Automating Next Generation Sequencing Workflows for xGen™ Custom Hyb Panels: High quality library preparation and target enrichment with xGen DNA library preparation and hybridization capture on the Biomek Systems

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Introduction

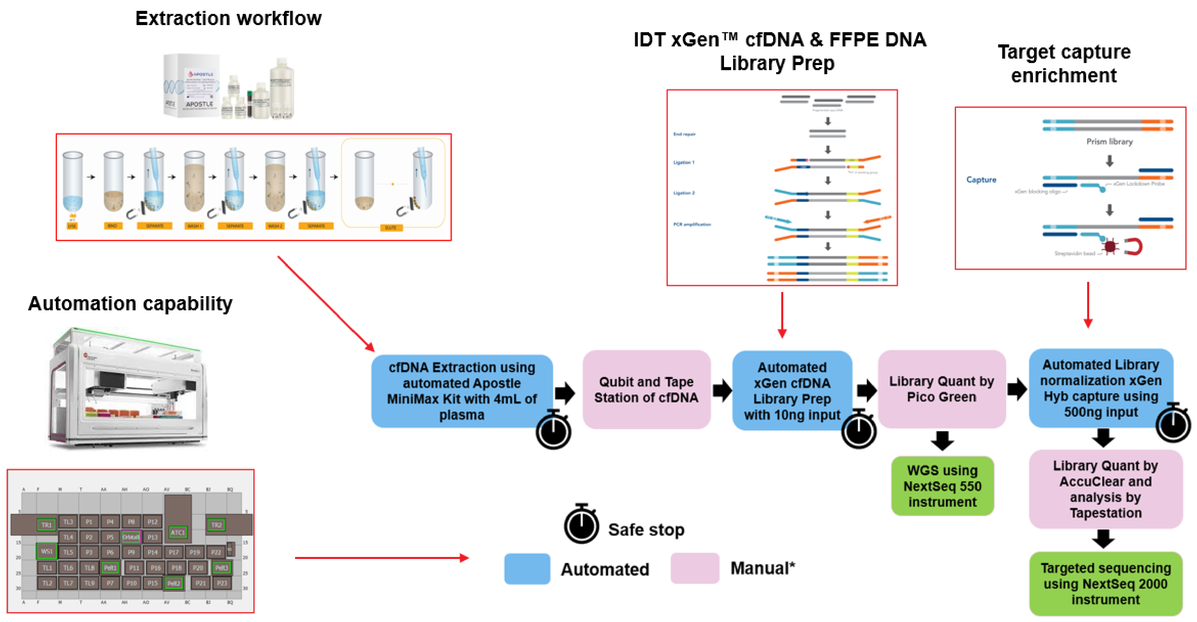

Figure 1. Complete NGS sample preparation workflow for cfDNA.

Automation in laboratories is advantageous to generate reproducible next generation sequencing (NGS) sample libraries. Different automation instruments and deck layouts can require a significant amount of time for optimizing before being able to begin generating NGS libraries. In addition to this optimization, these automation workflows need to work well and consistently for some research, e.g., cancer research utilizing low input and/or degraded samples such as cell-free DNA (cfDNA) from plasma or damaged DNA extracted from formalin-fixed paraffin-embedded (FFPE) biopsies. Together Integrated DNA Technologies (IDT) and Beckman Coulter Life Sciences provide a flexible walk-away NGS automation solution for these research samples. Flexibility includes small sample batch sizes in a closed and controlled instrument on the Biomek NGeniuS Next Generation Library Prep System or high-throughput processing on the Biomek i-Series i7 automated workstation. This also provides a solution for laboratories to minimize technician hands-on time, errors and rework while improving flexibility. IDT's xGen cfDNA & FFPE DNA Library Prep kit utilizes novel chemistry to maximize sample input conversion, suppress adapter-dimer formation, and facilitate consensus analysis. IDT's xGen Hybridization Capture products maintain high library diversity, obtain high on-target, and provide consistent and uniform sequencing coverage regardless of panel size.

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 (Biomek i7 n = 96; Biomek NGeniuS n = 16). 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 (Figure 5).

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. thermal cyclers)

Figure 3. Biomek NGeniuS Next Generation Library Prep System. Instrument can run 4 to 24 samples. The NGeniuS Portal can be used for batch setup and remote run monitoring using a customer PC enabled with Microsoft Edge or Google Chrome. Batch setup does not include method writing and requires no programming skills from the lab. After batch creation, a work aid is generated for reagent and deck setup.

