An Improved Circular Consensus Algorithm with an Application to Detect HIV-1 Drug Resistance Associated Mutations (DRAMs)

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Introduction

Confident resolution of heterogeneous, complex populations continues to rely on cost- and labor-intensive Sanger sequencing methods.

Long, single-molecule sequencing reads can now be analyzed using a redesigned circular consensus sequencing algorithm (CCS2) to generate high-quality reads across longer insert lengths.

This new approach was validated with the characterization of the HIV-1 K103N drug resistance-associated mutation in both clonal and patient samples.

The K103N DRAM is clinically relevant, but has proven technically challenging to resolve due to regional sequence context.

Materials

- A region spanning ~1.3 kb of the RT region of the pol gene of HIV was PCR-amplified from clonal and several primary samples.

- Detection of this variant was tested using two sample sets (Table 1): Clonal wild-type and K103N variant amplicons, and Sequential primary samples from an individual who had failed an NNRTI-containing therapy.

Algorithm Development

- Sequencing data were analyzed using the new CCS2 algorithm, called Arrow.
- Arrow uses a hybrid discriminative/generative probabilistic model to polish consensus sequences for high accuracy.
- Arrow model achieves high accuracy by adjusting the model parameters using fixed covariates taken from the sequencing data of each molecule (Fig 2).
- The new hybrid-Arrow-based CCS2 algorithm achieves high accuracy as coverage increases (Fig 3).

Methods

- Sequencing was performed using a PacBio RS II with P6-C4 chemistry and 4 hour movies.
- Degenerate consensus reads were amplified from clonal and several primary samples.
- The K103N drug resistance-associated mutation frequency estimated at 49.7% in the mixed sample, consistent with orthogonal data.
- 0% miscall rate observed in both unmixed samples.
- K103N mutation frequency estimated at 49.7% in the mixed sample, consistent with orthogonal data.

Results

- Tertiary analysis of >10,000 sequences shows viral population evolution and the K103N emergence in several well-supported clusters.
- Suggests K103N mutation arose in more than one backbone.

Conclusions

- We demonstrate high-quality consensus for single molecules by modeling the sequencing reaction using the new Arrow modeling framework.
- We achieve accurate quantification of K103N DRAM mixes, in both a well-characterized mix and primary samples, and tracks parallel evolution of the K103 mutation in response to therapy.

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Figure 1. Schematic of HIV-1 genome, with amplified region in red.

Figure 2. Diagram of the “Arrow” model over an example AGG template context.

Branch and stick refer to homopolymeric and non-homopolymeric insertion events, respectively, while dark is synonymous with deletion. Transition parameters vary based on fixed per-reaction covariates.

Table 1. Table showing the homopolymeric region surrounding the clinically important K103N variant in the RT gene.

<table>
<thead>
<tr>
<th>Sample</th>
<th>100</th>
<th>101</th>
<th>102</th>
<th>103</th>
<th>104</th>
<th>105</th>
<th>RT as Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild control</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>N</td>
<td>amino acid</td>
</tr>
<tr>
<td>K103N control</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>N</td>
<td>amino acid</td>
</tr>
<tr>
<td>p24-1</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>N</td>
<td>amino acid</td>
</tr>
<tr>
<td>p24-3</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>N</td>
<td>amino acid</td>
</tr>
</tbody>
</table>

Table 2. Results of K103N quantification from synthetic 50/50 mixture and primary samples from subject 24.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TIME</th>
<th>PACBio RsII</th>
<th>ILLUMINA</th>
<th>SANGER</th>
<th>MISEQ</th>
<th>% K103N DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/50 Mix</td>
<td>50.4%</td>
<td>49.7%</td>
<td>56.0%</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 24</td>
<td>15.3%</td>
<td>19.1%</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Arrow vs. Quiver per-read empirical QV by coverage.

- Arrow achieves a per-read empirical QV30 at 15-fold coverage.
- Previous Quiver-based algorithm unable to model sequencing reactions on a per-molecule basis, and therefore could not achieve QV30 single-molecule consensus accuracy at any coverage.

Figure 4. Phylogenetic relationships of sequences at baseline (Green), and after K103N failure (blue) in subject p24. Topology generated using a GTR+gamma evolutionary model in FastTree.