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

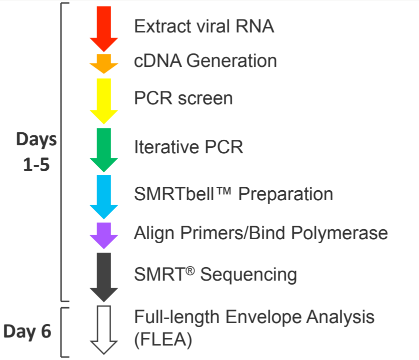

Figure 1. Streamlined end-to-end workflow for the isolation, amplification, preparation, sequencing and analysis of full-length HIV *env* amplicons.

Antibodies

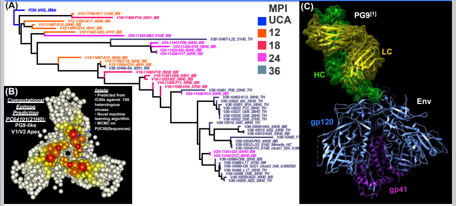

Figure 2. (**A**) Phylogeny of monoclonal antibodies showing progressive divergence from unmutated common ancestor (UCA). MPI = Months Post-Infection. (**B**) Computational prediction of PG9-like V1/V2 apex epitope from heterologous neutralization data. (**C**) PG9 docked into trimer, showing region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

HIV-1 env Phylogeny

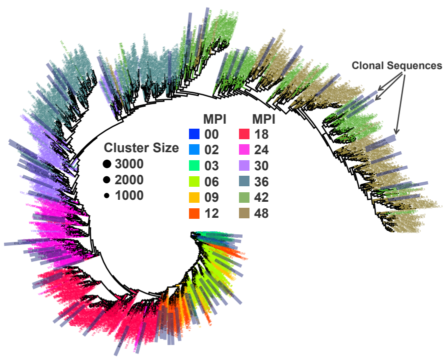

Figure 3. Phylogeny of HIV *env* SMRT sequences collected from PC64 at 12 visits, compared to previously generated clonal sequences (grey boxes). Sequences with >99% similarity were collapsed, and such clusters are represented by black circles.

V2 Epitope Kinetics

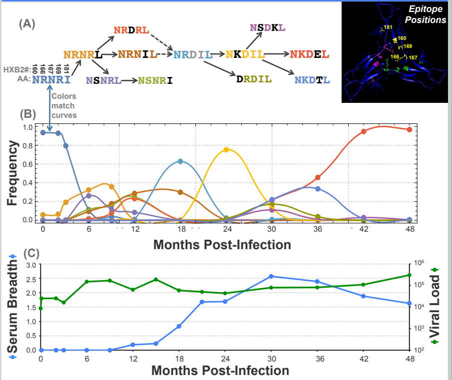

Figure 4. (**A**) V2/apex epitope configurations, comprising residues of interest for the primary antibody lineage. Colors serve as a key for the frequency curves in (**B**), where concurrent epitope diversity (3-12 MPI) is followed by sequential selective sweeps. (**C**) Serum breadth and viral load 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

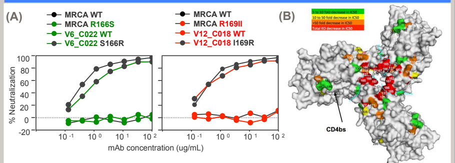

Figure 5. Figure 6. (**A**) Neutralization of PC64 autologous pseudoviruses by a PC64V36 mAb (**B**) Color-coded decrease in neutralization IC50 for single aa JRCSF mutant pseudovirus (AlaScan) compared to wildtype by PC64V36 mAbs, displayed on trimer structure[2].

HIV-1 env Longitudinal Selection Pressure

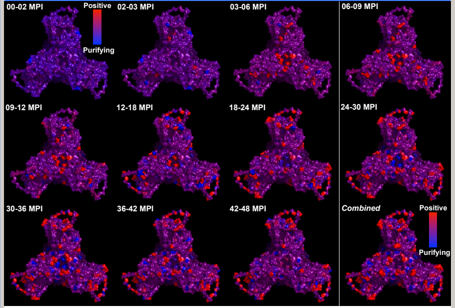

Figure 6. Selection at sites and over time. For each pair of contiguous samples, strength of selection was inferred using FUBAR[3], plotted from purifying selection (blue) to positive selection (red). As early as 3 to 6 MPI, strong positive selection can be seen at the trimer apex.

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.

ACKNOWLEDGEMENTS: This research was supported in part by the National Institutes of Health (AI090970, AI100665, AI036214, U01GM110749, GM093939), and the UCSD Center for AIDS Research (Developmental Grant, AI36214, Bioinformatics and Information Technologies Core)

For Research Use Only. Not for use in diagnostic procedures. Pacific Biosciences, the Pacific Biosciences logo, PacBio, SMRT, SMRTbell and Iso-Seq are trademarks of Pacific Biosciences of California, Inc. All other trademarks are the property of their respective owners. © 2015 Pacific Biosciences of California, Inc. All rights reserved.