Development of a Single-Cell Multiomic Assay to Phenotype SIV/SHIV Infected Rhesus Macaque Cells

Jayme M.L. Nordin[1,2], Vincent H. Wu[1,2], M. Betina Pampena[1,2], Michael R. Betts[1,2]

1 Department of Microbiology, Perelman School of Medicine, University of Pennsylvania (Philadelphia, PA, USA); 2 Center for AIDS Research, University of Pennsylvania (Philadelphia, PA, USA)

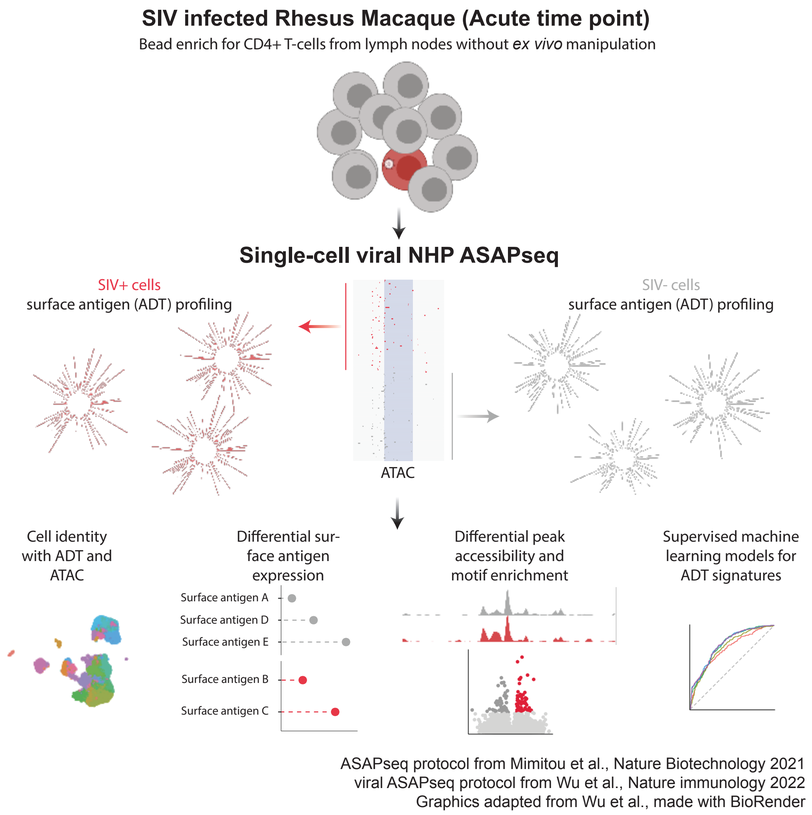
Background

Non-human primates (NHPs) infected with the simian immunodeficiency virus (SIV) and its genetically engineered derivatives, such as chimeras between HIV-1 and SIV (SHIV), are indispensable for understanding the effectiveness of HIV cure strategies. In contrast to clinical trials, NHPs are selected and are infected under controlled experimental conditions with a pretitrated dose of a well-characterized viral isolate or a viral clone. The macaque model of AIDS is therefore most suited to study virus dissemination and viral reactivation. However, the phenotype and epigentics of SIV infected cells at single-cell resolution remains to be defined.

Aims

Adapt the single-cell viral Assay for Transposase Accessible Chromatin with Select Antigen Profiling by sequencing (viral ASAPseq) to rhesus macaque SIV/SHIV infection, to characterize SIV/SHIV reservoir cellular epigenetics and phenotype.

