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SMRT SEQUENCING OF FULL-LENGTH POL AMPLICONS TO INVESTIGATE HIV-1 DRUG RESISTANCE

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Background

- Antiretroviral therapy (ART) reduces HIV viral loads to undetectable levels by targeting the three enzymes encoded by HIV-1 *pol* :
  - Reverse Transcriptase (RT), Protease (PR), and Integrase (IN)
- Broad implementation of ART will be key to reducing worldwide HIV-1 transmission, but will also likely lead to increased prevalence of drug resistance mutations (DRMs).
- Current DRM testing relies on Sanger sequencing which is unable to detect variants that comprise less than 20% of the viral population.
- Deep sequencing platforms such as MiSeq (Illumina) can lower the limit of detection to 1% of the viral population, but can only sequence short reads and may not capture DRM across the whole gene.

Objective

To use the long-read Single Molecule Real Time (SMRT, Pacific Biosciences®) deep sequencing platform to investigate linkage of HIV-1 DRMs across full-length *pol* sequences.

Methods

- Participants were selected from the San Diego Primary Infection Resource Consortium (SD PIRC) who had:
  - At least two longitudinal study visits (one before and one after ART initiation)
  - Blood viral load of at least 1000 copies/ml at the post-ART visit
- HIV-1 RNA was extracted from blood plasma samples, cDNA generated, and coding regions within *pol* (HXB2 1736-5074) were PCR-amplified.
- Amplicons were sequenced on a PacBio RSII machine to produce circular consensus sequences (CCS).
- Fastq files were filtered and processed using in-house variant reconstruction algorithms, inferring high quality consensus sequences (HQCSs), which were used for phylogeny and frequency analysis
- Sequences were analyzed for DRMs using the Stanford HIV Drug Resistance Database: (https://hivdb.stanford.edu/).
- Samples also underwent standard bulk sequencing of partial *pol* (ViroSeq) for comparison to HQCSs.

Single Molecule Real-Time (SMRT) Sequencing (PacBio ®)

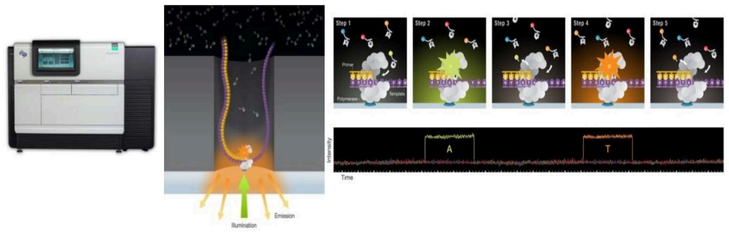

Table 1. Participant Characteristics

Participant ID	ART start (months after EDI)*	Time from EDI (months)*		Log HIV RNA (copies/ml)
		Pre-ART sampling	Post-ART sampling	
222-47n	14	13		5.61
			24	5.60
222-3yn	2	2		6.92
			61	4.55
222-5d9	1	1	NS	6.99
222-46m	31	6		4.77
			42	4.60
222-4d6	4	3	NS	6.00
222-47c	3	NS	34	4.86
222-433	22	3	NS	4.88
222-3mf	N/A	26	NS	4.68

