The Genetic Traits of Full-Length HIV sequenced from Memory T Cell Subsets

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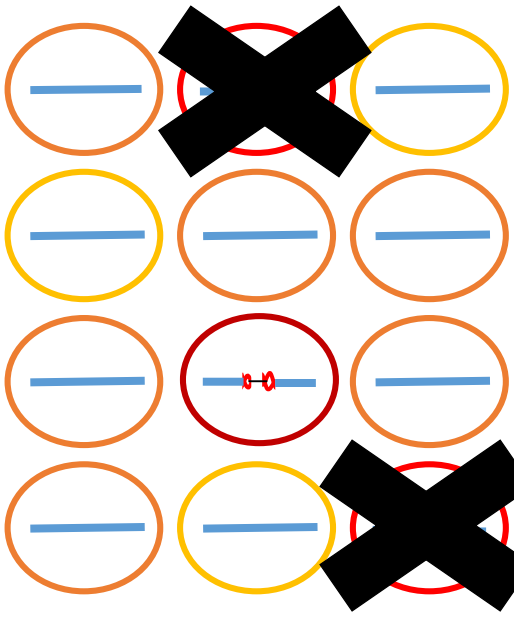
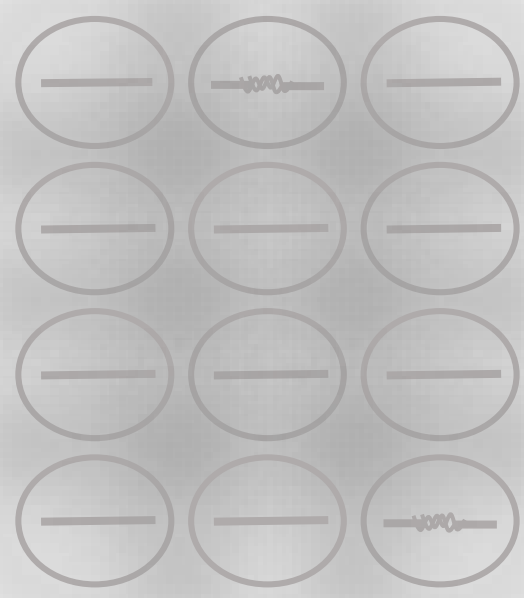
In an ideal world:



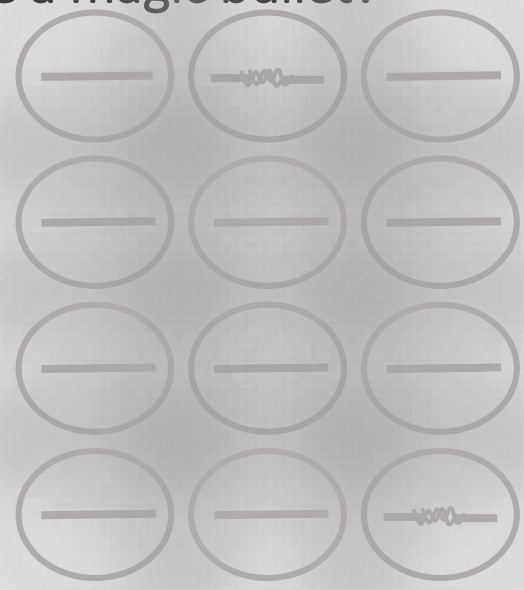
… We don't live in an ideal world

We know the reservoir is established early, and harboured largely by memory CD4+ T cells



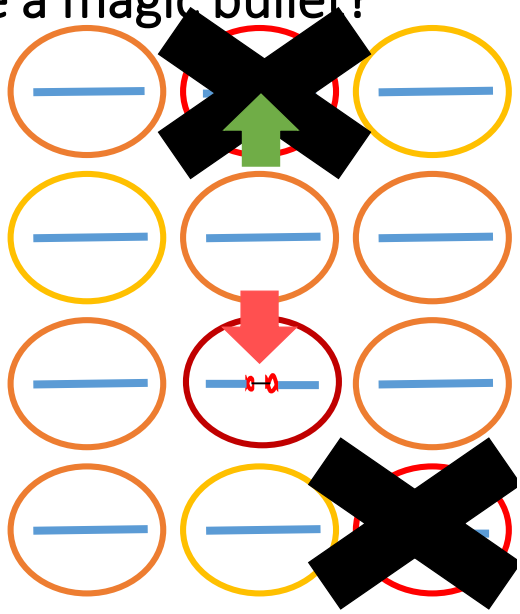
How can we make a magic bullet?

