High throughput end-to-end automation of Pan Cancer HuID workflow provides high quality, consistent results from plasma samples with various cancer mutations

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Introduction

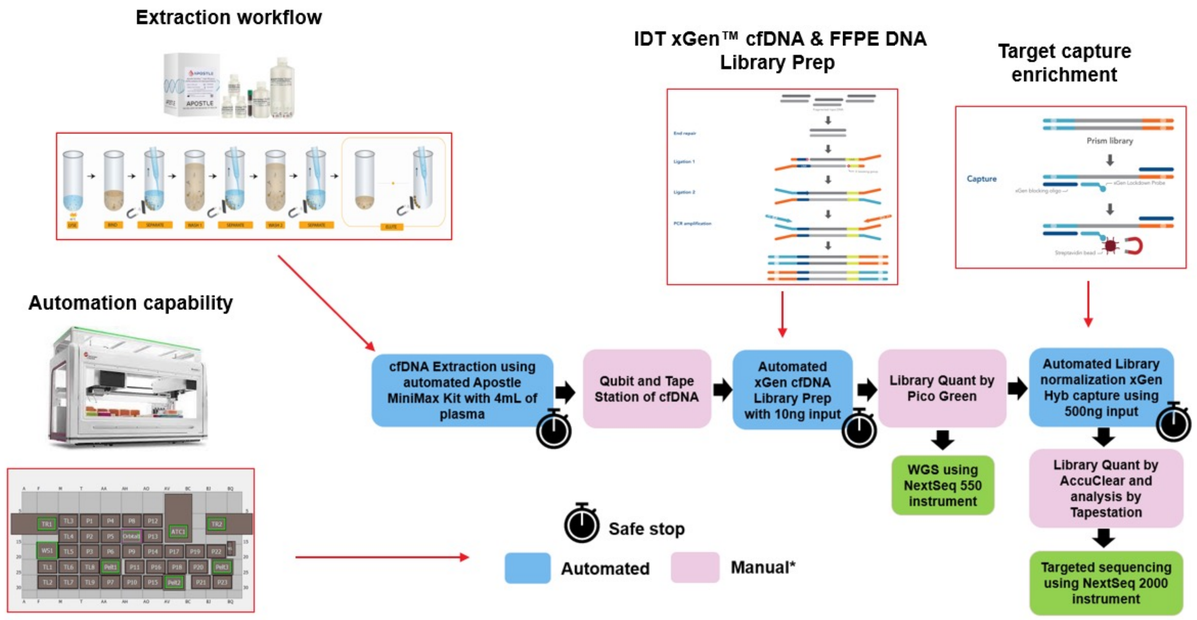

Figure 1. Complete NGS sample preparation workflow for cfDNA.

Due to the increased research into the development of Pan Cancer assays in precision genomics, automation in laboratories is advantageous to generate reproducible targeted sequencing while also increasing sample throughput. Together, Integrated DNA Technologies (IDT) and Beckman Coulter provide an end-to-end automation solution on Beckman Coulter Life Science's Biomek i7 Dual Hybrid (DH) Workstation allowing for the utilization of low input and/or degraded samples such as cell-free DNA (cfDNA) from plasma or formalin-fixed paraffin-embedded (FFPE) biopsies with a throughput of 96 samples per run. A custom IDT 2Mb xGen™ Hyb panel designed against gene targets that have mutations implicated in several cancers along with a Human ID spike-in panel to identify individual sample single nucleotide polymorphisms (SNPs) was used with IDT's xGen Hybridization Capture.

Figure 2. Biomek i-Series i7 Workstation
- Guided Labware Setup (GLS)
- DeckOptix Final Check to ensure accurate system setup
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip for multichannel pipetting to transfer custom array of samples
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with up to 45 positions
- Shaking, heating/cooling, and tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)

