Sensitive HIV Drug Resistance NGS Testing with and without Unique Molecular Identifiers

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Background

- Sensitive next-generation sequencing (NGS) HIV drug resistance (HIV DR) surveillance is needed for optimal future HIV treatment regimens (Fig 1).
- Adding a 10bp Unique Molecular Identifier (UMI) sequence to each HIV template in HIV DR NGS reduces polymerase fidelity error, PCR bias and sequence recombination.¹

Objective

- To perform an assessment of the 3 major steps likely to influence the sensitivity of a UMI-based versus a non-UMI-based assay: 1. Inefficient RT using primers with long UMI (Fig 2, Panels 2 & 3). 2. Skewed allelic amplification from PCR bias (Panel 4). 3. PCR recombination from MiSeq adapter addition (Panel 5) to determine if more affordable approaches could be employed to overcome limitations from non-UMI NGS artifacts.

Materials & Methods

- We used a previously characterized wild-type/drug-resistant HIV mixture panel (Panel 1-5) and a dataset of 33 HIV positive clinical samples (Panel 6) to determine the sensitivity of detecting minority HIV drug resistance templates with and without UMI-based consensus building.

Results

1. Sensitive detection of low frequency HIV DR mutations using a WT415A virus mixture panel with/without UMI consensus building.

2. Increased concentrations of RT primer improves limited RT efficiency in samples with polymorphic primer binding sites.

3. Increased concentrations of RT UMI primer (above) are not represented at levels to cause skewing of NGS data analysis.

4: Splitting samples into multiple PCR reactions does not improve the PCR bias in reactions where primers contain polymorphic bases (red circle).

5: PCR Recombination from the PCR addition of MiSeq elements does not cause recombination or skew NGS data.

Conclusions

- We observed limited RT efficiency with our current library prep protocol—particularly in sequences with primer-site polymorphisms—which can be improved 5 fold by increasing primer concentrations without significant risk of residual UMI contamination (Panels 2 & 3).

- Biased PCR amplification limited the total number of sequenced HIV templates in samples with PCR primer site polymorphisms; however, UMI consensus building was able to overcome data skewing from PCR amplicon preference (Panel 4).

- The PCR addition of MiSeq primers reduces assay complexity and cost, but does not cause recombination (Panel 5).

- In clinical specimens, the non-UMI based had similar level of sensitivity (low false negative mutations), but has a significant amount of false positives (low specificity) (Panel 6).

References
