PrEP exposure and the risk of low-frequency drug resistance assayed via deep sequencing

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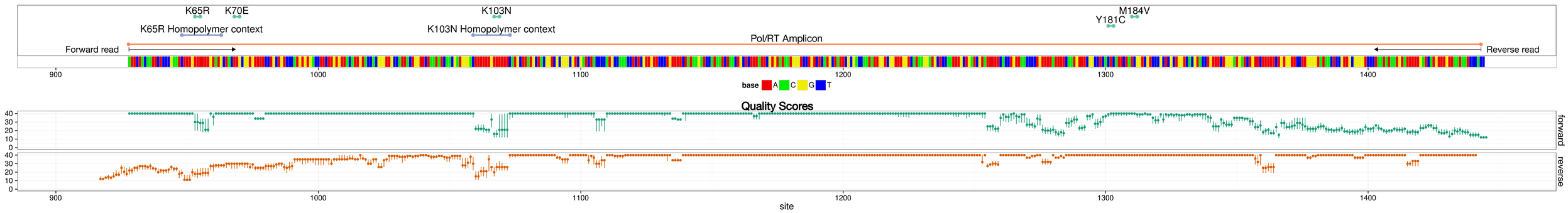


A 529bp amplicon in HIV pol and RT was sequenced to identify drug resistance mutations at three loci (top panel).
Sequence accuracy was highly dependent on position in read and context, particularly in homopolymeric regions around K65R (bottom panel) .

Abstract

Background

Pre-exposure prophylaxis (PrEP) reduces HIV acquisition. However, acquiring HIV while receiving PrEP introduces the potential for selecting drug-resistance mutation before seroconversion is detected. The level of resistance selected by PrEP may not be detectable with standard sequencing methods, which only detect resistance present at frequencies above 20% of the viral population. 454 deep sequencing can detect resistance at frequencies below 1%, but characteristic errors from the platform make error rates highly context dependent.

Methods / Results

In the Partners PrEP Study, a randomized trial of daily oral tenofovir disproxil fumarate (TDF) or tenofovir plus emtricitabine (FTC), plasma samples from the time of HIV seroconversion were tested for predefined mutations that could be selected by TDF (K65R and K70E) and FTC (K65R and M184IV) using 454 sequencing. Controls consisting of clonal wild-type populations, clonal mutant populations, and mixtures of wild-type and mutant strains were created and sequences to assess assay performance. A number of methods were considered and compared to detect minority drug-resistant variants within viral populations.

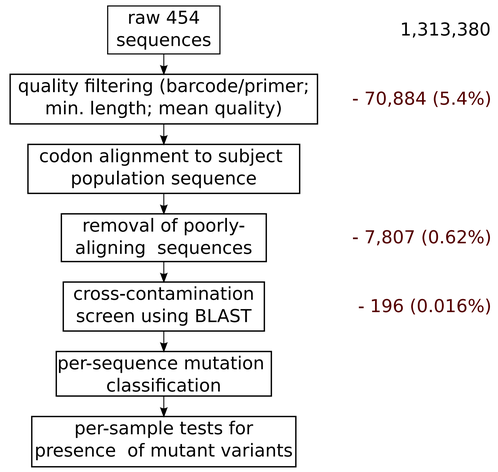
We used a rigorous computational pipeline combined with synthetic mixtures of mutant and wild-type variants to demonstrate sensitivity to detect mutations below 1%.

PrEP-related resistance mutations (K65R, K70E and/or M184IV) were detected in 9 subjects (7.4%) at levels >1%, 2 of which had previously been detected by standard sequencing. Mutations were most frequently observed in the FTC-TDF arm, where M184IV was observed in 5 of 25 (20%, p=0.024 vs placebo) participant samples.In seroconverters in the active PrEP arms, PrEP use was associated with higher risk of resistance: 6 of 23 (26%) with measurable drug levels versus only 1 of 39 (2.6%) without evidence of PrEP use had resistance >1% (p=0.009).

Conclusions

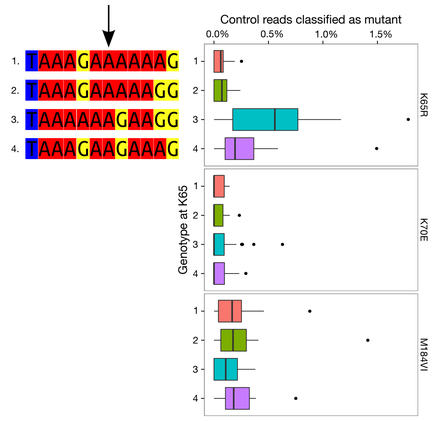
High-sensitivity resistance testing detected more resistance than standard sequencing in persons acquiring HIV who were exposed to PrEP, although resistance still occurred in a minority of subjects. More resistance was selected by FTC than TDF, as evidenced by the prevalence of M184IV compared to K65R.

1. Computational Pipeline

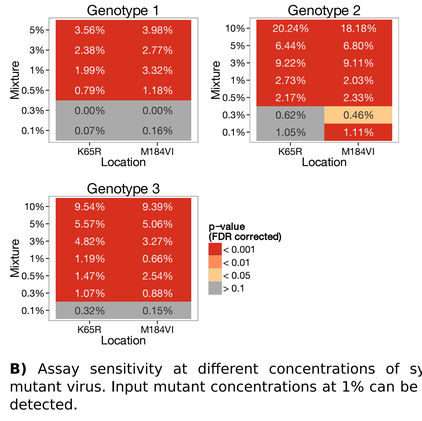


Raw 454 sequences were filtered to those with an exact match to a barcode and primer used in the study, at least 350bp long, and with a mean quality score of at least 25.0. Next, each sequence was pairwise codon aligned to a subject-specific population sequence with a reduced penalty for homopolymeric frameshifts. Sequences which aligned poorly to the population sequence or were identified as cross-contamination using BLAST were removed. Remaining sequences were classified as mutant or wild-type at each position. Fisher's exact test was used to identify samples with enriched mutation rates relative to genotype matched controls.

