus strand

Minus strand

Step 1: Outer PCR from U5 to U5

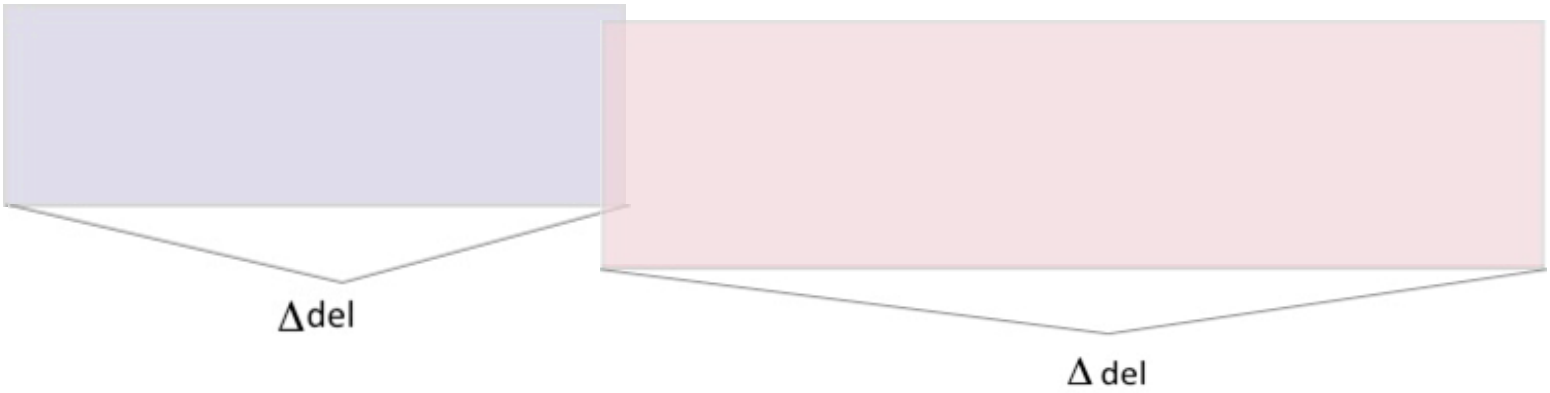
Step 2: *gag* and *env* inner PCRs to confirm clonal dilution

Step 3: Subject all wells to 6 inner PCRs, regardless of positivity for *gag* or *env* inner PCRs



Step 4: Visualize PCRs on a gel and directly sequence products to determine whether a provirus is genetically intact or defective

Methods



Step 4: Visualize PCRs on a gel and directly sequence products to determine whether a provirus is genetically intact or defective

Step 3: Subject all wells to 6 inner PCRs, regardless of positivity for *gag* or *env* inner PCRs