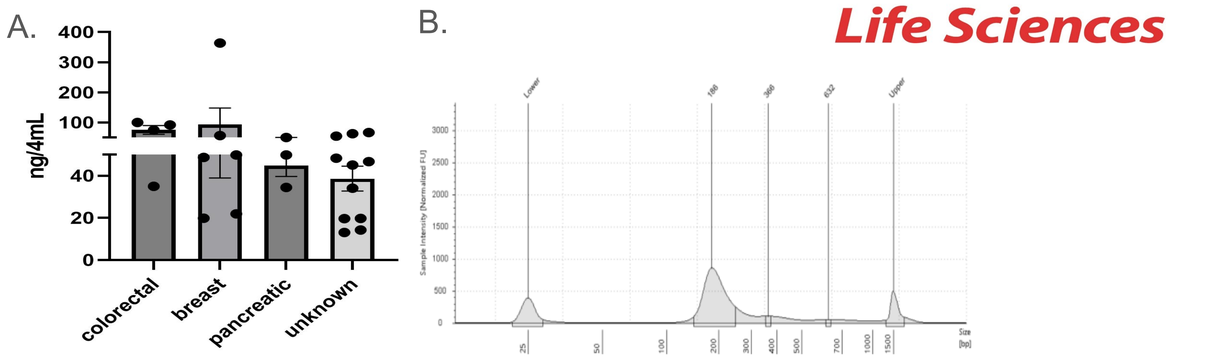

Figure 4. End-to-end workflow: Extraction, library preparation and hybridization capture on the Biomek i7 Hybrid Workstation. (A) Quantity of cfDNA after recovery using DNA extraction kits. Extraction of cfDNA from 4 mL of plasma samples using the Apostle MiniMax High Efficiency Isolation Kit. (B). cfDNA 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.

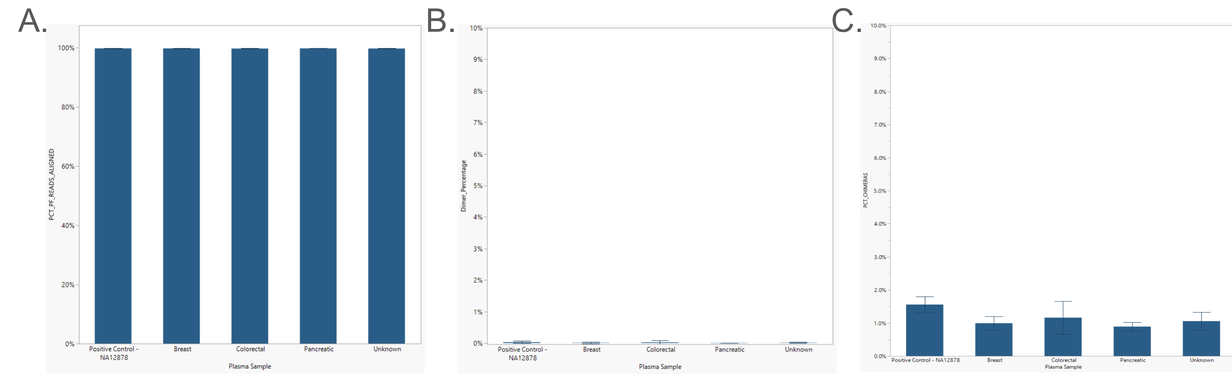

Figure 5. High-quality libraries generated from 10ng plasma samples. All plasma extraction types had 10ng input for library prep and subsampled to 3M reads (Biomek i7 n = 96; Biomek NGeniuS n = 16). Positive controls using Coriell gDNA NA 12878 was arrayed throughout the plate (n= 5). The xGen cfDNA & FFPE Library Prep kit demonstrated high mapping rates of ≥99.2% **(A)** almost zero dimer percentage at ≥0.2% **(B)** and low chimera molecules at ≥2% **(C)**. All metrics were calculated using the Broad Institute's Picard HsMetrics.

Hybrid Capture Results

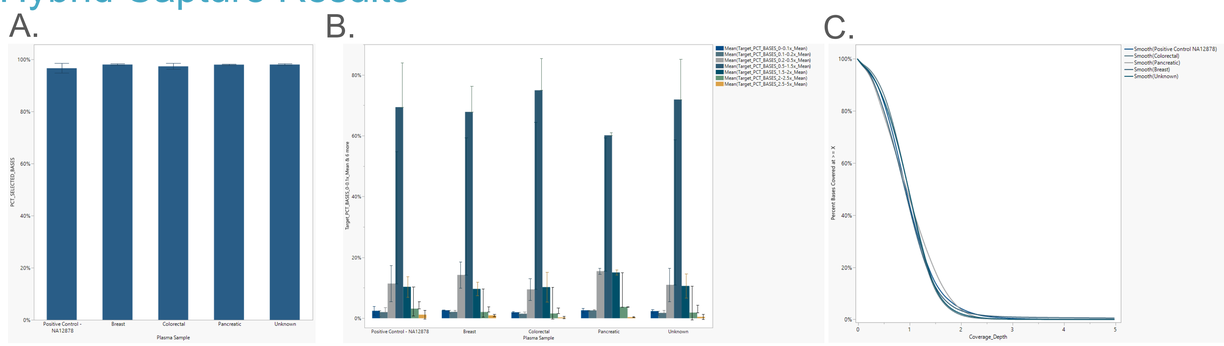

Figure 6. High On-Target percentage, uniformity across targets, and uniform coverage depth are important components for low frequency variant detection. (A) High on-target percentage (Picard HsMetrics) was obtained for all plasma extractions (n = 20) using a custom xGen Hyb Panel with a 2Mb design against 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.

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 in order 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 IDT's xGen cfDNA & FFPE Library Prep kit, xGen Hybridization Capture Core Reagents, and xGen Custom Hyb Panels on the Biomek platforms provide a reliable and consistent solution for analysis of precious low input and degraded samples.
- Apostle MiniMax™ High Efficiency cfDNA Isolation Kit generates high-quality cfDNA samples from breast, colorectal, plasma, and even unknown plasma collection samples.
- Libraries can be run on high-throughput automation platforms such as the Biomek i7 Workstation or in a closed and controlled instrument such as the NGeniuS Next Generation Library Prep System.

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