A complete automation and reagent workflow for analysis of cfDNA: from plasma to variants

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Introduction

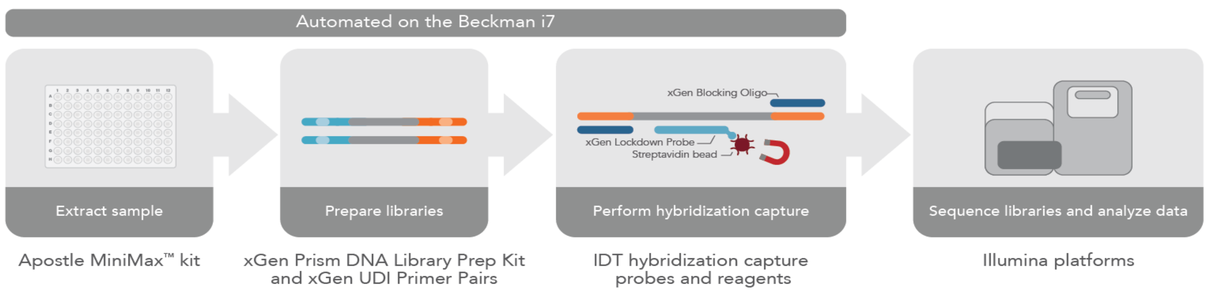

Figure 1: Complete NGS sample preparation workflow for cfDNA

As cost of sequencing continues to drop, the throughput and complexity of NGS assays have risen precipitously. At the same time, the types of samples being used for these assays have expanded as researchers find value in studying low input, degraded biological samples, such as cell free DNA (cfDNA). However, efficient extraction of cfDNA from bodily fluids is challenging due to the very low concentrations of nucleic acid. Manual protocols involved in the NGS workflow – DNA extraction, library preparation, and target enrichment – are often laborious and require significant hands on time, which can increase variability in the downstream analysis. Thus, complete automation workflows can increase throughput, reduce hands-on time, and minimize error. Integrated DNA Technologies (IDT) and Beckman Coulter (BC) have teamed up to provide a complete automation and reagent workflow for analysis of low frequency variants in cfDNA. The Apostle MiniMax™ High Efficiency Isolation Kit from BC is a magnetic nanoparticle based kit that extracts cfDNA from all bodily fluids. IDT's xGen™ Prism DNA library prep kit provides novel chemistry to maximize conversion, suppress adapter-dimer formation, reduce chimera rates, and facilitate UMI-based error correction to call ultra-low frequency variants. IDT's xGen™ target enrichment products maintain high library diversity and on-target rates to enable low frequency variant calling regardless of panel size. The combination of these reagents on the Biomek workstations provides a robust and reproducible solution for the analysis of cfDNA.

Automation

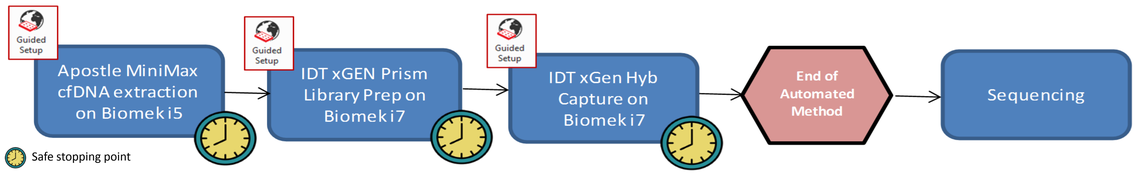

Figure 2: An end to end automated workflow from extraction to hybridization capture

Automation on the Biomek platform provides reduced hands on time, increased levels of throughput, decreases potential pipetting errors, and enables ready to implement methods. There are features built into the modularized method that provides flexibility and ease of use. For easy and precise setup, the Method Options Selector (MOS) user interface enables selection of runtime options. Guided Labware Setup (GLS) software greatly reduces setup errors providing dynamic reagent calculation and graphical guidance for setting up the deck. DeckOptix Final Check software utilizes the on-board camera system to automatically detect and notify the user of on-deck setup discrepancies. The deck utilizes Orbital Shaker for mixing and shaking incubations, as well at Static Peltier and on-deck thermocycler for incubations and PCR thus increasing walk away time for users.

