Customer Data

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cfDNA Extraction Efficiency Affects NGS Data

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Tissue biopsy remains the gold standard for cancer diagnosis and typically requires an invasive procedure. However, tissue biopsy only presents a snapshot of disease instead of a holistic view of cancer initiation and development. Liquid biopsy is an assay for non-solid biological tissue and offers the potential for noninvasive detection and real-time information about a tumor. Circulating cell-free DNA (cfDNA) is a small DNA fragment that sheds into the bloodstream in both healthy and cancer patients. An increasing number of researchers indicate that the use of cfDNA and circulating tumor DNA (ctDNA) for cancer detection, prognosis and monitoring positively impact the clinical management and outcomes of patients.

With the development of next-generation sequencing (NGS), it is now possible to screen most regions of the genome at ultra-deep sequencing depths to identify cancer mutations, even with very degraded samples such as cfDNA. However, cfDNA is at very low concentrations in bodily fluids; 1-50 ng cfDNA is typically obtained from 1 mL blood, among which, only 0.01 – 0.1% is ctDNA. How to increase cfDNA extraction efficiency remains one of the challenges for accurate NGS data, which in turn affects the accuracy of the liquid biopsy. The greatest challenge to the efficacy of liquid biopsy is the accuracy of NGS data, which can be overcome by increasing cfDNA extraction efficiency.

In order to understand how cfDNA extraction efficiency affects NGS data, we present Institute B titration data. EGFR L858R standards with an allele frequency (AF%) of 0.3%, 1.0%, and 3.0% were spiked into plasma and isolated using either Apostle MiniMax™ High Efficiency cfDNA Isolation or Product A. The cfDNA was analyzed via NGS and AF% was calculated by Institute B. cfDNA isolated by Apostle MiniMax cfDNA isolation kit shows concordance between detected AF% and expected AF%.

For cancer samples, the allele frequency represents the percentage of sequence reads carrying a mutant allele of an individual patient's cancer. Researchers often set thresholds for defining the variant allele frequency as mutation; therefore, low cfDNA recovery efficiency can decrease sensitivity and lead to an increase in false negatives for mutation calling.

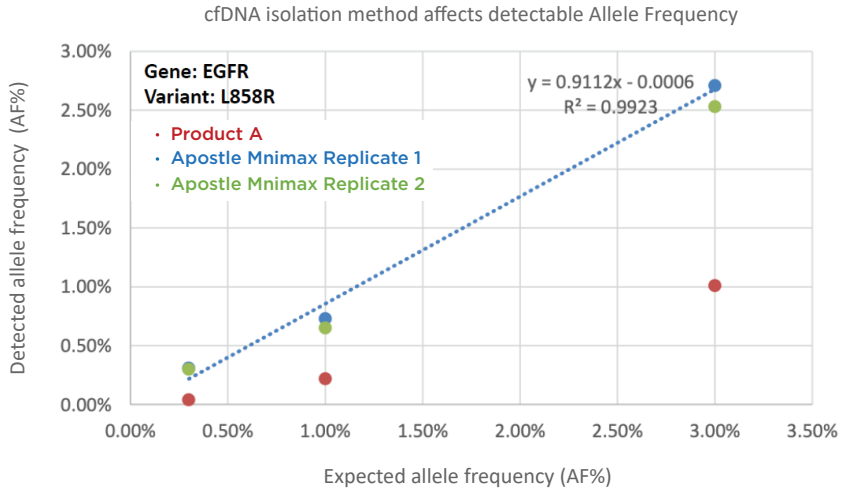

Figure 1. *cfDNA extraction method affects allele frequency (AF%). EGFR L858R standards with AF% of 0.3%, 1.0%, and 3.0% were spiked into plasma and isolated using Product A (red) or Apostle MiniMax cfDNA isolation kit (blue and green). The eluates were analyzed by NGS (performed by the Institute B) following standard protocol and AF% calculated using their established workflow. cfDNA isolated by Apostle MiniMax cfDNA isolation kit shows higher concordance between detected AF% and expected AF%.*

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AAG-659602.20