*N/A=Not applicable, NS=Not sequenced

Figure 1. Full-length *pol* sequences match bulk sequences

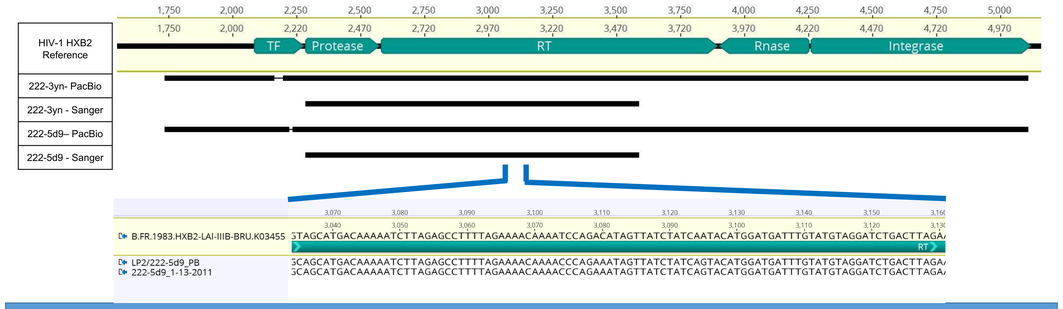

Figure 2. DRM linkage across full-length HIV-1 *pol* sequences

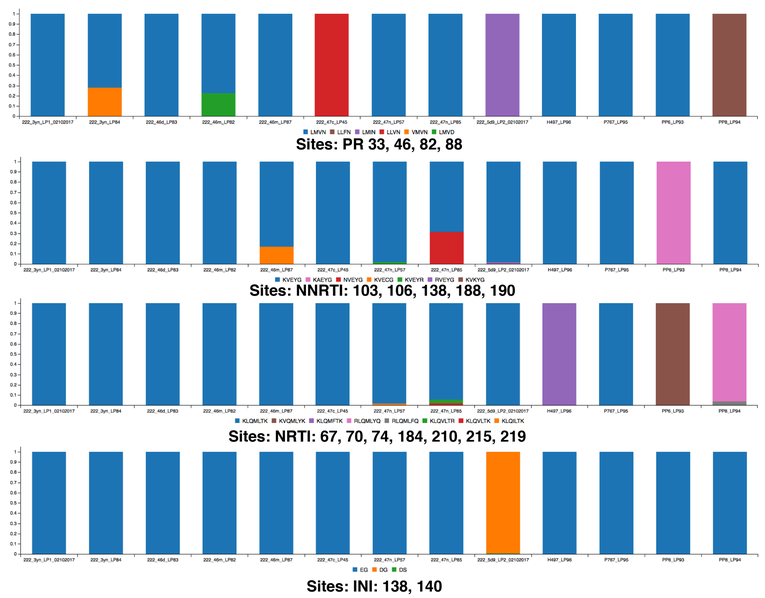

Figure 2. Linkage analysis of DRM sites across *pol*. Sequences from 11 participants and 2 panel samples (PP6 and PP8) with mutations at protease (PI), non-nucleoside RT inhibitor (NNRTI), nucleoside RT inhibitor (NRTI), and integrase inhibitor (INI) DRM sites were analyzed for evidence of resistance. Within each drug class, common DRM sites were grouped into motifs and represented as different colors.

Results

- We produced full-length *pol* sequences from multiple participants.
- The consensus sequences of HQCSs from two participants matched bulk Sanger sequencing (Figure 1).
- Sequences submitted to the Stanford HIV drug resistance database revealed mutations at DRM sites in 6 participants (Figure 2).
- We were able to link mutations at sites of drug resistance from all four drug classes targeting RT, PR, and IN.

Figure 3. Full-length *pol* HQCSs produced by SMRT PacBio sequencing from multiple participants

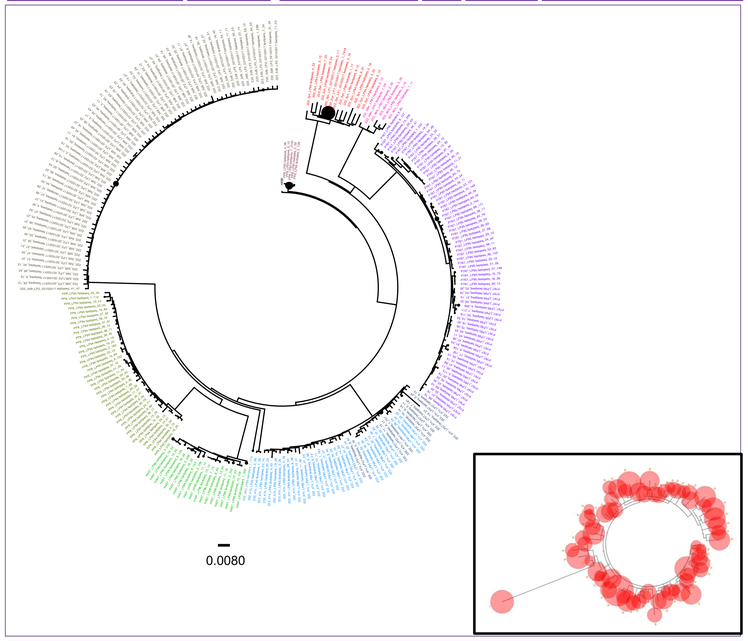

Figure 3. A phylogeny of *pol* sequences, colored by participant, estimated by maximum likelihood from full-length *pol* high quality consensus sequence (HQCS). Bubbles represent the proportion of circular consensus sequences that mapped to each HQCS. Inset: A phylogenetic tree of variation at position 184 in reverse transcriptase of 222-47n (pre-ART). We detected the M184I drug resistance mutation on a single outlying haplotype variant, which also had evidence of 18 APOBEC-mediated hypermutations.

Conclusions

- SMRT sequencing is a high-throughput approach to providing full-length sequences of the HIV-1 *pol* gene.
- PacBio HQCSs matched bulk Sanger sequencing of partial *pol* regions.
- Full-length *pol* sequences provided a method to detect and link DRMs across the entire length of *pol*.

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Acknowledgments

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