- What do we want to hit?
  - Where is the intact virus?

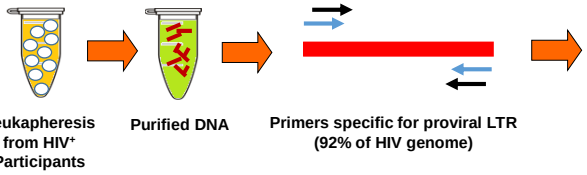


How can we make a magic bullet?

- What do we want to hit?
  - Where is the intact virus?
- What is our strategy?
  - What does it look like?
    - e.g What is it's tropism?
  - How is it surviving? Is it replicating? Is the host cell?
- How do we scope it out?
  - Are all assays of the reservoir adequate to tell us if a provirus could be infectious?



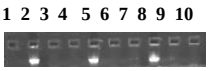
Sequencing whole HIV Genomes: The FLIPS Assay



Leukapheresis from HIV+ Participants

Purified DNA

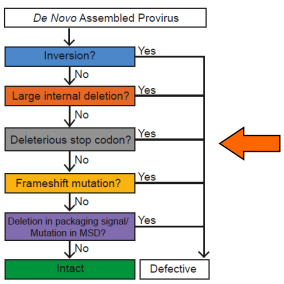
Primers specific for proviral LTR (92% of HIV genome)



Two Rounds of Nested PCR at Limiting Dilution

Next Generation Sequencing

De Novo Sequence Assembly, Alignment



Hiener et al. 2017
Cell Reports

Participants

Participant (SCOPE ID)	Viral load (copies/mL)	Time before initiation of therapy (months)	ART duration (years)
ACUTE PARTICIPANTS			
2302	<40	3.4	4.6
2115	<40	0.6	17.3
2275	<40	0.8	15.3
CHRONIC PARTICIPANTS			
2452	<40	24.4	3.2
2026	<40	117	17.7
2046	<40	70	16.3

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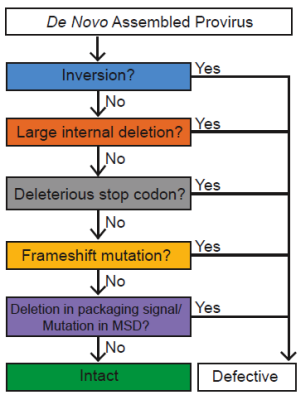
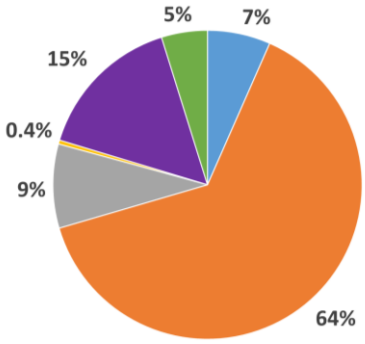
5% (35) of 728 HIV-1 proviruses sequenced were intact

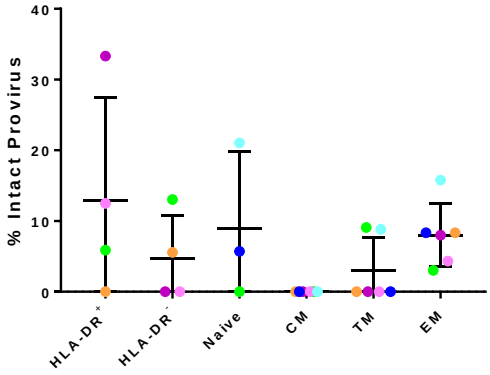




n = 728

Horsburgh et al.
manuscript under review

The proportion of genetically-intact provirus found in different cell subsets was significantly different (p = 0.001)



