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

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

![Subject Information Diagram]

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

Day 1
- Extract viral RNA
- cDNA Generation

Day 2
- PCR screen

Day 3
- Iterative PCR

Day 4
- SMRTbell™ Preparation

Day 5
- Align Primers/Binder Polymerase

Day 6
- SMRT Sequencing
- Full-length Envelope Analysis (FLEA)

HIV env SMRT Sequences Match Clonal envs

![HIV env SMRT Sequences Match Clonal envs Diagram]

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.

<table>
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<tr>
<th>MPI</th>
<th>Viral Load (IU/mL)</th>
<th>Raw Reads</th>
<th>CCS (6-pass)</th>
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SMRT Sequencing

![SMRT Sequencing Diagram]

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

Mapping Viral Escape in HIV env

![Mapping Viral Escape in HIV env Diagram]

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

![Functional Validation of Viral Escape Diagram]

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

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