Automated Liquid Biopsy Workflow for Low Frequency Variant Detection

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Abstract

Automated liquid biopsy assays enable non-invasive profiling of circulating, cell-free DNA (cfDNA) and circulating tumor cell DNA to assist in early-stage diagnosis of disease and monitoring treatment response. Since high sequencing depth is required to profile cfDNA for low frequency variants, most liquid biopsy assays use targeting to cost-effective areas to achieve deep coverage of target loci for pathogenic variant detection as low as 1% allele fraction. An assay that produces uniform coverage from clinical relevant DNA quantities is critical for obtaining the necessary sensitivity. We developed a liquid biopsy workflow for low frequency variant detection from 10 mL of blood combined with Accel-NGS® library preparation. Whole blood samples were collected in Streck Cell-Free DNA BCT® vials from patients with late stage cancer and cfDNA was extracted with the Promega Maxwell RSC. A total of 10 ng cfDNA was used to make an Accel-NGS® 25 by library followed by hybridization capture using IDT xGen® Pan Cancer probes. Molecular barcodes were incorporated to label each library molecule uniquely prior to amplification. Sequencing was performed to a minimum of 8000x mean bait coverage. The Accel-NGS® 25 Hybrid Kit exhibited 90% conversion with cfDNA provided 100% complex libraries with uniform target coverage (> 99% of bases covered > 100x). Molecular barcodes enabled removal of PCR duplicates while preserving fragmentation and shared duplicates to maximize coverage. Sensitive and precise detection of variants was achieved down to 0.5% allele frequency. We developed a liquid biopsy workflow for low frequency variant detection from clinically-relevant quantities of cfDNA can be used to identify tissue-of-origin. This approach provides powerful methods for detecting, identifying, and monitoring disease.

MIDs Label Unique Library Molecules

Uniform Incorporation of MIDs

Increased Specificity with MIDs

Increased Throughput with Automation

Conclusions

- Labeling unique library molecules with MIDs prior to amplification allows for the removal of sequencing and PCR induced errors during data analysis and results in an increase in coverage across samples.
- Inclusion of MIDs in Swift library preparation improves low frequency variant calling by increasing sensitivity and specificity and is shown here to facilitate detection of known variants present at 1% and 0.5% allele frequencies.
- The use of MIDs for de-duplication results in increased data retention through the accurate distiction of PCR duplicates from fragmentation and complementary strand duplicates.
- The automated Biomark FX® method provides flexible workflow options, incorporation of on-deck incubations and thermal cycling, minimal user interventions and optimized pipetting which minimize consumable use and sample loss and increase lab efficiencies with a walk-away solution capable of generating up to 96 PCR-free libraries in approximately 4.5 hours.
- Automated Accel-NGS® kits require no adapter titration or adjustments to PCR cycles, generating data equivalent to manual preparation, for up to 96 samples simultaneously.