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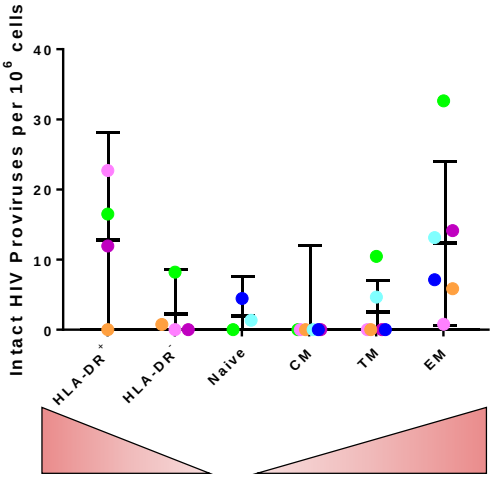
Intact HIV was found most frequently in EM and HLA-DR$^+$ CD4$^+$ T cells



What do we want to hit?

In these patients, the EM and the HLA-DR+ are the cells we should target – but not exclusively

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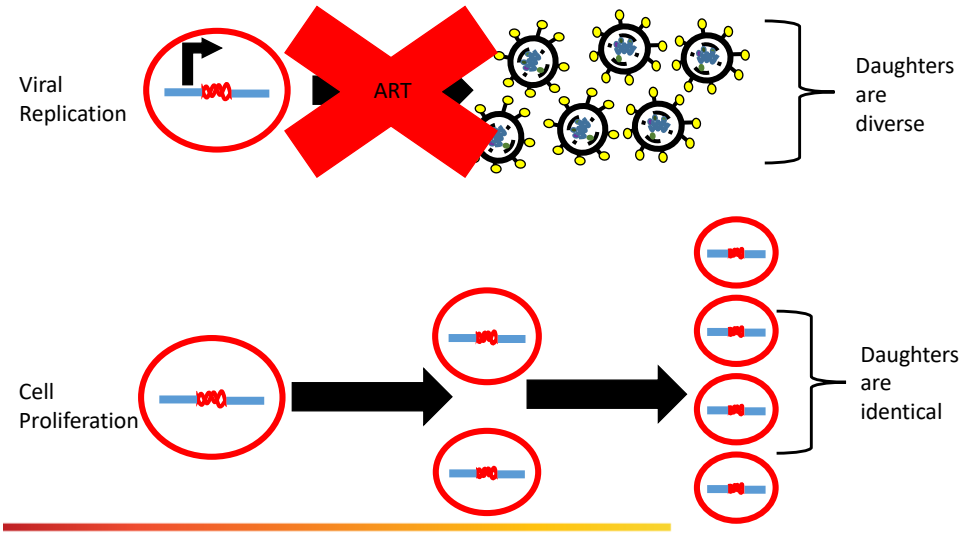
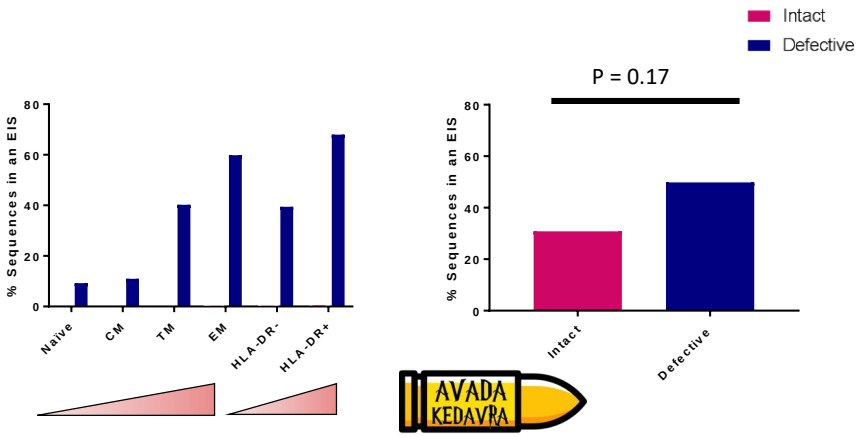
Maintaining HIV in the Reservoir



