Abstract #1877

Next Generation Sequencing of Full-Length HIV-1 *env* During Primary Infection

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Introduction

The use of next-generation sequencing (NGS) to examine circulating HIV *env* variants has been limited by *env* gene length (~2.6 kb), indel polymorphism, GC deficiency, and long homopolymeric regions.

Objective

To develop and standardize protocols for plasma viral RNA isolation, RT-PCR amplification, single-molecule real-time (SMRT®) Sequencing, and bioinformatics analysis of circulating HIV-1 *env* variants to evaluate viral diversity in primary infection.

Methods

PCR

Day 1 — Virus pelleted by high-speed centrifugation through a sucrose cushion

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Viral RNA extracted (QIAGEN kit), cDNA generated (SuperScript III pol)

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FL *env* amplification (Q5 Pol, NEB)

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Day 2 — Analyze PCR by gel electrophoresis and BioAnalyzer instrument

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Day 3 — Iterative PCR (500 ng product required)

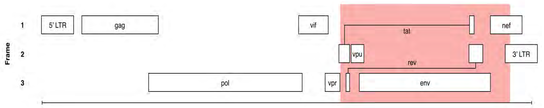


Figure 1. HIV genome, with our amplicon highlighted in red. Primers bracket the *env* coding sequence, amplifying an expected product of 3.2 kb. Primer sequences are as follows:
EnvA (F): GCTTAGGCATCTCCTATGGCAGGAAGAA
EnvN (R): CTGCCAATCAGGGAAGTAGCCTTGTGT

Methods

SMRT Sequencing

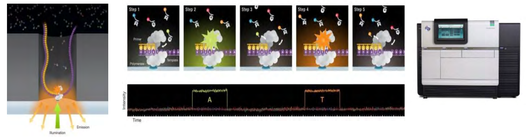


Figure 2. HIV *env* amplicons were sequenced on the PacBio RS II instrument using commercially available P4-C2 chemistry and protocols.

Results

PCR Amplification of 3.2kb HIV-1 *env*

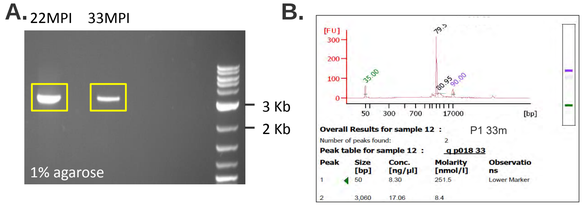


Figure 3. (A) 3.2 kb *env* amplicon from subject P1 at 22 and 33 months post-infection. (**B**) Bioanalyzer quantitation of final P1, 33 month *env* amplicon prior to SMRTbell library construction.

SMRT Sequencing of FL HIV-1 *env* Amplicons

Subject ID	Months PI	Viral Load (log IU/mL)	CCS reads	Raw reads	Diversity
P1	3	5.65	11,541	75.0K	0.74%
	22	4.55	11,316	67.6K	1.15%
	33	4.54	12,234	82.1K	2.0%
P9	3	4.36	9,246	57.4K	0.77%
H4	28	4.22	7,322	35.5K	1.3%
K4	12	4.57	5,098	32.8K	0.8%
Q8	6	5.51	8,775	63.7K	0.31%

Table 1. Summary of full-length HIV-1 *env* SMRT Sequencing. Diversity was measured as the mean nucleotide pairwise distance among circular consensus sequence (CCS) reads.

Results

Coverage and Variation of HIV-1 *env*

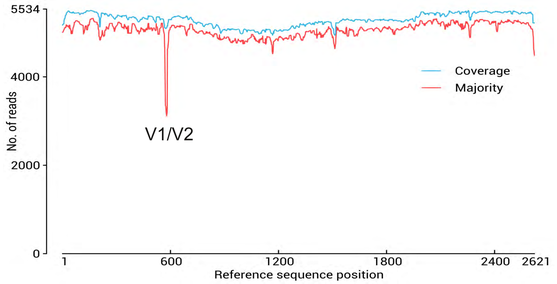


Figure 4. Examples of CCS coverage (blue) and sequence count with majority residue (red) over *env* for subject P1 at 3 months post-infection, compared to the in-sample consensus.

HIV-1 *env* Evolution

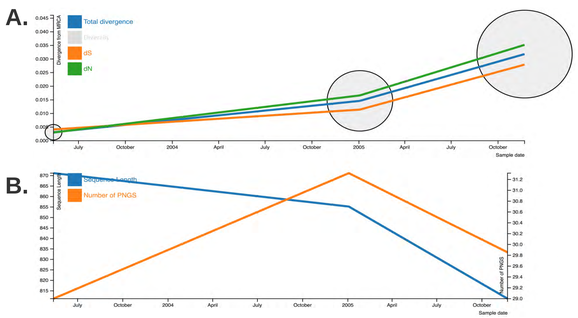


Figure 5. (**A**) Temporal evolution of *env* sequences in P1: mean synonymous, non-synonymous and total divergence (from the imputed ancestral strain), and mean within-sample nucleotide diversity. (**B**) Examination of sequence length and putative N-linked glycosylation sites (PNGS) in FL HIV-1 *env* from Subject P1 throughout primary infection (3, 22 and 33 months post-infection).

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Results

HIV-1 *env* Phylogeny

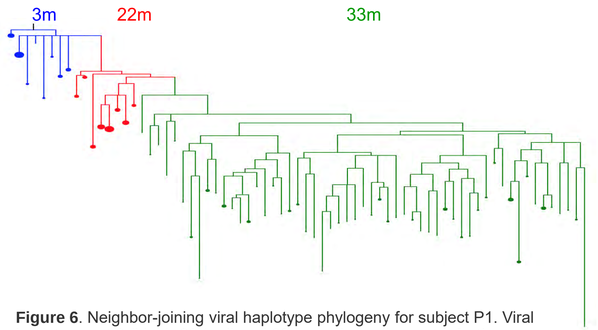


Figure 6. Neighbor-joining viral haplotype phylogeny for subject P1. Viral haplotypes at 3 (blue), 22 (red) and 33 (green) months post-infection were estimated by hierarchically clustering circular consensus sequences (CCS) and constructed using a windowed partial order alignment.

Conclusions

- This study developed a standardized procedure using PacBio SMRT technology to deep sequence full-length HIV *env* variants from the circulating viral population, achieving good coverage, and confirming the pattern of low *env* diversity during primary infection that increased over the course of disease progression.
- The number of reconstructed viral haplotypes increased from 8 to 55 throughout primary infection. Haplotype diversity increased from 0.74% (3 months) to 1.15% (22 months) and to 2.0% late in infection (33 months).
- The long, accurate reads obviate the need for short-read-based computational haplotype reconstruction, increasing our confidence in the results.
- The sequencing methodology and analysis tools developed here are immediately useful for any setting in which full-length HIV *env* analysis would be applicable.

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