Apostle Minimax provides higher quantity and quality cfDNA extraction

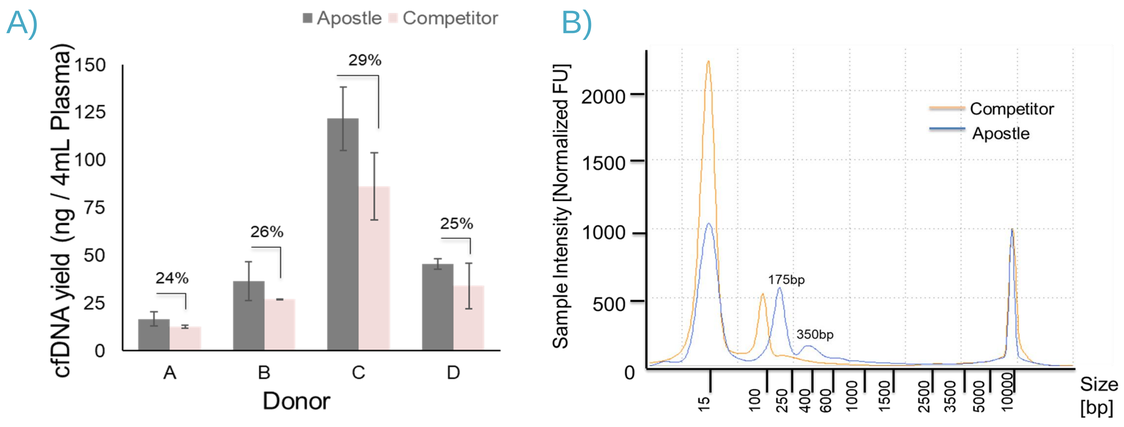

Figure 3: (A) Extraction of cfDNA from 4mL of plasma samples using the Apostle MiniMax™ High Efficiency Isolation Kit obtained higher recovery compared to a competitor kit. (B) cfDNA size distribution was analyzed by Agilent High Sensitivity D5000 ScreenTape. cfDNA extracted from Apostle MiniMax has typical mononucleosome peaks at 175bp and dinucleosome peak at 350bp (blue).

Methods

Blood samples were drawn from healthy donors (N=4) into K_2EDTA tubes. 4mL of plasma was harvested by centrifugation and cfDNA was extracted using Apostle MiniMax™ High Efficiency Isolation Kit or Supplier Q cfDNA kit using manufacturer's recommendations. cfDNA yield was measured by qPCR. Sequencing libraries were generated from 5ng of Apostle MiniMax extraction samples with xGen Prism DNA Library Prep kit or Supplier K kit according manufacturer's instructions. 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 75kb custom panel of xGen Lockdown™ probes. To calculate sensitivity and PPV, ground truth was determined by ultra-deep sequencing of 100% non-mixture samples.