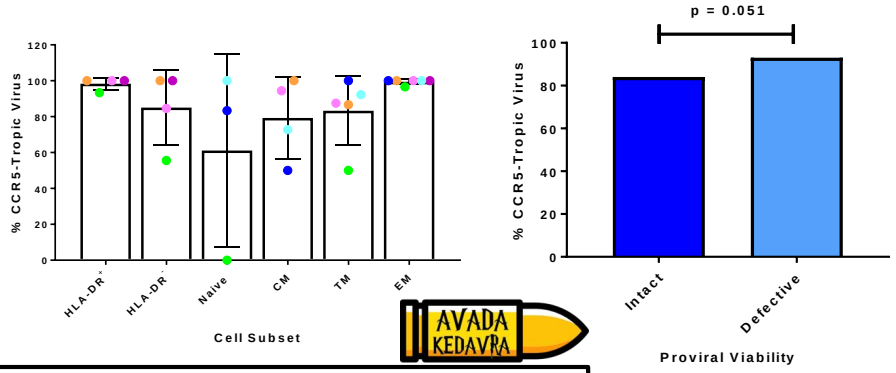
Identical Sequences: An Indication of Cell Proliferation



CELLULAR ACTIVATION & PROLIFERATION

What's our strategy? How is it surviving?
Any cure strategy needs to halt **cellular proliferation**

The majority of proviruses used the CCR5 co-receptor



AVADA KEDAVRA

What's our strategy? What does it look like?
It's largely CCR5-tropic: the reservoir is seeded very early during infection, and this true even after long-term ART

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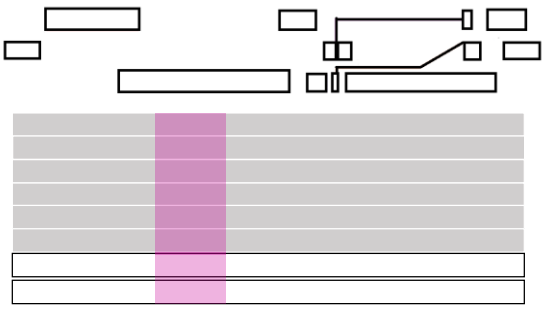
Cell-associated RNA matches DNA derived from FLIPS



FLIPS derived
Whole HIV Genomes

Single Genome
Sequencing (SGS) of
P6-RT RNA (*E. Lee)*

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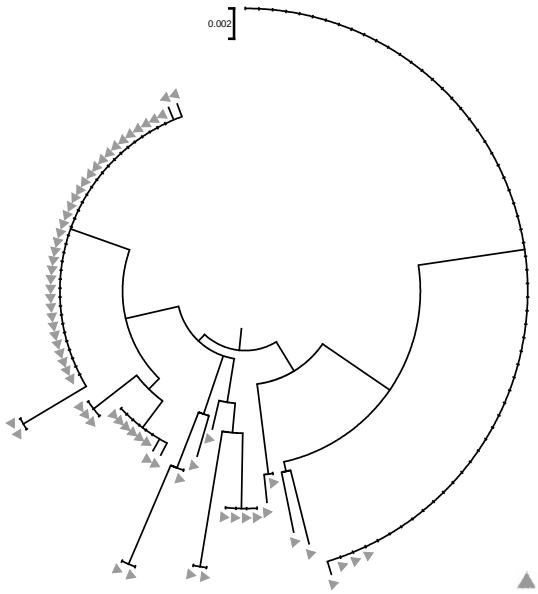
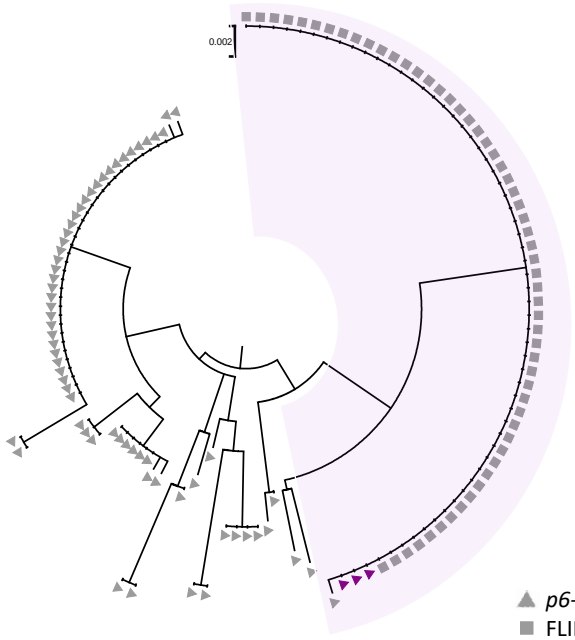
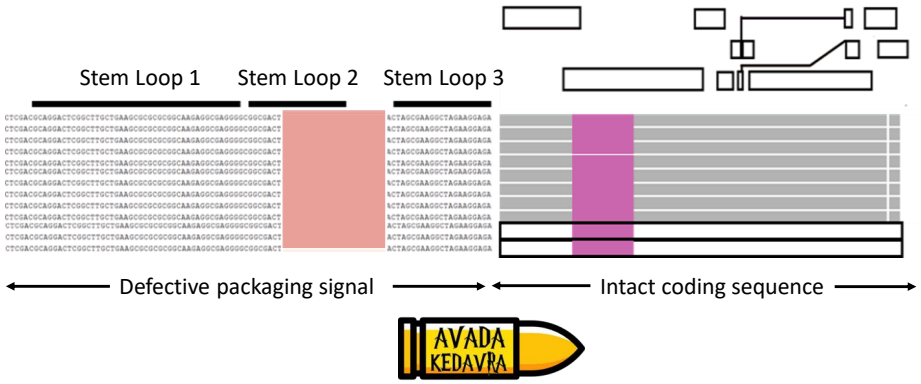


