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

Anand Sethuraman, Michael P. S. Brown, Colleen Ludka, Yan Guo, and Ellen E. Paxinos
Pacific Biosciences, Menlo Park, CA

Abstract #1956

Genotyping of the HIV-1 virus is an important aspect of patient therapy and requires assays capable of detecting the entire spectrum of viral variants. 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.

Materials and Methods
- Two PCR products were generated by BC-CIE sequenced that covered region of the HIV-1 RT gene region amplified. The smaller 400 bp amplicon is shown highlighted in yellow. NNRTI resistance-associated mutations are shown in RED.
- Amplons were received in 2 shipments (March 2013, Oct 2013) and prepared as SMRTbell™ templates for sequencing.
- PacBio Reads Span Entire 1.8 kb Region
- Two Experiments with 1.8 kb S74 Amplicon

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.

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 dilution 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).

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.

Acknowledgments
The authors would like to thank the laboratory of Dr. Richard Horvitz, British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, CANADA, for providing the S74 sample.