Higher coverage and complexity enables accurate low frequency variant detection

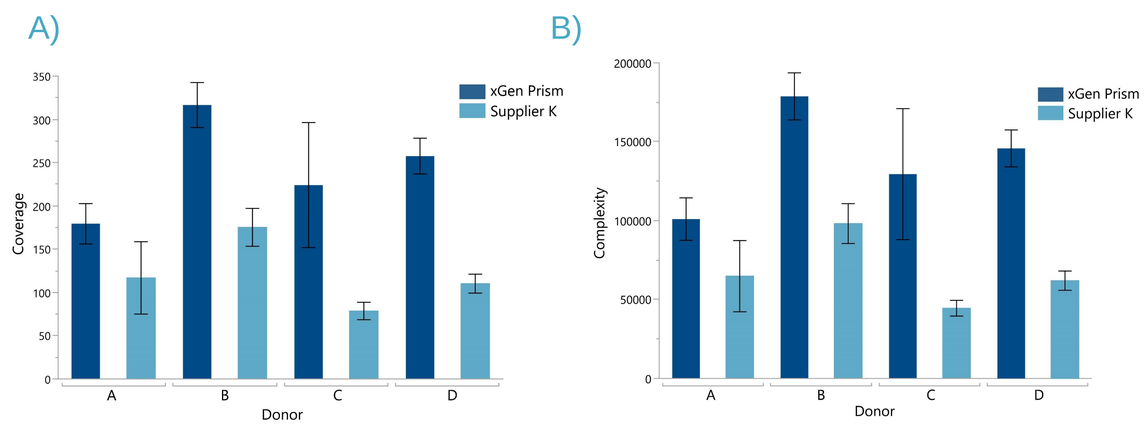

Figure 4: xGen Prism DNA Library Prep libraries have higher coverage and complexity than a competitor kit. For matched 5ng samples into library prep and subsampled to 8.3M reads (A) 2-fold higher coverage and (B) 1.5-2 fold higher complexity was measured with xGen Prism DNA library prep kit compared to Supplier K.

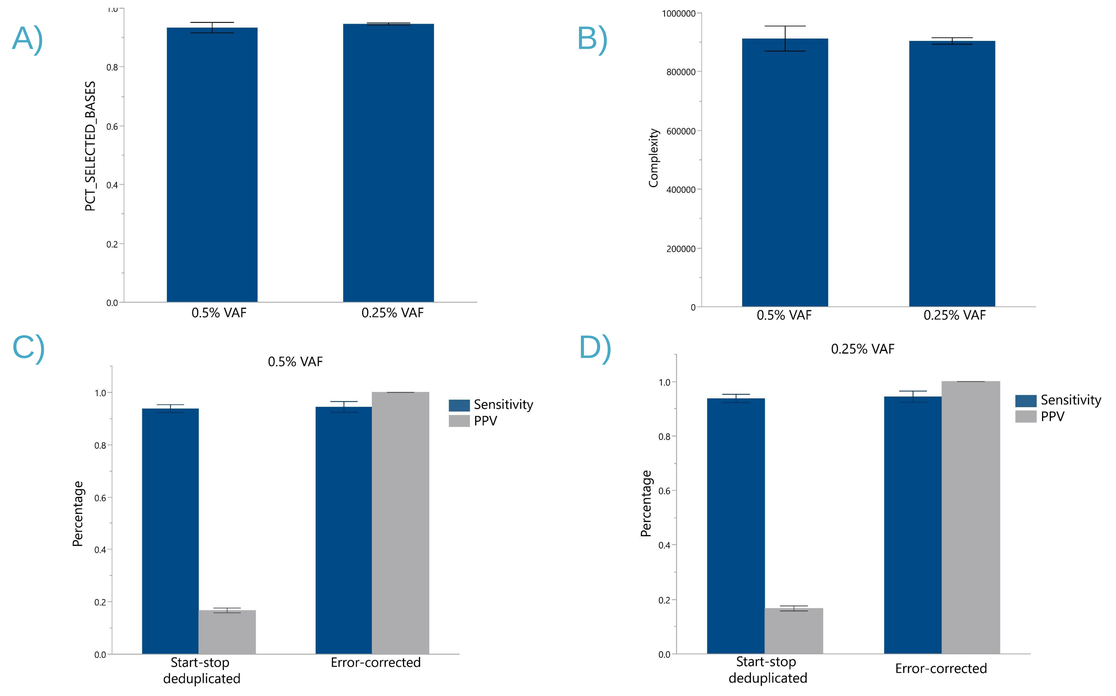

Figure 5: Apostle MiniMax and xGen Prism DNA library prep together enables low frequency variant detection of cfDNA (A) High on-target rates (Picard) were obtained using the xGen hybridization capture protocol with a 75kb size custom panel of xGen Lockdown probes. (B) Library complexity was calculated from Picard HsMetrics after downsampling to 60M reads. (C) High sensitivity (blue) and specificity (gray) in cfDNA samples was obtained with UMI based error correction. (D) Sensitivity and specificity for 0.25% variant allele frequency.

Conclusion

- Apostle Minimax, xGen Prism, and xGen hybridization capture provide a complete automated solution from plasma to sequencing on the Beckman Coulter Biomek platforms
- Apostle Minimax provides higher quantity and quality cfDNA extraction compared to a competitor kit
- xGen Prism is specifically designed for low input, degraded samples, such as cfDNA and generates higher coverage and complexity compared to competitor kits
- UMI-based error correction can be used to eliminate nearly all false positive calls and enables accurate low frequency variant calling