Abstract #1956

Characterization of NNRTI Mutations in HIV-1 RT Using Single Molecule, Real-Time SMRT Sequencing

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Introduction

Genotypic testing of chronic viral infections is an important part of patient therapy and requires assays capable of detecting the entire spectrum of viral mutations. Single Molecule, Real-Time (SMRT®) Sequencing offers several advantages to other sequencing technologies, including superior resolution of mixed populations and long read lengths capable of spanning entire viral protein coding regions. We examined detection sensitivity of SMRT Sequencing using a mixture of HIV-1 RT amplicons containing NNRTI mutations.

Objective

Examine detection sensitivity of SMRT Sequencing from amplicons derived from a mixture of HIV-1 clones containing single NNRTI mutations.

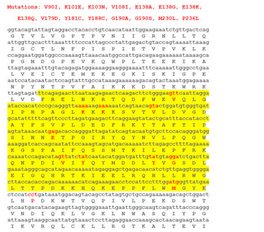


Figure 1. Sequence of 1.8 kb region amplified. The smaller ~400 bp amplicon is shown highlighted in yellow. NNRTI resistance-associated mutations are shown in RED.

Materials and Methods

- A prospective reference material being developed by BC Center of Excellence for HIV/AIDS (BC-CfE) contained a mixture of fifteen infectious viruses with single NNRTI resistance mutations (*viz* V90I, K101E, K103N, V108I, E138A/G/K/Q, V179D, Y181C, Y188C, G190A/S, M230L, and P236L) built upon the HIV-1LAI molecular clone.
- Two PCR products were generated by BC-CfE sequenced that covered region of *pol* associated with NNRTI resistance.
  - S74-S: ~400 bp, (HXB2 2813-3151 +barcodes +adapters for Miseq and 454)
  - S74-L, 1.8 kb, (HXB2 2469-4295)
- Amplicons were received in 2 shipments (March 2013, Oct 2013) and prepared as SMRTbell™ templates for sequencing.
- Replicate SMRT Cells were sequenced on two instrument configurations (PacBio® *RS* & RS II) with P4-C2 chemistry, minimum 4 SMRT Cells/condition.
- For variant detection, we used highly accurate Circular Consensus Sequences (CCS). The filtering cutoffs were: minimum full pass = 1; minimum predicted accuracy = 90%.
- Relative abundances of the mutant viruses were estimated using a custom-built codon-aware analysis pipeline.
- Any reads with local alignment issues near the site of detection were also filtered out.

SMRT Sequencing Method

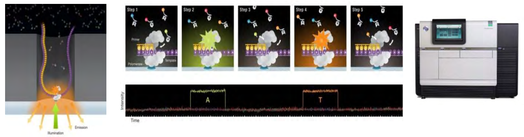
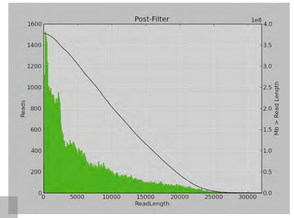


Figure 2. HIV *env* amplicons were sequenced on the PacBio RS II instrument using commercially available P4-C2 chemistry and protocols.

Results from 1 SMRT Cell

Sequencing of these templates produced average read lengths of 5.0 kb (>2 passes across the amplicon), comprising averaged 17,500-fold coverage across the 1.8 kb amplicon on a single SMRT Cell.

Figure 3a. Post-filter read length
- Maximum >24kb
- Average ~6kb
- Half of data in reads: >11kb (6 passes across amplicon)
- Yield/SMRT Cell ~350MB



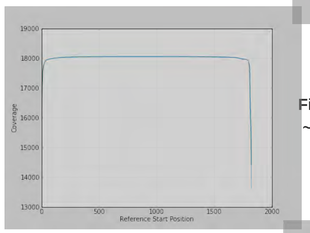
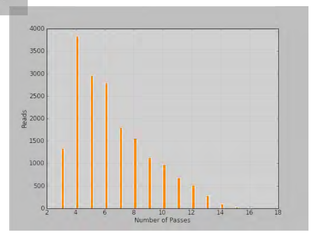


Figure 3b. CCS Coverage Plot
~ 18,000 CCS reads

Figure 3c. CCS Histogram
Many CCS with >10 Passes



PacBio Reads Span Entire 1.8 kb Region

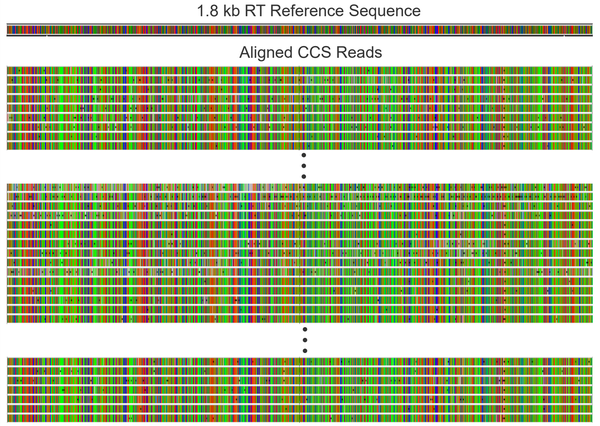


Figure 4. Long PacBio reads cover the 1.8 kb amplicon from end to end.

Two Experiments with 1.8 kb S74 Amplicon

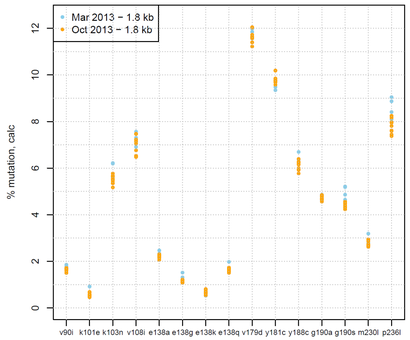


Figure 5a. Profile of the frequencies of the NNRTI mutations detected using the 1.8 kb amplicon, from two instrument configurations and two PCR dates.

All the expected mutations in the mixture of mutant viruses were accurately identified. Frequencies of NNRTI variants estimated ranged from 0.5% to 12.5%.

NNRTI Mutation Frequencies for Two Amplicon Sizes

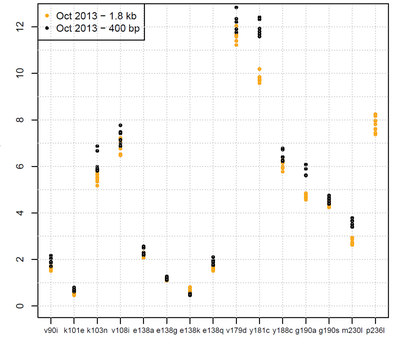


Figure 5b. Profile of the frequencies of the NNRTI mutations detected from 2 different amplicon sizes, sequenced at same time, using the same machine configuration.

Several positions show different estimated frequencies.

Conclusions

- Codon analysis revealed a number of variants across the amplicon with highly consistent results across SMRT Cells.
- Long PacBio reads allowed us to accurately determine all of the 15 expected mutations.
- Long polymerase reads and high accuracy reads make it possible to call variants from just a few molecules. SMRT Sequencing can identify species comprising a mixed viral population, with granularity and low cost of consumables, allowing for smaller multiplexing of samples and first-in-first-out processing.
- Results were consistent across 2 batches (March, October) and two instrument configurations (PacBio *RS* and PacBio RS II).
- Minor variant frequencies estimated from the same material differed by amplicon size, with 400 bp amplicon frequencies matching those determined by Miseq and 454 in Gonzalez-Serna *et al* CROI poster 2014.

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