An end-to-end automated workflow for low-frequency variant detection in cfDNA

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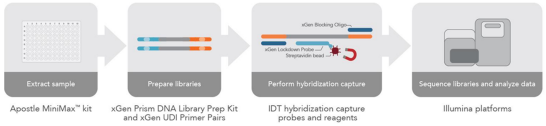
Introduction

Liquid biopsy, as a tool to detect tumor-associated variants in cell-free DNA (cfDNA) from plasma, is a powerful non-invasive approach for biomarker discovery in oncology research. Integrated DNA Technologies (IDT) and Beckman Coulter Life Sciences (BECLS) have teamed up to provide a comprehensive end-to-end automated solution on the Biomek workstations for low frequency variant detection in cfDNA using next generation sequencing. The combined workflow including reagents for cfDNA extraction from plasma, NGS library preparation and hybrid capture, provides a robust and reproducible solution analyzing cfDNA samples.

Integrated reagent workflow for cfDNA



Materials and Methods

Blood samples were drawn from four healthy donors and stored into K2EDTA tubes. 4 mL of plasma was harvested by centrifugation and cfDNA was extracted using Apostle MiniMax High Efficiency Isolation Kit (BECLS) or competitor cfDNA kit by following manufacturer's recommendations. cfDNA yield was measured by qPCR. cfDNA standards, used in benchmarking studies, were obtained from Horizon. Libraries were generated with IDT xGen Prism DNA Library Prep Kit or competitor library prep kit according to manufacturer's instructions, captured using the IDT xGen capture protocol, and sequenced on a NextSeq500. After demultiplexing and mapping to hg19, deduplication, coverage and complexity metrics were calculated using Picard. Relative conversion rates were calculated from mean target coverage. To evaluate variant calling, mixtures were created by spiking Donor C cfDNA into Donor B samples at 0.25% and 0.5%. Libraries were captured in 4-plex reactions using a custom xGen panel.. For the cfDNA study, to calculate sensitivity and positive predictive value (PPV), ground truth was determined by ultra-deep sequencing of 100% non-mixture samples. Variants were called using Vardict.

Apostle Minimax provides higher quantity and quality cfDNA post extraction

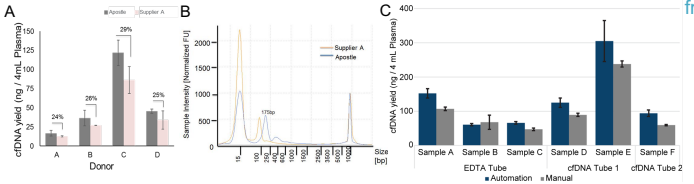


Figure 1. Quantity and quality of cfDNA after recovery using the Apostle MiniMax™ High Efficiency Isolation Kit from BECLS. (A) Higher cfDNA recovery using Apostle MiniMax High Efficiency Isolation Kit. (B) The size distribution of cfDNA. (C) Consistent cfDNA yields were obtained when extracting the same plasma samples either manually (gray) or using the Beckman Biomek i7 Hybrid Workstation (blue) using Apostle MiniMax kit. Samples were collected using EDTA tubes (Sample A-C), cfDNA tube 1 (Samples D and E) or cfDNA tube 2 (Sample F).

xGen Prism provides higher rate of conversion, higher diversity and an option for UMI based error correction

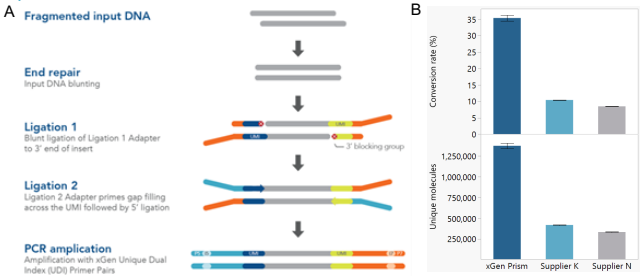


Figure 2. IDT xGen Prism DNA Library Prep kit enables the generation of libraries from low input cfDNA samples. (A) The workflow shows a single-strand ligation strategy with a novel ligase that maximizes library complexity, reduce adapter-dimer and chimera formation. In addition, UMI-based error correction enables ultra- low variation detection for cfDNA samples. (B) xGen Prism libraries had higher conversion rate and higher complexity compared to competitor kits

Apostle MiniMax and xGen Prism DNA library prep together enables low frequency variant detection in cfDNA samples

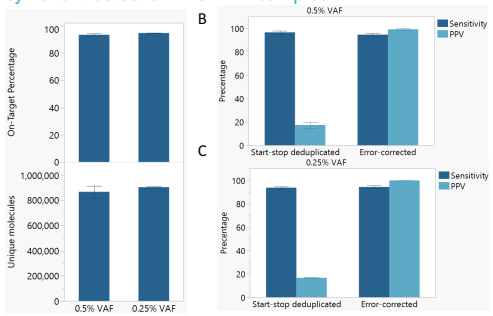
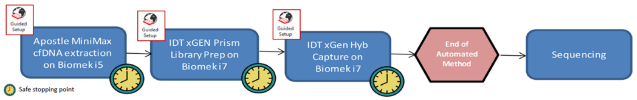


Figure 3. Variant calling. (A) High on-target rates and high library complexity were observed from cfDNA libraries captured with a 75 kb xGen Lockdown Probe custom panel. High sensitivity were observed for both 0.5% VAF (B) and 0.25% VAF (C) mixtures. UMI-based error correction, eliminates nearly all false positive calls, leading to high PPV.

An end-to-end automated NGS workflow



Conclusions

- We provide an end-to-end automation and reagent workflow from plasma to cfDNA analysis that includes Apostle Minimax, xGenPrism DNA Library Prep Kit, and xGen hybridization capture.
- Apostle MiniMax™ High Efficiency Isolation Kit provides high yield extraction of cfDNA.
- The xGen™ Prism DNA library preparation kit, optimized for low input and degraded samples such as cfDNA, provides sensitive and accurate detection of ultra-low frequency variants.

All authors are employees of IDT or BECLS.