# Early Evaluation Site Experience with a Liquid Biopsy Kit designed for Next Generation Sequencing of Circulating Tumor DNA

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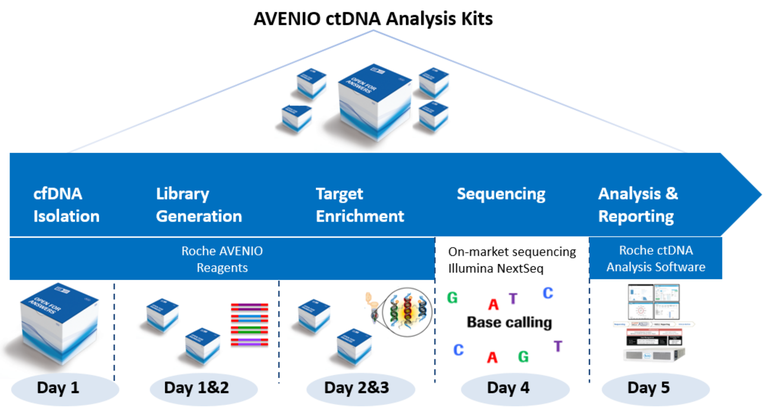
## Introduction

DNA-based microarray technology is useful for providing information about gene copy-number and chromosomal aneuploidy. FFPE (formalin fixed paraffin embedded) tissue is an important yet difficult sample type to work with in molecular assays. The chemical fixation process intended to preserve specimens for long term storage causes genetic information to be lost through DNA/protein cross linking and the creation of apurinic sites. Further compounding the problem, long-term storage of blocks before molecular analysis contributes to nucleic acid degradation. Here we describe a method for reference size matching, amplification and fluorescent labeling of FFPE specimens that yields high quality material for downstream DNA-based microarray analysis of this difficult sample type.

## Methods

In the current study, our CAP/CLIA accredited laboratory was selected to participate in an Early Evaluation Program (EEP) of the research use only (RUO) AVENIO ctDNA Analysis Targeted and Expanded Kits (Roche Sequencing Systems, Pleasanton, CA). The Targeted and Expanded kits interrogate cancer personalized profiles by deep sequencing (CAPP-Seq) and integrated digital error suppression (IDES) technology to overcome key challenges of liquid biopsy and provide clinical laboratories with an end to end capture-based NGS liquid biopsy solution on the NextSeq platform (Illumina, San Diego CA.) In the EEP, conducted across three sites including our own, 48 normal plasma and contrived cell line DNA samples with known mutations were evaluated for intra and inter-laboratory accuracy at allele frequencies of 1%, 0.5%, and 0.25% using 30 ng of input DNA for both kits.




**Scheme1. Workflow for AVENIO ctDNA Analysis Kits.** *AVENIO ctDNA products leverage Roche assets and expertise to provide customers with a comprehensive end-to-end solution.* Product 1 (P1) – The *AVENIO ctDNA Targeted Kit**: designed to detect 17 genes including those in the NCCN Guidelines for solid tumors plus additional variants relevant for therapy selection. Product 2 (P2) – The *AVENIO ctDNA Expanded Kit**: designed to detect 77 genes associated with solid tumor cancers including variants relevant for clinical trial research.

## Samples used for Evaluation

42 samples per panel for each laboratory

- 12 healthy donor plasma
- 12 healthy donor cfDNA
- 12 SNV samples (cell lines and contrived cfDNA samples)
- 6 Fusion samples (cell lines)

12 - 16 samples per NextSeq run (~40 M reads/sample)

6 Fusion samples:

- Cell line blend Fus1 (2 replicates): 12.5%
- Cell line blend Fus2 (2 replicates): 2%
- Cell line blend Fus3 (2 replicates): 1%

32 cell line blend

- SNV1 (2 replicates): undiluted ~ 1.5% AF
- SNV2 (2 replicates): 3x dilution ~ 0.5% AF
- SNV3 (2 replicates): 6x dilution ~ 0.25% AF

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## Results and Conclusions

**Results:** All three sites showed 100% sensitivity and specificity at 1% and 0.5% allele frequencies and > 90% at 0.25%. Median coverage across regions of interest was 2,700-6400x for both the Expanded and Targeted kits. The kits integrated seamlessly into our laboratory's five-day NGS workflow from DNA extraction through reporting

**Conclusions:** The ctDNA kit solution has enabled our laboratory to perform accurate NGS analysis of liquid biopsy clinical research samples using existing equipment and staff with minimal disruption of our routine NGS workflow. Future directions include an expanded 60 sample intra-laboratory evaluation of the ctDNA kit at allele frequencies below 0.25% using contrived samples prepared from normal human plasma and fragmented DNA with known SNV's created from our onsite repository of over 100 characterized cell lines.

