Comparison of HIV Drug-Resistant Mutant Detection by NGS with and without Unique Molecular Identifiers (UMI)

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Introduction

Background

Sequencing error compromises the sensitivity of NGS for detection of HIV drug-resistant mutants.

Consensus building with UMIs can reduce sequencing artifacts and quantify the true sampling depth. UMI-based consensus building has not been universally adopted for drug-resistant surveillance because it adds technical and bioinformatics challenges with uncertain gain.

![Figure 1: Consensus building from sequences derived from individual cDNA templates that are tagged with UMIs (left); A Million Combinations (middle) using (UMI) synthesized to overcome PCR and sequencing problems and to quantify the depth of mutation and wild-type template sampling](image1.png)

Materials & Methods

Mixture Panel

We created a mixture panel of recombinant wild-type and mutant viruses that were spiked into HIV-negative blood.

NGS Library Prep and Analysis

NGS libraries were constructed using the ultrasensitive single-genome sequencing method as previously described.

- The Zhou method was used for UMI consensus building and UMI bioinformatic analysis.

- PASeq v1.4 was used for non-UMI NGS analysis (https://www.paseq.org)

Clinical Data Set

A UMI-NGS dataset derived from plasma samples from viremic donors with HIV acute infection was re-analyzed without consensus building.

Results

Summary

- We detected 0.5% drug-resistant associated mutations with and without UMI-based sequence consensus building, indicating that both methods are generally robust.

- False negative error rates are higher in non-UMI-based NGS in samples with limited template sampling.

- Non-UMI NGS had unrepeatable background mutations (<5%) in clinical samples, which lowered the specificity relative to the UMI assay.

![Figure 2: Patient derived HIV-1 Subtype C RT was cloned into a cDNA and the HIV-1 integrations: K103N, Y181C and M184V were derived from an antiretroviral drug-resistant isolate. Sensitivity and Specificity for non-UMI analysis were calculated by the number of different samples relative to UMI-based NGS analysis](image2.png)

![Figure 3: MiSeq Libraries were prepared from the HIV-1 RT region (261 codons 80-157, R2 codons 151-214) of archived isolated from HD viremic donors with HIV acute infection. Sensitivity and Specificity for non-UMI analysis were calculated by the number of different samples relative to UMI-based NGS analysis](image3.png)

Conclusions

- UMI-based NGS should be used when calling mutations at frequencies below 5%.

- This is predominantly true for samples that are likely to have limited sampling depth from low viral inputs (e.g. from DBS) or with diverse samples (polymorphic primer binding sites).

References


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