Full-length HIV-1 *env* Deep Sequencing in a Donor with Broadly Neutralizing V1/V2 Antibodies

Melissa Laird[1], Ben Murrell[2] Elise Landais[3], Caroline Ignacio[2], Terri Wrin[4], Sergei Pond[2], Douglas Richman[2,5], Pascal Poignard[3,6], Davey Smith[2], Ellen Paxinos[1]

[1]Pacific Biosciences, Menlo Park, California, [2]University of California San Diego, La Jolla, California, [3]The International AIDS Vaccine Initiative Neutralizing Antibody Center, La Jolla, California, [4]Monogram Biosciences, LabCorp, South San Francisco, California. [5]VA San Diego Healthcare System. [6]The Scripps Research Institute, La Jolla, California.

Introduction

Understanding the co-evolution of HIV populations and broadly neutralizing antibodies (bNAbs) 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

To use full-length HIV *env* SMRT® Sequencing to examine viral dynamics and immune escape in an HIV-1 subtype A-infected individual who developed potent, broadly neutralizing antibodies targeting the V1/V2 loop.

Subject Information

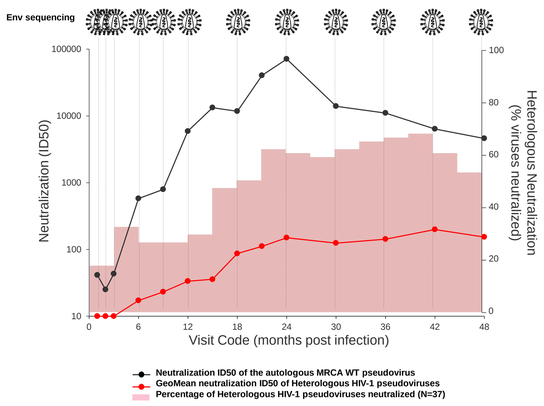


Figure 1. Donor PC64 developed potent, broadly neutralizing antibodies, peaking at 30 months post-infection (MPI). Samples were collected from enrollment in this study to 48 MPI.

Workflow

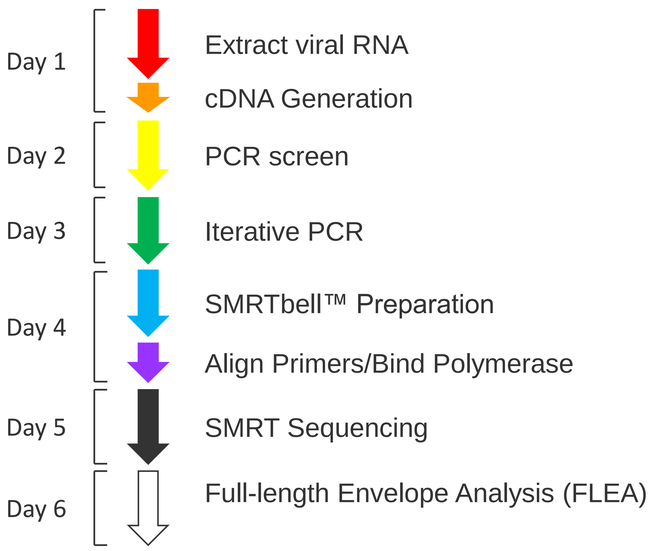


Figure 2. Streamlined end-to-end workflow for the isolation, amplification, preparation, sequencing and analysis of full-length HIV *env* amplicons.

SMRT Sequencing

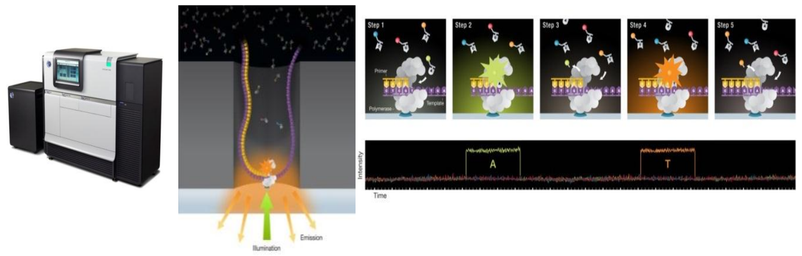


Figure 3. HIV *env* amplicons were sequenced on the PacBio® RS II using P5-C3 chemistry and standard protocols

PC64 Full-Length HIV *env* Sequences

MPI	Viral Load (IU/mL)	Raw Reads	CCS (6-pass)
0	25200	8,521	1770
2	25500	28,819	6019
3	16300	37,669	7957
6	150000	46,325	9439
9	169000	43.571	8611
12	64000	9,731	1813
18	59300	61,688	12438
24	43600	34,718	7272
30	79600	44,387	9670
36	81543	67,307	14727
42	109506	41,175	8892
48	303680	47,855	11479

Table 1. Summary of full-length HIV *env* SMRT Sequencing. Circular consensus sequences (CCS) comprised 6 passes or more over the read of insert were used for further analysis.