▲ *p6-RT* RNA
■ FLIPS-derived DNA



▲ *p6-RT* RNA
■ FLIPS-derived DNA

Cell-associated RNA matches defective provirus



How do we scope it out?
Cell-associated RNA is not a good measure of the intact reservoir, and must be used with caution

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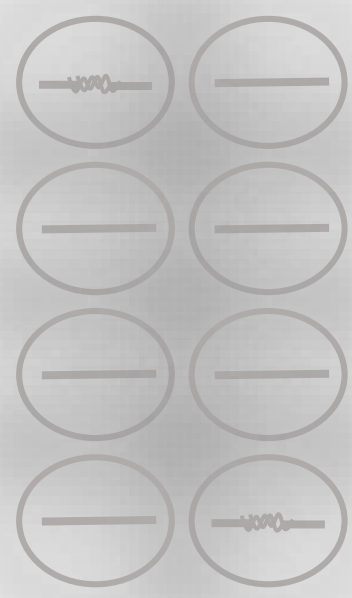
Conclusions

- Genetically intact (and likely replication-competent) HIV is enriched in HLA-DR+ and EM cells



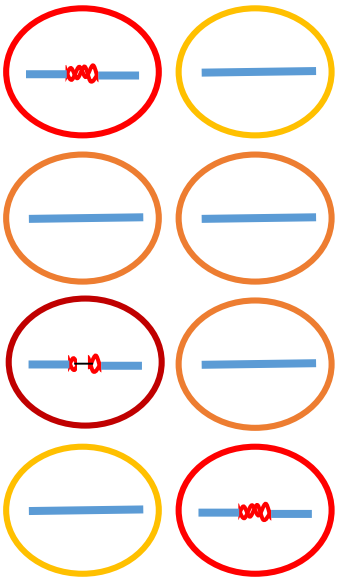
Conclusions

- Genetically intact (and likely replication-competent) HIV is enriched in HLA-DR+ and EM cells

- Cell proliferation is maintaining intact and defective HIV
- The reservoir is largely CCR5-tropic

-> This indicates that the latent HIV reservoir is established early and maintained by T cell proliferation and differentiation

- Intracellular RNA is produced by defective provirus



How are we getting to that magic bullet now?

