Abstract 2744

Inter-site comparison of performance of an ultra-sensitive sequencing technology for circulating tumor DNA

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Introduction

The combination of liquid biopsies and ultra-sensitive next-generation sequencing (NGS) holds tremendous promise in oncology for the detection and surveillance of somatic mutations that correlate with diagnosis, prognosis, response prediction, resistance monitoring and tumor burden.

We have developed a capture-based NGS workflow that provides ultra-sensitive detection of four mutation classes - single nucleotide variants (SNVs), insertions/deletions, copy number amplifications (CNAs) and fusions - from low input cell-free DNA isolated from plasma.

To evaluate inter-site robustness of our workflow, we designed an early evaluation program to measure performance of the Roche AVENIO ctDNA Analysis Kits at four testing sites.

Footnotes:

[1] See definitions of QC metrics in table below.

[2] The EEP used a draft version of the AVENIO kits from early in the development process. The final AVENIO kit performance for launch has been improved, including the on-target rates.

Definitions of QC metrics reported

Median Depth: the unique depth across bases in the targeted region

% Error Rate: the fraction of non-reference bases observed at positions that are likely reference

% Uniformity: the % of bases in two-fold range of the median depth

% On-Target: the percentage of aligned reads to the human genome that are within the targeted region

Study Design

Four laboratories received early evaluation versions of Roche AVENIO ctDNA Analysis Kits (for Research Use Only) (**Table 1**). Each lab performed the assay on 48 samples that included contrived DNA blends with known mutations validated by digital PCR and normal plasma samples (**Table 2**).

Table 1. Kits evaluated by each lab

AVENIO ctDNA Analysis Kit	Laboratory			
	1	2	3	4
Targeted	✓	✓		
Expanded	✓	✓	✓	
Surveillance	✓			✓

Table 2. Samples assessed per kit

Sample Type		No. of Samples
Healthy donor	cfDNA	12
	plasma	12
SNV	cell lines	6
	cfDNA spikes	6
Fusion	cell lines	6
CNV	cell lines	6

Each lab sequenced the prepared samples on the Illumina NextSeq 500. We included up to 40 million reads per sample in downstream bioinformatics analyses to normalize QC metrics comparisons between samples and labs.

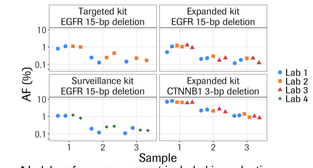
Utilizing the AVENIO ctDNA Analysis Software, labs generated QC metrics and variant calls for each sample. To characterize lab-to-lab variability, we compared key metrics such as median depth and coverage uniformity across different labs for the same samples.

We also evaluated the sensitivity and specificity of each kit on different mutation classes. Sensitivity was calculated by variant for each sample. We report the range of values across two technical replicates per sample per lab. Specificity was calculated by variant across healthy donor samples (24 biological replicates per kit per lab).

Key Findings

- **QC metrics such as depth and uniformity were highly consistent for the same samples prepared at different testing sites.**
- On-target rates[2] ranged from 31-71% (5th-95th percentile) for the Targeted kit, 33-70% for the Expanded kit, and 57-77% for the Surveillance kit.
- Median coverage for the Targeted kit was 4300-5100X, which was greater than that for the Expanded kit (2700-3200X) and Surveillance kit (3500-3700X). This was expected given the Targeted kit covers a smaller region and all samples had a similar number of sequencing reads.
- **Testing sites achieved 100% sensitivity for SNVs and fusions at allelic frequencies of 0.5% and 1%, respectively, from a targeted amount of 30 ng of input DNA with all three kits.**
- Specificity was 99.94-99.99% for hotspot SNVs in healthy donor samples across the three kits. The specificity for fusion detection was 100% for all three kits.
- At an expected 5X or 8X copy number amplification for multiple genes, all three kits were 100% sensitive. Overall, the three kits were 87.5-100% specific per gene.
- **We demonstrate that the AVENIO ctDNA Analysis Kits for plasma and NGS applications provide robust performance for the simultaneous detection of four* different mutation classes.**

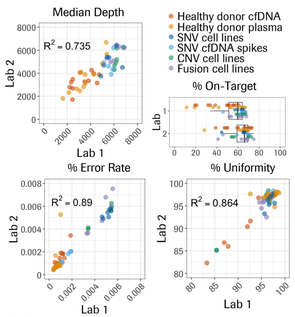
Figure 1. Indels detected per kit



* Indel performance was not included in evaluation program; however, the SNV cell lines contained indels that were detected down to 0.1% allelic frequency (**Figure 1**).

AVENIO ctDNA Targeted Kit

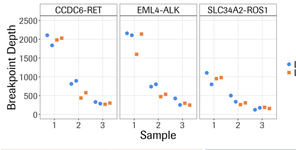
QC metrics[1]



The AVENIO ctDNA Targeted Kit was designed to detect mutations in 17 genes, across 81 kb, included in NCCN Guidelines for solid tumors in addition to variants relevant for therapy selection.

Fusion performance

Sensitivity and specificity was evaluated on ALK, RET, and ROS1.



Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2
1	AF~12.5%	100%	100%	100%	100%
2	AF~2%	100%	100%		
3	AF~1%	100%	100%		

SNV performance

Sensitivity was evaluated on 22 cancer hotspot variants in cell line blends. Healthy donor samples were used to measure specificity across >300 cancer hotspot variants.

Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2
1	AF~1.5%	100%	100%	99.95 %	99.97 %
2	AF~0.5%	100%	100%		
3	AF~0.25%	100%	95.5 - 100%		

CNV performance

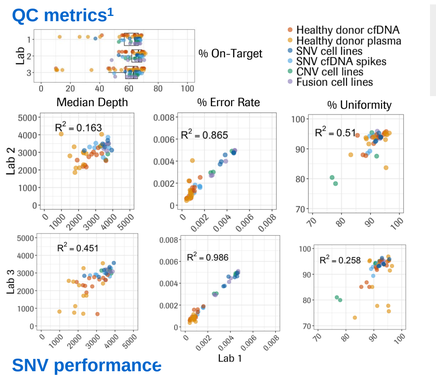
Sensitivity and specificity was evaluated on EGFR, ERBB2, and MET.

Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2
1	≥5 copies	100%	100%	97.2%	100%
2	≥2.6 copies	33%*	33-100%*		
3	≥2.3 copies	33%*	33%*		

* MET sensitivity was 100%.

AVENIO ctDNA Expanded Kit

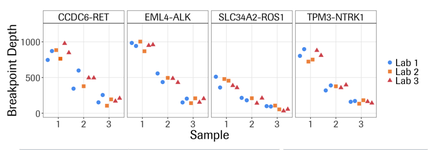
QC metrics[1]



The AVENIO ctDNA Expanded Kit was designed to detect mutations in 77 genes, across 192 kb, associated with solid tumor including variants relevant for clinical trial research and included in NCCN Guidelines.

Fusion performance

Sensitivity and specificity was evaluated on ALK, NTRK1, RET, and ROS1.



Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Sensitivity Lab 3	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2	Specificity (N=24 per lab) Lab 3
1	AF ~ 12.5%	100%	100%	100%	100%	100%	100%
2	AF ~ 2%	100%	100%	100%			
3	AF ~ 1%	100%	100%	100%			

SNV performance

Sensitivity was evaluated on 26 cancer hotspot variants in cell line blends. Healthy donor samples were used to measure specificity across >500 cancer hotspot variants.

Sample (N = 2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Sensitivity Lab 3	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2	Specificity (N=24 per lab) Lab 3
1	AF~1.5%	100%	100%	100%	99.98%	99.94%	99.99%
2	AF~0.5%	92.3 - 96.2%	100%	96.2 - 100%			
3	AF~0.25%	88.5 - 96.2%	88.5 - 92.3%	88.5 - 92.3%			

CNV performance

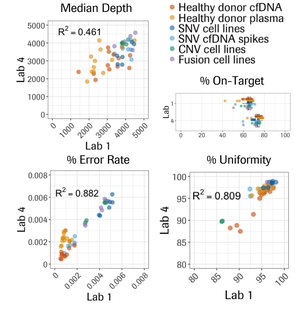
Sensitivity and specificity was evaluated on EGFR, ERBB2, and MET.

Sample (N = 2 per lab)		Sensitivity Lab 1	Sensitivity Lab 2	Sensitivity Lab 3	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 2	Specificity (N=24 per lab) Lab 3
1	>=5 copies	100%	100%	100%	100%	100%	100%
2	>=2.6 copies	33%*	100%	33%*			
3	>=2.3 copies	0%	0%	33%*			

* MET sensitivity was 100%.

AVENIO ctDNA Surveillance Kit

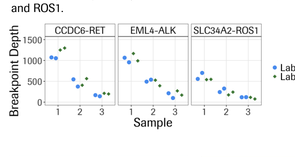
QC metrics[1]



The AVENIO ctDNA Surveillance Kit was designed to detect mutations in 197 genes, across 198 kb, for residual disease monitoring and included in NCCN Guidelines.

Fusion performance

Sensitivity and specificity was evaluated on ALK, RET, and ROS1.



Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 4	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 4
1	AF~12.5%	100%	100%	100%	100%
2	AF~2%	100%	100%		
3	AF~1%	100%	100%		

SNV performance

Sensitivity was evaluated on 25 cancer hotspot variants in cell line blends. Healthy donor samples were used to measure specificity across >300 cancer hotspot variants.

Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 4	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 4
1	AF~1.5%	100%	100%	99.99 %	99.96 %
2	AF~0.5%	100%	100%		
3	AF~0.25%	92 - 96%	100%		

CNV performance

Sensitivity and specificity was evaluated on EGFR, ERBB2, and MET.

Sample (N=2 per lab)		Sensitivity Lab 1	Sensitivity Lab 4	Specificity (N=24 per lab) Lab 1	Specificity (N=24 per lab) Lab 4
1	≥5 copies	100%	100%	97.2%	87.5%
2	≥2.6 copies	33%*	100%		
3	≥2.3 copies	33%*	33%*		

* MET sensitivity was 100%.

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