Methods



ASAPseq protocol from Mimitou et al., Nature Biotechnology 2021
viral ASAPseq protocol from Wu et al., Nature immunology 2022
Graphics adapted from Wu et al., made with BioRender

NHP Sample information

Rhesus ID	Sex	Days post Infection	Total cells passing QC	SIV+ cells (% of total cells)	Plasma Viral Load (RNA)*
RDw14	M	13	15,334	81 (0.5%)	3.32e06 copies/mL
REs14	M	13	35,158	370 (1.05%)	1.20e07 copies/mL

*Data published in Roberts et al., *PLoS Pathog* (2016)

Detection of SIV infected cells via proviral alignment

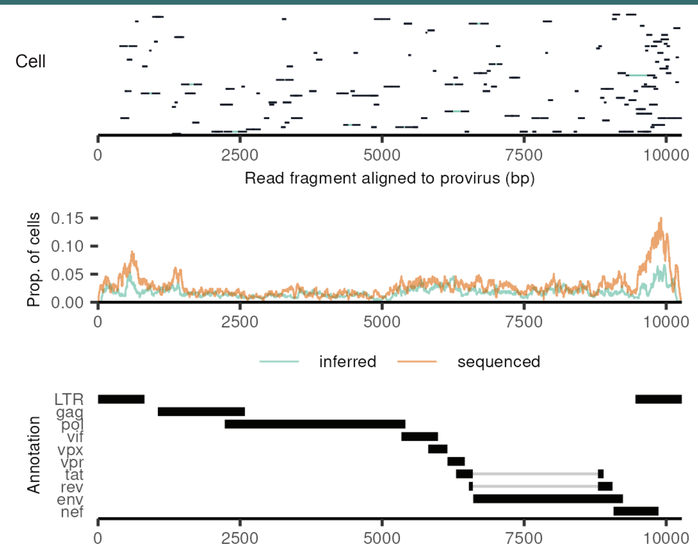


Figure 1: (Top) Proviral fragments aligned to SIVmac251, each line corresponds to one cell, figure shows a representative subset of the reads captured and aligned per cell. (Middle) Aggregated proviral read fragment alignment from all 451 cells. (Bottom) Annotation and gene order of SIVmac251.

Annotation of cell identity through surface antigen expression and gene accessibility

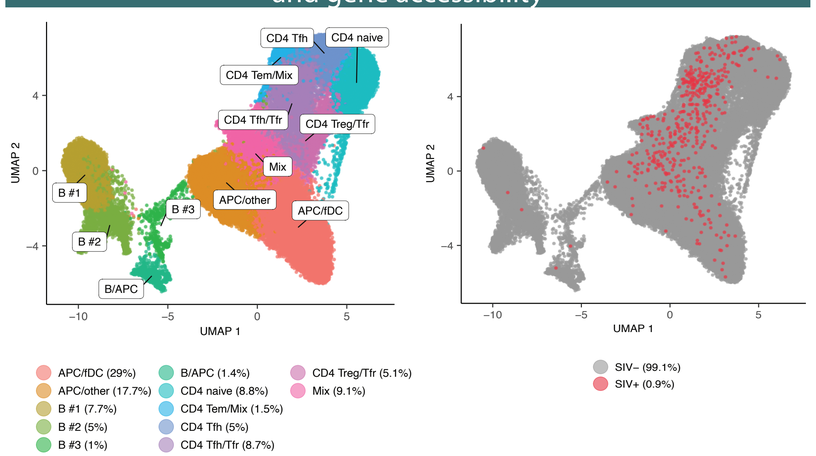


Figure 2: (Left) UMAP representation of ATAC component colored by manually annotated cell phenotypes. (Right) UMAP representation of ATAC component colored by detection of SIV reads.

Distribution of SIV+/- cells in each cluster

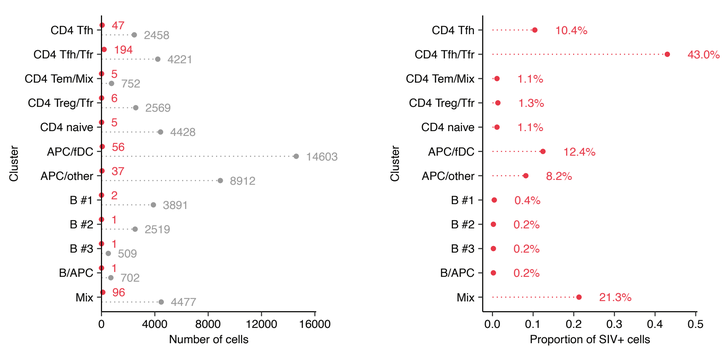


Figure 3: (Left) Absolute count of SIV+ and SIV- cells in each manually annotated cluster. (Right) Percentage of SIV+ cells found by cluster.