Methods

The Apostle MiniMax™ High Efficiency cfDNA Isolation Kit isolates cfDNA and circulating tumor DNA (ctDNA) from plasma collected from blood collection tubes containing EDTA and other collection tube types such as serum and urine. Proteins in cell-free plasma are digested and cfDNA is captured using Apostle's proprietary magnetic nanoparticles. Contaminants are removed from the samples through several simple washes, leaving high-quality cfDNA samples that are ready for elution. The kit produces high-quality extracted cfDNA that can be used in downstream genomic assays. Here blood samples were drawn from unhealthy donors who were diagnosed with either colorectal, breast, or pancreatic cancer (n=22). 10ng of cfDNA extraction was used as input into the xGen cfDNA & FFPE DNA Library Prep kit (n = 96). The xGen cfDNA & FFPE DNA Library Preparation kit is designed specifically for generating libraries from 1 to 250ng of degraded samples. The method features a proprietary ligation strategy that maximizes molecule conversion while also suppressing formation of adapter-dimers and chimeras creating greater library complexity compared to traditional TA ligation-based library methods. Libraries were QC'd via Accuclear Ultra High Sensitivity dsDNA Quantification kit on a Spark 10M Fluorescent Plate Reader. Libraries were also run on Agilent's 4200 TapeStation. Libraries were sequenced on Illumina's NextSeq 550 instrument for WGS analysis (Figure 4).

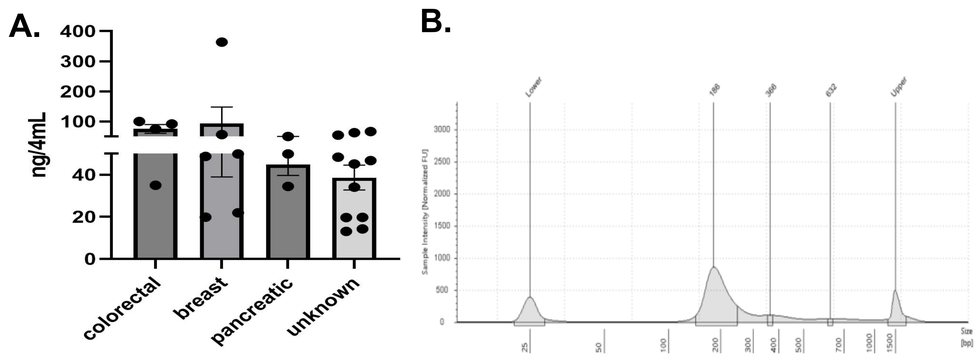

Figure 3. End-to-end workflow: Extraction, library preparation and hybridization capture on the Biomek i7 Hybrid Workstation. (A) Quantity of cfDNA after recovery using DNA extraction kits. Extraction of cfDNA from 4 mL of plasma samples using the Apostle MiniMax High Efficiency Isolation Kit. (B). cfDNA extraction size distribution was analyzed by measuring fluorescence units (FU) on Agilent TapeStation High Sensitivity D1000 ScreenTape. Mononucleosome peak at 186bp, dinucleosome peak at 366bp, and trinucleosome peak at 632bp.

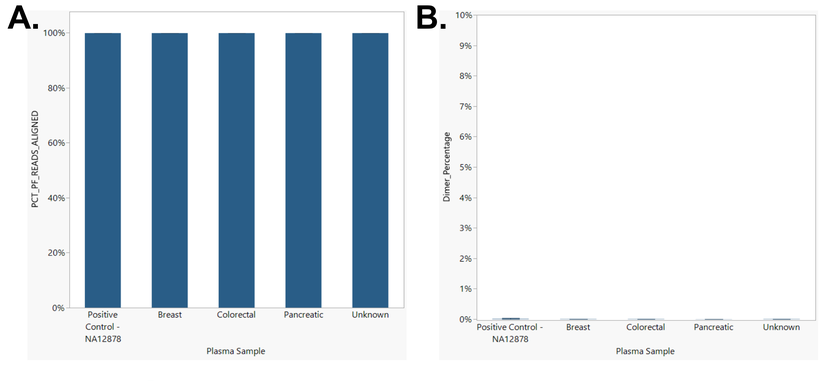

Figure 4. High-quality libraries generated from 10ng plasma samples. All plasma extraction types had 10ng input for library prep and subsampled to 3M reads (n = 96). Positive controls using Coriell gDNA NA 12878 was arrayed throughout the plate (n= 5). The xGen cfDNA & FFPE Library Prep kit demonstrated high mapping rates of ≥99.2% **(A)** and almost zero dimer percentage at ≥0.2% **(B)**. All metrics were calculated using the Broad Institute's Picard HsMetrics.

