# Advancing Cancer Genomics with Agilent Avida DNA Panels for Liquid Biopsy Samples: A Breakthrough in Targeted Sequencing

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## Introduction

Liquid biopsy is a minimally invasive technique that has transformed oncology research by enabling the ultra-sensitive detection of cancer-related genomic alterations from circulating cell-free DNA (cfDNA). Despite its potential, the use of liquid biopsy samples faces challenges such as low input amounts, PCR bias, and complex, time-consuming workflows.

Optimized to address these issues, the Agilent Avida target enrichment technology (Figure 1) offers highly sensitive DNA variant detection. Avida DNA libraries can be generated with inputs of cfDNA as low as 1 ng, and up to 100 ng DNA input, with high sample recovery that scales linearly with input amount.

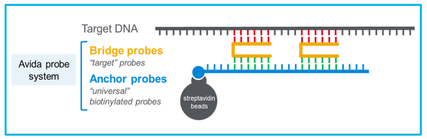


Figure 1: The Agilent Avida technology is based on a synergistic, indirect hybridization chemistry that uses two probe types: short, target-specific bridge probes and a biotinylated anchor probe for high affinity capture with streptavidin beads. This enables efficient and specific target capture that is especially suited for low input samples.

The Avida DNA workflow generates sequencing-ready libraries in five hours (Figure 2). Eliminating the need for pre-capture PCR, the Avida DNA workflow is fast, simple, and efficient. Additionally, the Avida technology can capture methylation targets from the same sample for multiomic insights.

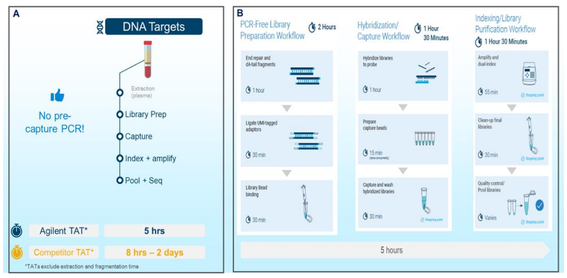


Figure 2: The Agilent Avida DNA workflow to generate sequencing-ready libraries. (A) The five-hour workflow enables sample to sequencer in a single shift, for a faster turnaround time (TAT) compared to conventional target hybridization methods. (B) Detailed steps for the Avida DNA workflow are shown.

The OncNGS Consortium, a collaborative initiative of eight institutions across five European countries, has incentivized the market to research and develop novel, affordable NGS solutions for profiling tumor and lymphoma samples, with a particular focus on liquid biopsy. Based on the consortium requirements, Agilent has developed three new Avida DNA panels targeting key cancer genes for tumor and lymphoma samples. Here we describe the performance of these new Avida DNA panels based on sequencing metrics and the detection of key variant types from reference samples with different amounts of input and allele frequencies.

## Experimental

Three Agilent Avida DNA panels were designed to detect cancer-associated variants (SNVs/indels, CNVs, translocations) for key genes based on OncNGS Consortium requirements (Table 1): the Agilent Avida Onco DNA LB panel focuses on Tier 1 & 2 targets in ESCAT and OncoKB Levels 1 & 2; the Agilent Avida DNA Onco LB Plus panel combines the Avida DNA Onco LB panel with additional content for a more comprehensive approach; the Agilent Avida DNA Lymphoma panel targets key genes for researching hematologic malignancies.

| Panel | Target Size (kb) | Total Gene Count | Gene count per coverage type | | |
|---|---|---|---|---|---|
| | | | Full coding regions | Select exons/hotspots | Translocations |
| Avida DNA Onco LB | 879 | 164 | 107 | 57 | 27 |
| Avida DNA Onco LB Plus | 1,765 | 437 | 362 | 75 | 34 |
| Avida DNA Lymphoma | 383 | 86 | 37 | 49 | 18 |

Table 1: Agilent Avida DNA panels designed based on OncNGS Consortium requirements

The performance of these panels was evaluated using a broad set of reference materials: Seraseq ctDNA Mutation Mix v4, Seraseq ctDNA Lymphoma Mix, and human plasma cfDNA isolated from a pool of healthy/normal donors (Biochain p/n Z2121001). Libraries were prepared using the Avida DNA workflow and sequenced on Illumina NovaSeq 6000 to assess capture specificity and target region coverage. Variant analysis was done using Agilent's R&D software analysis pipeline which incorporates Vardict (version 1.8.3), PureCN (version 2.8.0) and GeneFuse (version 0.8.0) tools for SNV, CNV, and TL analysis, respectively. Avida technology leverages unique molecular identifiers (UMIs) to tag each DNA molecule prior to PCR amplification, enabling accurate molecular counting and error correction, thereby enhancing variant detection and quantification.