Surface marker enrichment within the Tfh/Tfr cell subset

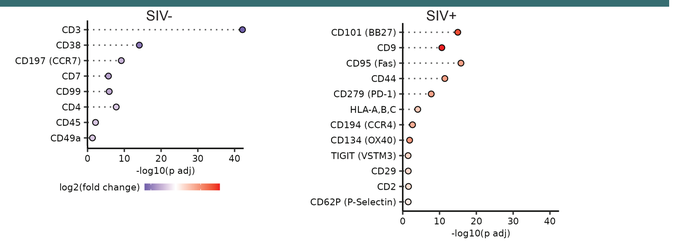


Figure 4: Differential expression of surface antigens on SIV+ CD4+ T-cells grouped by Tfh and Tfr phenotypes; (Left) surface antigens enriched in SIV- Tfh/Tfr, (Right) surface antigens enriched in SIV+ Tfh/Tfr. (Assessed using DESeq2 method in Seurat; two-sided with multiple comparison adjustment using Bonferroni–Hochberg method)

Accessible motif enrichment within the Tfh/Tfr cell subset

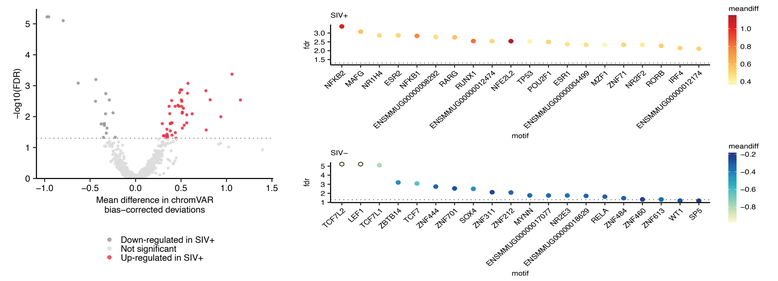


Figure 5:(Left)Comparison of motifs associated with accessible chromatin regions of SIV+ Tfh/Tfr cells versus SIV– Tfh/Tfr cells. The volcano plot displays the differentially enriched motifs from chromVAR, with a threshold of FDR <0.05 indicating statistical significance. Mean difference values greater than zero indicate an enrichment in SIV+ cells, while negative mean difference values indicate enrichment in SIV– cells. (Right) The top 20 motifs (ordered by FDR) are shown for SIV+ Tfh/Tfr cells (right top) and SIV- Tfh/Tfr cells (right bottom).

Conclusions

- NHP viral single-cell ASAPseq can be used to identify SIV+ cells using DNA sequencing reads aligned to the provirus
- We identified 451 SIV+ cells out of 50,492 cells (0.9%) each with 1 or more reads aligned to multiple regions of the provirus
- Using both the epigenetic and surface antigen profiles, 55% of infected cells had characteristics of T follicular helper, T follicular regulatory, or T regulatory cells.
- Identified candidate surface markers and chromosomal motifs to validate as a cell signature associated with SIV+ cells.

Future Directions

- Assess the SIV cellular reservoir in SIV infected NHP samples suppressed on ART and after LRA reactivation
- Use the cross-reactive antibody cocktail titrated in this assay in the tri-modal assay, DOGMAseq, for NHP sample analysis

Acknowledgments

We thank the current and previous members of the Betts lab for their assistance, the University of Pennsylvania Flow Core, and the University of Pennsylvania Next-Generation Sequencing Core and Interius Therapeutics. MRB is supported by the NIH grants (P01-AI31338, R21-AI172629, and U19-A1-149680-02). MRB is further supported by the Penn Center for AIDS Research (P30-AI045008) and UM-1AI164570 (BEAT-HIV Collaboratory) which is co-supported by the National Institute of Allergies and Infectious Diseases (NIAID), the National Institute of Mental Health (NIMH), the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute on Drug Abuse (NIDA), and the Robert I. Jacobs Fund of The Philadelphia Foundation.