HIV *env* SMRT Sequences Match Clonal *envs*

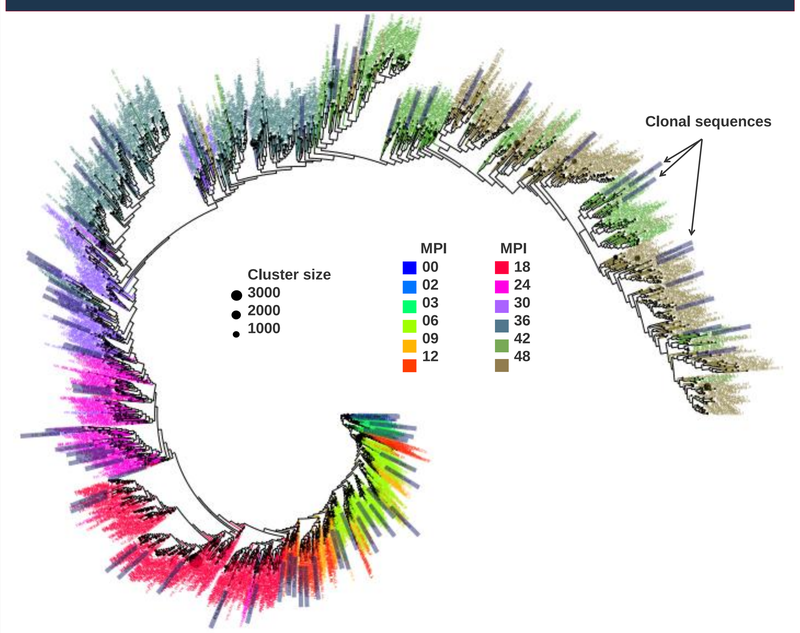
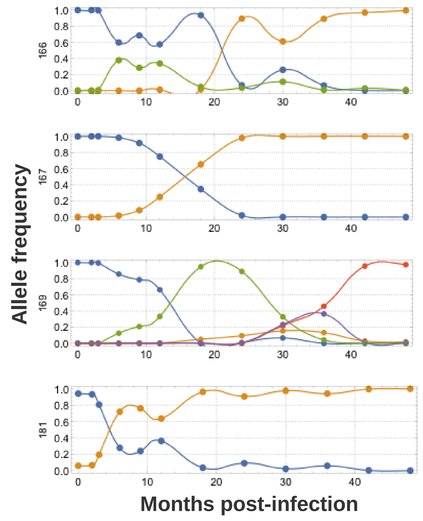


Figure 4. Phylogeny of HIV *env* SMRT sequences collected from PC64 at 12 visits, compared to previously generated clonal sequences (grey boxes). Sequences with >99% identity were collapsed and such clusters are represented by black circles.

Mapping Viral Escape in HIV *env*



3-6m: N166S emerges
18m: N166K emerges and fixes

9m: N167D emerges and fixes

6-18m: 169I fixes in population
≥24m: Eventual fixation of 169E

6m: I181L appears and fixes

Figure 5. Amino acid dynamics throughout infection in PC64 at particular residues within epitopes in HIV *env* under strong selective pressure from V1/V2 neutralizing antibodies.

Functional Validation of Viral Escape

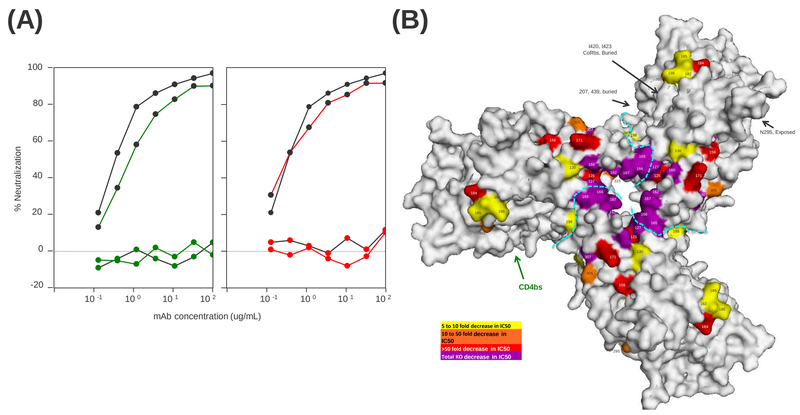


Figure 6. (A) Neutralization of PC64 autologous pseudoviruses by a PC64V36 mAb (B) Color-coded decrease in neutralization IC50 for single aa JRCSF mutant pseudovirus (AlaScan) compared to WT by PC64V36 mAbs, displayed on the BG505-SOSIP 3D structure.

Conclusions

- Full-length HIV *env* SMRT sequences provide an unprecedented view of HIV *env* dynamics throughout the first four years of infection.
- Longitudinal full-length HIV *env* deep sequencing allows
  - Accurate phylogenetic inference
  - Detailed view of epitope escape dynamics
  - Identification of minor variants.
- These data will prove critical for understanding how HIV *env* evolution drives development of antibody breadth and potency

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 (DevelopmentalGrant, AI36214, Bioinformatics and Information Technologies Core)