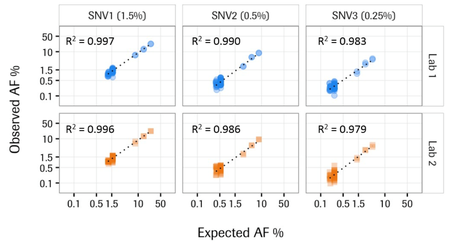
References:

1. Diaz, J. R., & Bardelli, A. (2014, February 20). Liquid biopsies: genotyping circulating tumor DNA. Retrieved October 27, 2017, from https://www.ncbi.nlm.nih.gov/pubmed/24449238
2. Newman, A. (2014, April 06). An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Retrieved October 27, 2017, from https://www.nature.com/articles/nm.3519
3. Offin, M., (2017). Capturing Genomic Evolution of Lung Cancers through Liquid Biopsy for Circulating Tumor DNA. Retrieved October 27, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5368362/

## Assay Performance

**A**

### P1 SNV Calls Linearity




### P1 SNV Calls Summary

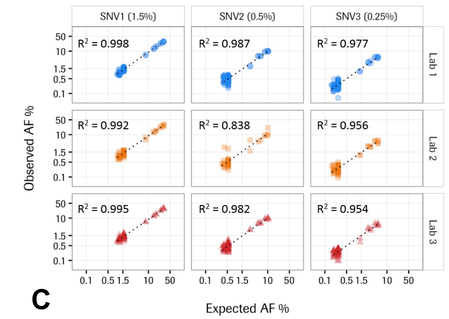
| Sample (2 reps each) | Sensitivity | | Median Coverage | |
|---|---|---|---|---|
| | Lab 1 | Lab 2 | Lab 1 | Lab 2 |
| SNV1(1.5%) | 100% | 100% | 6449 | 5462 |
| SNV2(0.5%) | 100% | 100% | 4996 | 4221 |
| SNV3(0.25%) | 100% | 95.5% - 100% | 5493 | 5390 |

| Specificity (sample based) | |
|---|---|
| Lab 1 | Lab 2 |
| 100% | 100% |

| Specificity (variant based) | |
|---|---|
| Lab 1 | Lab 2 |
| 100% | 100% |

**B**

### P2 SNV Calls Linearity




### P2 SNV Calls Summary

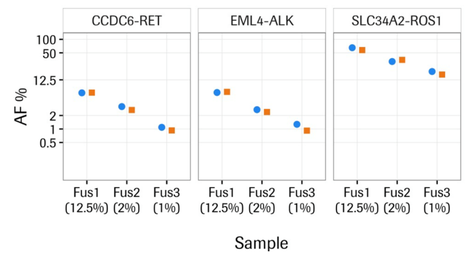
| Sample (2 reps each) | Sensitivity | | | Median coverage | | |
|---|---|---|---|---|---|---|
| | Lab 1 | Lab 2 | Lab 3 | Lab 1 | Lab 2 | Lab 3 |
| SNV1(1.5%) | 100% | 100% | 100% | 3821 | 3400 | 3454 |
| SNV2(0.5%) | 100% | 100% | 100% | 2730 | 3104 | 2808 |
| SNV3(0.25%) | 87.5% - 100% | 91.7% - 95.8% | 95.8% - 100% | 3469 | 3394 | 3069 |

| Specificity (sample based) | | |
|---|---|---|
| Lab 1 | Lab 2 | Lab 3 |
| 100% | 91.7% | 100% |

| Specificity (variant based) | | |
|---|---|---|
| Lab 1 | Lab 2 | Lab 3 |
| 100% | 99.7% | 100% |

**C**

### P1 Fusion Calls

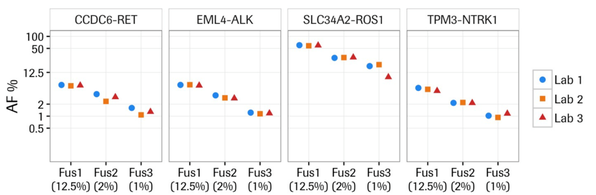



| Sample | Sensitivity | |
|---|---|---|
| | Lab 1 | Lab 2 |
| Fus1(12.5%) | 100% | 100% |
| Fus2(2%) | 100% | 100% |
| Fus3(1%) | 100% | 100% |

| Specificity | |
|---|---|
| Lab 1 | Lab 2 |
| 100% | 100% |

**D**

### P2 Fusion Calls




| Sample | Sensitivity | | | Specificity | | |
|---|---|---|---|---|---|---|
| | Lab 1 | Lab 2 | Lab 3 | Lab 1 | Lab 2 | Lab 3 |
| Fus1(12.5 %) | 100% | 100% | 100% | 100% | 100% | 100 % |
| Fus2(2%) | 100% | 100%* | 100% | | | |
| Fus3(1%) | 100% | 100% | 100% | | | |

Figure 1:A- Linearity and calls summary for P1,B- Linearity and calls summary for P2,C- Fusion calls for P1, D- Fusion calls for P2