- Can we narrow the cellular location of the reservoir down even further?
  - Future work will be looking at subdivisions of these subsets, to see if we can narrow in our focus
- Are these expansions of intact provirus stable over time?
  - Longitudinal samples will reveal if such expansions remain after 4 extra years of therapy

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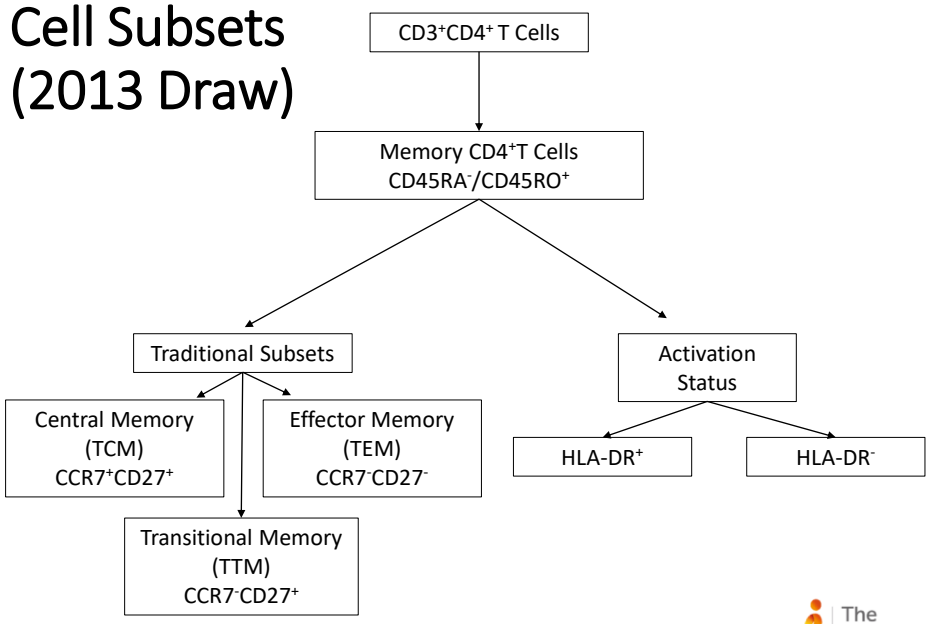
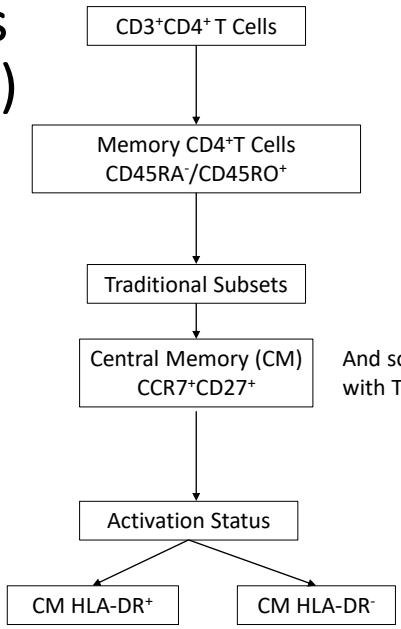
Palmer Lab c. 2017
Thank you Ciccy!

We acknowledge with gratitude the participants of this study

Cell Subsets (2013 Draw)



Cell Subsets (2017 Draw)



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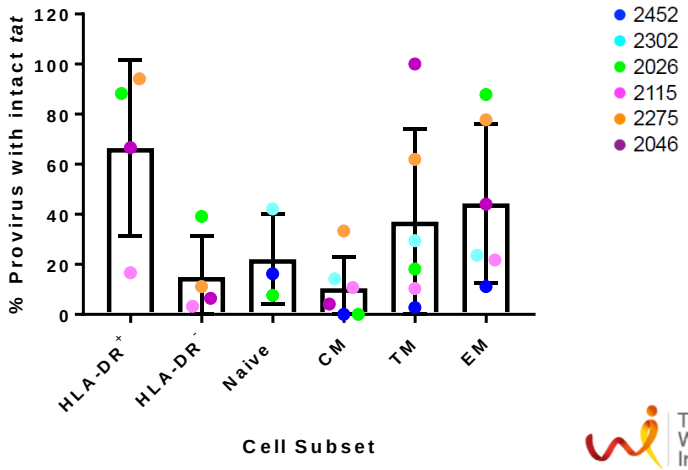
Provirus found in HLA-DR+ and EM CD4+ T cells is significantly more likely to have an intact Tat coding sequence (p = 0.0259)



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