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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.

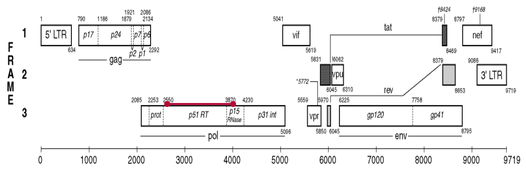


Figure 1. Schematic of HIV-1 genome, with amplified region in red.

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

Methods

- Sequencing was performed using a PacBio RS II with P6-C4 chemistry and 4 hour movies.

SAMPLE	100	101	102	103	104	105	RT aa Position
"wild" control	T T A A A A C A G	A A A A A A	T C A	base
	L	K	Q	K	K	S	amino acid
K103N control	T T A A A A C A G	A A C A A A	T C A	base
	L	K	Q	N	K	S	amino acid
p24-t1	T T A A A A A A G	A A A A A A	T C A	base
	L	K	K	K	K	S	amino acid
p24-t3	T T A A A A A A G	A A C A A A	T C A	base
	L	K	K	N	K	S	amino acid

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

- > 5000 1.3 kb consensus sequences with a collective empirical quality of ~QV40 were generated for each sample.

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)**

Arrow Model & Enhanced Accuracy

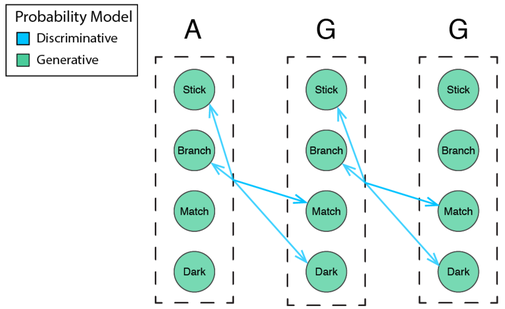


Figure 2. Diagram of the "Arrow" model over an example AGG template context.

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.

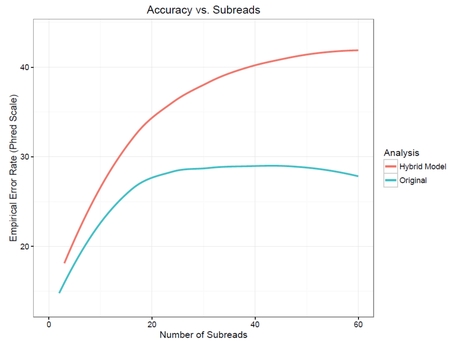


Figure 3. Arrow vs. Quiver per-read empirical QV by coverage

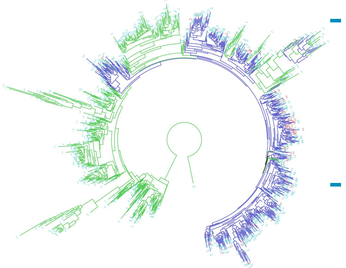
- Arrow achieves a per-read empirical QV30 at 15-fold coverage.
- Previous Quiver-based algorithm unable to model sequencing reactions on a per-molecule basis, and therefore could not achieve QV30 single-molecule consensus accuracy at any coverage.

HIV K103N DRAM Detection

- 0% miscall rate observed in both unmixed samples
- K103N mutation frequency estimated at 49.7% in the mixed sample, consistent with orthogonal data

		% K103N DETECTED			
SAMPLE	TIME POINT	PACBIO RSII QUIVER	PACBIO RS II ARROW	ILLUMINA MISEQ	SANGER DETECTION?
50/50 Mix		50.4%	49.7%	56.0%	Y
Subject 24	T1	0	0	0	N
	T3	15.3%	15.3%	19.1%	2

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



- 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

Figure 4 Phylogenetic relationships of sequences at baseline

(t=1 in Green), and after K103N failure (t=3, in blue) in subject p24. Topology generated using a GTR+gamma evolutionary model in `FastTree`.

Conclusions

- We demonstrate high-quality consensus for single molecules by modeling the sequencing reaction using the new Arrow modeling framework.
- We achieve accurate quantitation 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.

Acknowledgements

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