Evaluation of Two Workflows for Variant Detection in Cell-Free DNA

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Introduction

- The application of cell-free DNA (cfDNA) as a bio marker for early cancer detection has led to the availability of numerous cfDNA library preparation kits.
- This study compares the performance of two commercial NGS library kits / workflows for their detection of low allele frequency variants in a commericially available reference material Seraseq ctDNA.

Table 1. Key features of the two NGS workflows for cfDNA.

	Workflow I	Workflow II
Chemistry	Amplicon-based	Capture-based
Fragmentation required	✓	✗
Custom Panel	✓	✓
UMI & UDI	✓	✓
TAT (Library Prep)	One day	Two days

Methods

Table 2. Experiment design.

		Workflow I	Workflow II
Samples (input)	VAF	Panel Size (bp)	Panel Size (bp)
Seraseq® ctDNA Complete™ Reference Materials (50 ng)	5%	2.2M	1.9M
	2.5%		
	1.25%	0.1M	
	0.5%		
	0% (wt)	2.2M	

- For fair comparison, data was processed with the smallest BED file, and VCF intersected with the smallest panel so that all the variants are from the same region of interest.

Results

QC Comparison

- BioAnalyzer traces shows the library sizes from the two workflows match the expectation.
- There were no major QC concerns on further comparison.

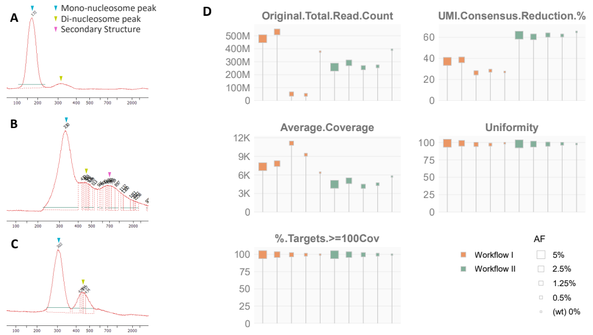

Fig 1. BioAnalyzer traces of the original ctDNA (A), and the final library from Workflow I (B) and Workflow II (C). (D) Key QC metrics of those ctDNA libraries from pipeline. Note that due to the panel size difference, total reads vary among different samples.

Sensitivity & Accuracy

- All 18 expected variants were detected by both workflows at the lowest AF of 0.5%.
- Workflow I struggles to get the correct AF of vaiant BRCA1 c.1961delA.

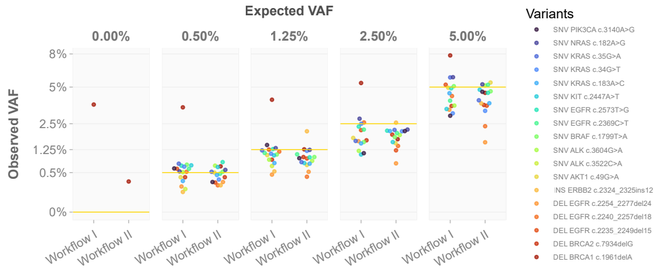

Fig 2. Observed VAF vs Expected VAF of the 18 variants in Seraseq ctDNA with the two workflows. Yellow horizontal lines in each panel denote the expected VAF.

Specificity / Background Noise

- In the same intersected target region, Workflow I has ~1000x as many variant calls as Workflow II.
- Most of those variant calls in Workflow I are in the lower VAF range, suggesting more background noise in Workflow I.

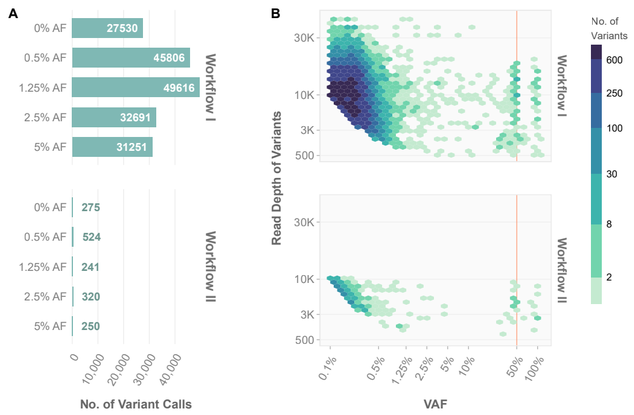

Fig 3. (A) Number of variant calls in the ctDNA samples with various expected VAF by each workflow. (B) Distribution of all the variant calls by their VAF (x-axis) and depth (y-axis) by each workflow. Darker color denotes more variant calls.

Precision

- Workflow II has better repeatability/precision of variant calling than Workflow I in the VAF range from 0.5% - 100%.

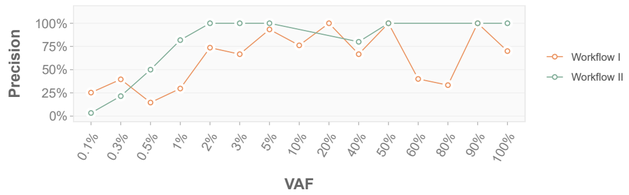

Fig 4. Precision (intra) comparison of the two assays at different VAF interval. The precision was calculated based on the occurrence of the same variant in three Seraseq ctDNA of the same genomic background.

Summary

	Workflow I	Workflow II
Sensitivity (VAF 0.5%)		✓
Accuracy		✓
Specificity / Background Noise		✓
Uniformity		✓
Precision		✓
Library Conversion Rate	✓	
Ease of Use	✓	
TAT	✓	
Automation	✓	
Cost	✓	

Conclusion

- Although workflow I offers a simpler process, shorter turnaround time, and lower cost, workflow II exhibited superior performance in terms of accuracy , uniformity, precision, and less background noise.
- These advantages of workflow II make it particularly valuable for accurate and confident variant calling at low allele frequencies in cfDNA.