## Results and Discussion

### Comprehensive performance assessment of Avida DNA panels across varying DNA input amounts

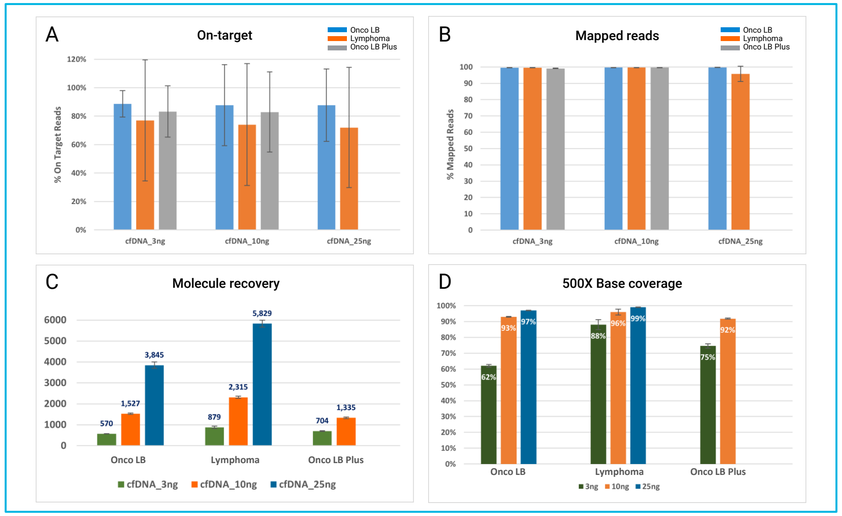


Figure 3: Assay specificity and sequencing accuracy are demonstrated by a high percentage of (A) on-target reads and (B) mapped reads across various DNA input amounts. (C) Efficient recovery of input material, measured as the number of informative unique molecules recovered per target (from both DNA strands) on average (25-50% of the expected value), is depicted by the deduplicated median span. (D) Robust coverage performance is shown by the proportion of bases achieving at least 500X unique reads. The cfDNA sample (Biochain p/n Z2121001) was tested using the Avida DNA Onco LB and Avida DNA Lymphoma panels at input amounts of 3 ng (n=4), 10 ng (n=4), and 25 ng (n=3). The Avida DNA Onco LB Plus panel was tested with 3 and 10 ng DNA inputs only.

### Copy number gains for all CNVs were consistently detected across Avida DNA Onco LB and Onco LB Plus panels

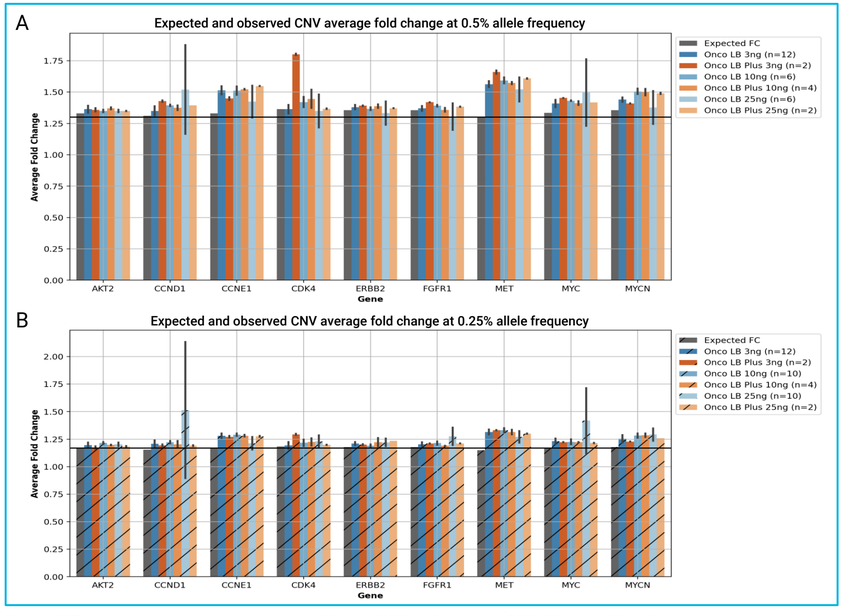


Figure 4: The average observed fold change (FC) for CNVs in Seraseq ctDNA Mutation Mix v4 at the gene level for each DNA input amount (3, 10, 25 ng) tested with the Avida DNA Onco LB and Onco LB Plus panels are shown along with expected fold change at (A) 0.5% allele frequency (AF) (2.68 CN) and (B) 0.25% AF (2.34 CN). The solid horizontal line corresponds to the average expected FC for all 9 CNVs.

