Abstract #1877

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

Melissa E. Laird1, 2, Lance Hepler1, 2, Colleen Lucka1, 2, Michael Brown2, Stephen Espitia1, Ben Murrell1, Yan Guo2, Douglas D. Richman3, 4, 5, 6, Sergei L. Kosakovsky Pond2, 4, 5, 6, Ellen E. Paxonos2, 4, 5, 6, Davey M. Smith1, 2

1 Department of Medicine, University of California, San Diego, 92093; 2Pacific Biosciences, 1380 Willow Road, Menlo Park, CA 94025; 3Veterans Affairs San Diego Healthcare System, San Diego, CA 92161

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.

Objectives

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

1. Viral RNA extracted (QIAGEN kit), cDNA generated (SuperScript II RT)
2. FL env amplification (PCD: NEB)
3. Analyze PCR by gel electrophoresis and BioAnalyzer instrument
4. PCR Amplification of 3.2kb HIV-1 env

Subject ID Months PI Viral Load (log 10/mL) CCS reads Raw reads Diversity
P1 22 4.59 11,316 67.9% 0.74%
P2 33 5.48 12,234 82.1% 2.0%
P3 9 3.65 9,246 57.4% 0.77%
P4 28 4.22 7,322 35.5% 1.3%
P5 12 4.57 5,058 32.6% 0.8%
P6 8 5.51 8,775 63.7% 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

HIV-1 env Evolution

Coverage and Variation of HIV-1 env

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. Haplotypic 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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