Hybrid Capture Results

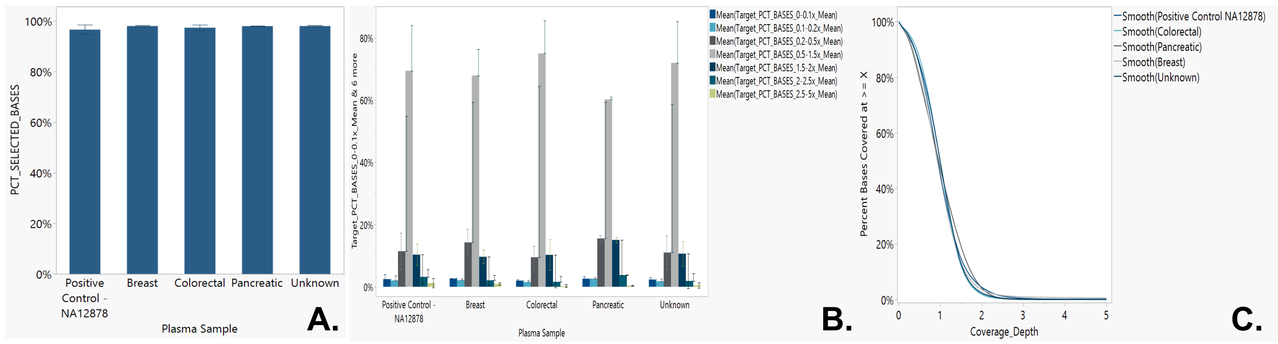

Figure 5. High On-Target percentage, uniformity across targets, and uniform coverage depth are important components for low frequency variant detection. (A) High on-target percentage (Picard HsMetrics) was obtained for all plasma extractions (n = 20) using a custom xGen Hyb Panel with a 2Mb design against mutated gene targets implicated in several cancers. Positive control samples using Coriell NA12878 was arrayed across the Biomek i-Series i7 plate (n= 4). **(B)** Clean base coverage histogram by bins of target bases with X mean coverage (Picard HsMetrics). **(C)** Uniform sequence coverage across mutated gene target regions.

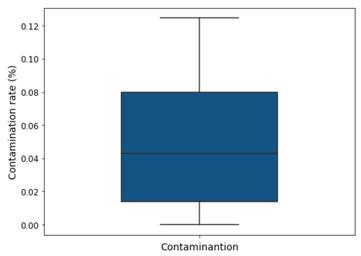

Figure 6. Contamination percentage. Low contamination percentage was obtained for all plasma extractions (n = 20) using xGen Human Identification hybridization panel.

Libraries from Figure 3 were then taken into xGen Hybridization Capture on the Biomek i-Series i7 platform (n = 96). A 2Mb xGen Custom Hyb panel designed against mutated gene targets across several cancer types was used for target pull down. The xGen Human Identification (ID) hybridization panel was also used as a spike in to enable identification of individual samples. 24 capture samples were selected for sequencing on a NextSeq2000 instrument to achieve deeper coverage depth (positive control Coriell NA12878 n = 4, colorectal n = 4, pancreatic n = 3, breast n = 7, and unknown n = 5). Regardless of cfDNA type, results from an end-to-end workflow achieved high on-target percentage and uniform coverage against the custom pan cancer design.

Conclusions

- The combination of Apostle MiniMax™ High Efficiency cfDNA Isolation Kit, IDT's xGen library preparation and xGen Hybridization capture workflows on the Biomek i7 DH Workstation provides a high throughput end-to-end workflow for analysis of precious low input and degraded plasma samples for laboratories performing precision genomics research.
- Apostle MiniMax™ High Efficiency cfDNA Isolation Kit generates high-quality cfDNA samples from breast, colorectal, plasma, and even unknown plasma collection samples.
- High flanked on target (≥97%), target mean coverage (≥438), uniform evenness, and low contamination rate (median ~0.04%) was demonstrated on the Biomek i7 DH Workstation for a 2Mb xGen Custom Hyb panel with xGen Human ID spike – in.