## Results and Discussion

### Consistent performance of Avida DNA panels in detecting unique multiplexed variants

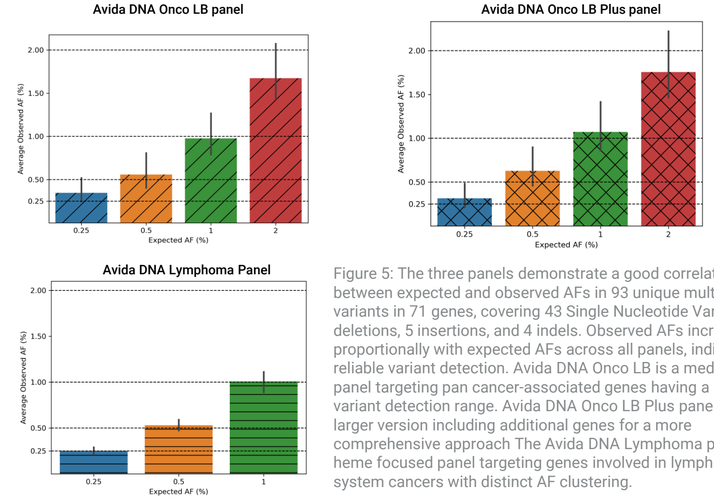


Figure 5: The three panels demonstrate a good correlation between expected and observed AFs in 93 unique multiplexed variants in 71 genes, covering 43 Single Nucleotide Variants, 19 deletions, 5 insertions, and 4 indels. Observed AFs increase proportionally with expected AFs across all panels, indicating reliable variant detection. Avida DNA Onco LB is a medium size panel targeting pan cancer-associated genes having a broad variant detection range. Avida DNA Onco LB Plus panel is a larger version including additional genes for a more comprehensive approach The Avida DNA Lymphoma panel is a heme focused panel targeting genes involved in lymphatic system cancers with distinct AF clustering.

### Assessment of translocation detection sensitivity across DNA inputs and allele fractions

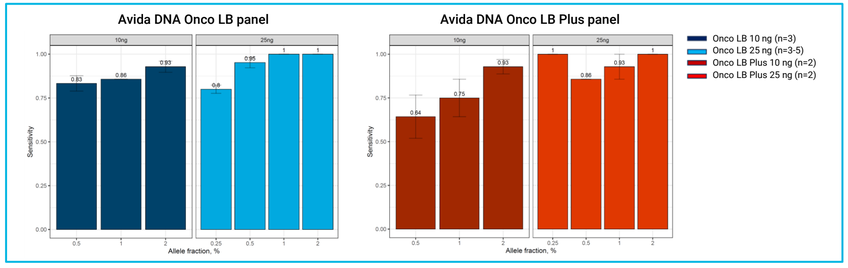


Figure 6: Efficacy of TL detection by Avida DNA Onco LB and Avida DNA Onco LB Plus panels. The panels effectively detect TLs at lower allele fractions, with improved detection performance as the input amount and allele fraction increase. TL analysis was done in Seraseq ctDNA Mutation Mix v4 across these panels covering 7 out of 10 known translocations (*ALK-EML4, BICC1-FGFR2, CD74-ROS1, ETV6-NTRK3, FGFR3-TACC3, NCOA4-RET, NTRK1-TPM3*).

## Conclusions

The newly developed Agilent Avida DNA panels described here demonstrate high sample recovery across a range of DNA input amounts, exceptional capture specificity, and robust target region coverage. These panels enable sensitive and reliable detection of cancer-associated genomic alterations, including SNVs/indels, CNVs, and translocations, across key oncogenes. Performance assessment across varying inputs confirms the high sequencing accuracy, efficient molecular recovery, and excellent coverage depth, facilitated by unique molecular identifiers (UMIs) for precise error correction and variant quantification. These panels expand the Avida DNA panel portfolio to offer flexible and modular solutions for ultra-sensitive genomic profiling from circulating cfDNA in liquid biopsy samples. This innovative approach supports liquid biopsy-based precision oncology applications and advances cancer research by evaluating the utility of non-invasive techniques for sample analysis.

ONCNGS PCP

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