Full-Length *env* Deep Sequencing in a Donor With Broadly Neutralizing V1/V2 Antibodies

Ben Murrell[1], Melissa Laird[2], Elise Landais[3], Caroline Ignacio[1], Kemal Eren[1], Pham Phung[4], Ellen E. Paxinos[2], Sergei L. Kosakovsky Pond[1], Douglas D. Richman[1], Pascal Poignard[3], Davey M. Smith[1]

[1]University of California San Diego, La Jolla, CA, United States; [2] Pacific Biosciences, Menlo Park, CA, United States. [3]The International AIDS Vaccine Initiative Neutralizing Antibody Center, La Jolla, CA, United States; [4]Monogram Biosciences, LabCorp, South San Francisco, CA, United States.

Ben Murrell, Ph.D.
University of California, San Diego
9500 Gilman Drive, MC0679
San Diego, CA 92093
E-mail: bmurrell@ucsd.edu

Introduction

Understanding the co-evolution of HIV populations and broadly neutralizing antibody (bNAb) lineages may inform vaccine design. Novel long-read, next-generation sequencing methods allow, for the first time, full-length deep sequencing of HIV *env* populations.

Objective

We developed a Pacific Biosciences single molecule, real-time sequencing protocol to deeply sequence full-length *env* from HIV RNA, and a bioinformatics pipeline to analyze such sequences. We longitudinally examined *env* populations (12 time points) from a subtype A infected individual from the IAVI primary infection cohort (Protocol C) who developed bNAbs (Serum ID50>50 for 62% of a diverse panel of 105 viruses) targeting the V1/V2 region.

Methods

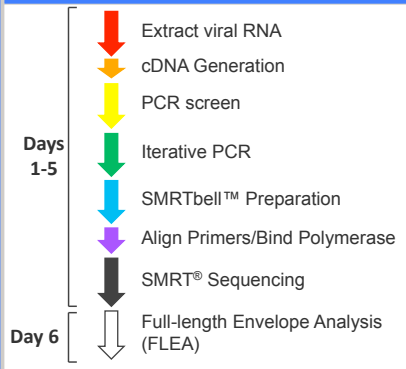

Figure 1. Streamlined end-to-end workflow for the isolation, amplification, preparation, sequencing and analysis of full-length HIV *env* amplicons.

Antibodies

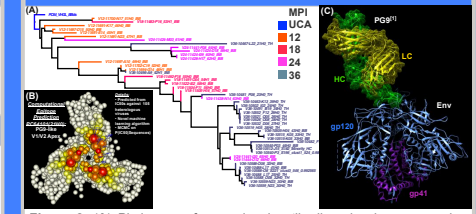

Figure 2. (**A**) Phylogeny of monoclonal antibodies showing progressive divergence from unmutated common ancestor (UCA). MPI = Months Post-Infection. (**B**) Computational prediction of PG9-like V1/V2 apex epitope from heterologous neutralization data. (**C**) PG9 docked into trimer, showing region of contact.

HIV-1 env Phylogeny

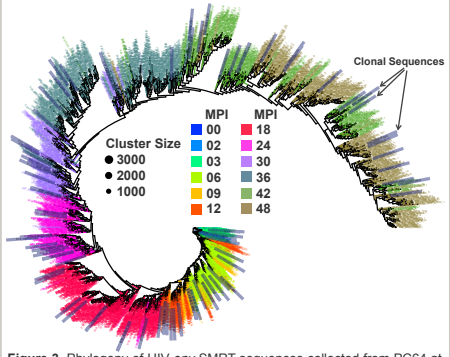

Figure 3. Phylogeny of HIV *env* SMRT sequences collected from PC64 at 12 visits, compared to previously generated clonal sequences (grey boxes). Sequences with >99% similarity were collapsed, and such clusters are represented by black circles.

V2 Epitope Kinetics

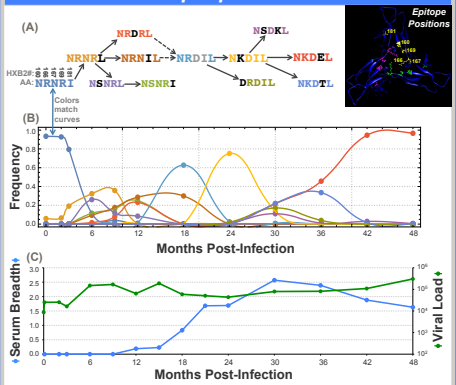

Figure 4. (**A**) V2/apex epitope configurations, comprising residues of interest for the primary antibody lineage. Colors serve as a key for the frequency curves in (**B**), where concurrent epitope diversity (3-12 MPI) is followed by sequential selective sweeps. (**C**) Serum breadth and viral load over time, showing initial development of heterologous neutralization after a period of dramatic concurrent diversity at the apex, followed by increases in breadth that may be a response to viral selective sweeps.

Functional Characterization

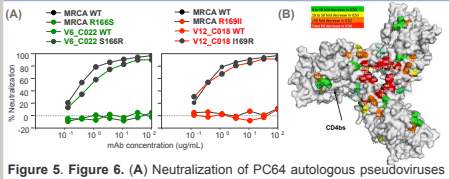

Figure 5. Figure 6. (**A**) Neutralization of PC64 autologous pseudoviruses by a PC64V36 mAb (**B**) Color-coded decrease in neutralization IC50 for single aa JRCSF mutant pseudovirus (AlaScan) compared to wildtype by PC64V36 mAbs, displayed on trimer structure[2].

HIV-1 env Longitudinal Selection Pressure

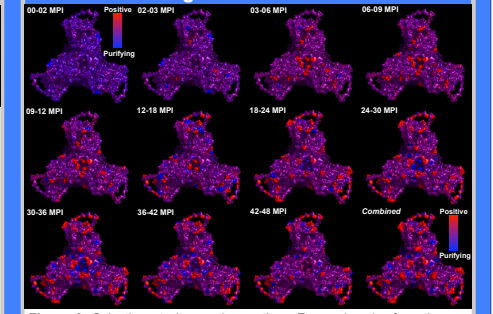

Figure 6. Selection at sites and over time. For each pair of contiguous samples, strength of selection was inferred using FUBAR[3], plotted from purifying selection (blue) to positive selection (red). As early as 3 to 6 MPI, strong positive selection can be seen at the trimer apex.

Conclusions

- Longitudinal full-length HIV *env* deep sequencing allows:
  - Accurate phylogenetic inference
  - Detailed view of epitope escape dynamics
  - Synthesis of observed Env proteins for functional characterization
- In PC64, concurrent epitope diversity (3-12 MPI) immediately preceded the development of heterologous serum activity. Waves of sequential escape preceded rapid breadth increases.

References

[1] McLellan, J.S., *et al.* (2013). *Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9.* Nature.
[2] Pancera, M., *et al.* (2014)., *Structure and immune recognition of trimeric pre-fusion HIV-1 Env.* Nature.
[3] Murrell, B. et al. (2013). FUBAR: A Fast, Unconstrained Bayesian AppRoximation for inferring selection. Molecular Biology and Evolution.

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