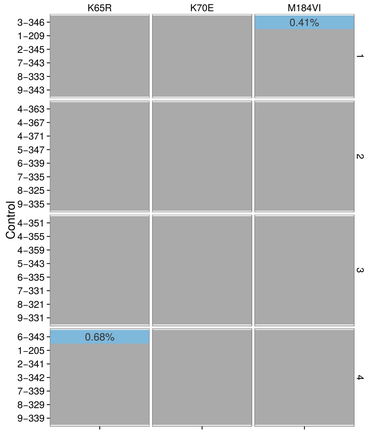
2. Assay Sensitivity and Specificity



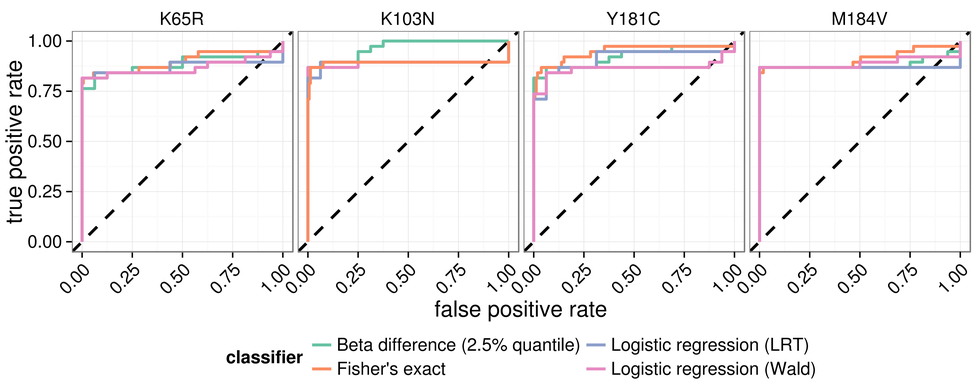
A) Error rates in wild-type controls depend on the genotype at K65 at the K65 locus only. For genotypes 3 and 4, mis-calling a homopolymeric region leads to an A to G mutation at the position of interest (arrow).



B) Assay sensitivity at different concentrations of synthetic mutant virus. Input mutant concentrations at 1% can be reliably detected.



C) 2/87 wild-type controls were falsely classified as having a drug resistance mutation (2.3%), both at less than 1%.



D) Receiver Operating Characteristic (ROC) curves for classifiers evaluated using 100% wild-type and mixture controls. Dashed black line indicates performance expected by chance alone.

Beta difference: the 2.5% quantile of 10,000 posterior draws from a
Beta(1 + mut, 1 + wt) - Beta(1 + ctrl_mut, 1 + ctrl_wt)
distribution.

Fisher's exact: Fisher's exact test

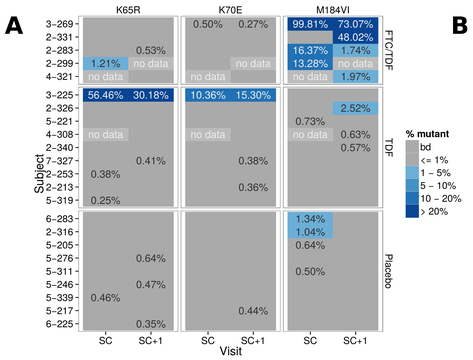
Logistic regression (LRT): likelihood ratio test between a model including a subject-specific term and one without.

Logisitic regression (Wald): Wald test for inclusion of subject-specific term.

Fisher's exact test was chosen due to superior performance at M184V and K65R.

3. Resistance in Partners PrEP cohort

A



B

Resistance above 1%	TDF	FTC/TDF	Placebo	All
Overall	2/38 (5.3%)	5/25 (20%)	2/58 (3.5%)	9/121 (7.4%)
Among subjects retrospectively found to be HIV+ at enrollment or re-randomization	1/8 (12.5%)	2/4 (50%)	0/6 (0%)	3/18 (16.7%)
Among subjects who acquired HIV after enrollment or re-randomization	1/30 (3.3%)	3/21 (14.3%)	2/52 (3.8%)	6/103 (5.8%)

A) Mutation levels for those participants with any PrEP-related mutation detected.

	Resistance >1% n / total	Resistance >1% %	Fisher's exact p-value
Treatment arm at SC			
FTC/TDF or TDF vs placebo	7/63 vs 2/58	11% vs 3.45%	0.167
FTC vs placebo	5/25 vs 2/58	20% vs 3.45%	0.024
TDF vs placebo	2/38 vs 2/58	5.2% vs 3.45%	0.65
FTC vs TDF	5/25 vs 2/38	20% vs 5.2%	0.1
Estimated days of PrEP after infection			
>30 vs <30 days	6/38 vs 1/19	16% vs 5.3%	0.4
Infected at PrEP start			
Yes vs no	3/18 vs 6/103	16.7% vs 5.8%	0.13
TDF concentration at SC (ng/ml)			
TDF >0.31 vs <0.31	6/23 vs 1/39	26% vs 2.6%	0.009

B) A summary table of resistance detected at levels above 1%.

Conclusion

454 sequencing can reliably detect drug resistance mutations at levels below 1% high specificity and sensitivity.

Resistance, including low-frequency resistance, selected by PrEP is rare.

Detection of PrEP drug in blood plasma was associated with an increased risk of resistance.

Risk of resistance was highest in the FTC/TDF arm due to M184IV mutations.

analyze our control data: