End-to-end workflow: Extraction, library preparation, and hybridization capture on the Biomek™ i7 Dual Hybrid Workstation

Introduction

In this app note we demonstrate an end-to-end automated workflow using the Apostle MiniMax™ kit to extract cfDNA from plasma and prepare libraries using IDT xGen™ cfDNA & FFPE DNA Library Prep followed by enrichment using IDT xGen Hybridization Capture on a Biomek i7 Dual Hybrid (DH) Automated Workstation. The Apostle extraction was performed in a pre-PCR space on a Biomek i7 DH Workstation as described previously [1]. Library preparation and enrichment were carried out on a Biomek i7 Workstation with a different deck layout which has been described previously [2,3].

The Apostle MiniMax High Efficiency cfDNA Isolation Kit (Beckman Coulter PN: C40603, C40604, C40605, C43459) isolates cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) from plasma collected from blood collection tubes containing EDTA and other blood collection tube types as well as from serum and urine. The kit produces high-quality extracted cfDNA that can be used in downstream genomic assays, such as PCR amplification and next generation sequencing. 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 xGen cfDNA & FFPE DNA Library Preparation Kit is designed specifically for generating libraries from 1–250 ng of degraded samples, such as cell-free DNA (cfDNA), or damaged DNA extracted from formalin-fixed paraffin-embedded (FFPE) samples. The method features a proprietary ligation strategy that maximizes conversion, suppresses adapter-dimer formation, and reduces chimera rates.

Hybridization capture is a target enrichment method that can provide disease specific information tuned to user needs. From small target spaces used to detect rare variants to exome-wide data, the IDT hybridization capture system can provide the flexibility needed by researchers. This workflow generates Illumina® platform-compatible libraries prepared from DNA or RNA using xGen Hyb Capture Panels. Users can achieve uniform coverage and robust capture performance across a broad range of xGen hybridization probe panels.

This workflow on the Biomek i7 platform provides:

- Reduced hands-on time
- Reduced potential for pipetting errors
- Quick install with ready-to-implement methods
- Knowledgeable support from IDT and Beckman Coulter Life Sciences

Spotlight

The Biomek i7 Hybrid (Multichannel 96, Span-8) Workstation System features deliver reliability and efficiency to increase user confidence and walk-away time as compared to manual library preparation.

- 1200 µL multichannel head with 1–1000 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip 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., Automated Thermal Cyclers [ATC])

Figure 1. Biomek i7 DH Workstation with optional enclosure on a Biomek Mobile Workstation. Deck layout in the lower image.

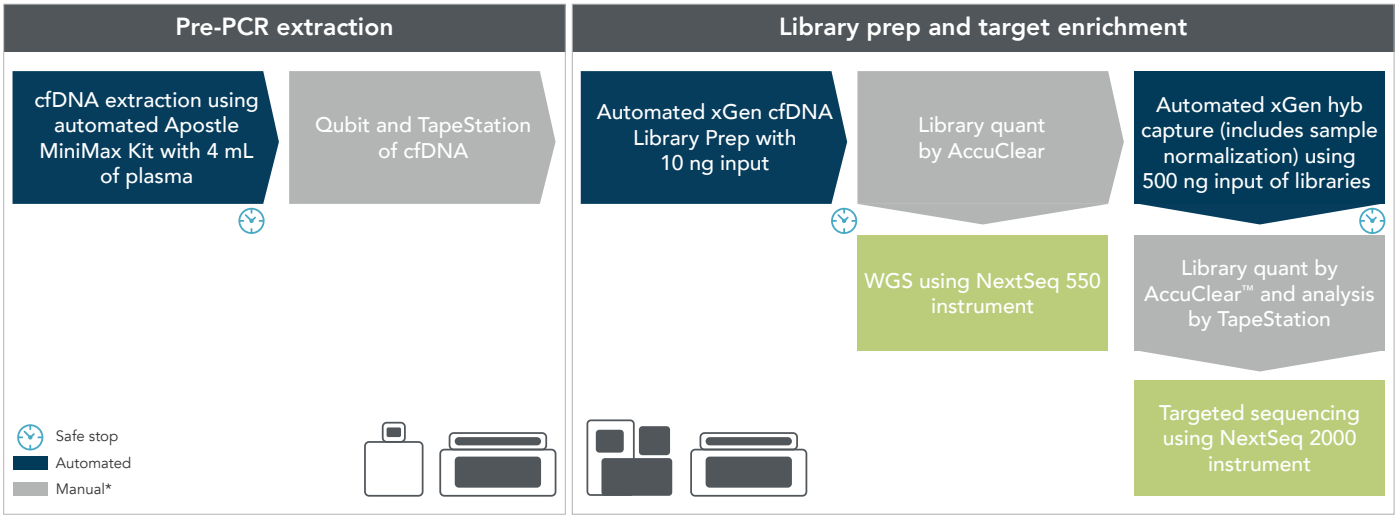

Figure 2. End-to-end automated workflow.

* Automated workflow for TapeStation™ setup and library quant by PicoGreen™ is available.

Automated method

Automation provides increased efficiency and reduction in human errors, with minimal hands-on time (Table 1).

Table 1. Estimated run times for automating the extraction, library preparation, and hybrid capture for 96 samples on the Biomek i7 DH Workstation.

Kit type	Apostle MiniMax High Efficiency cfDNA Isolation Kit	IDT xGen cfDNA & FFPE DNA Library Prep Kit	IDT xGen Hybridization Capture of DNA Libraries
Instrument setup time	30 min	20 min	30 min
Method run time	4 hr 50 min	4 hr 43 min	8 hr 13 min
Total time (with on-deck ATC)	5 hr 20 min	5 hr 3 min	8 hr 43 min

* Total timing estimates include thermocycling with on-deck ATC but do not include reagent thawing.

The automated methods can be run using Method Option Selector, which is an interactive user interface that supports modular design and logical start and stop points based on Beckman Coulter Life Sciences and IDT recommendations. Guided Labware Setup helps with ease of deck setup and reagent information, and DeckOptix Final Check software minimizes costly setup errors. The automated method provides flexibility to users in scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput.

The instruments have a static Peltier for chilling the reagents, and an Orbital Shaker, an incubator shaker as well as on-deck thermocycling capability.

The software provides several user-friendly features such as:

1. Biomek Method Launcher (BML)

   BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed.

2. Method Option Selector (MOS)

   MOS enables selection of plate processing and sample number options to maximize user method flexibility, adaptability, and the ease of method execution.

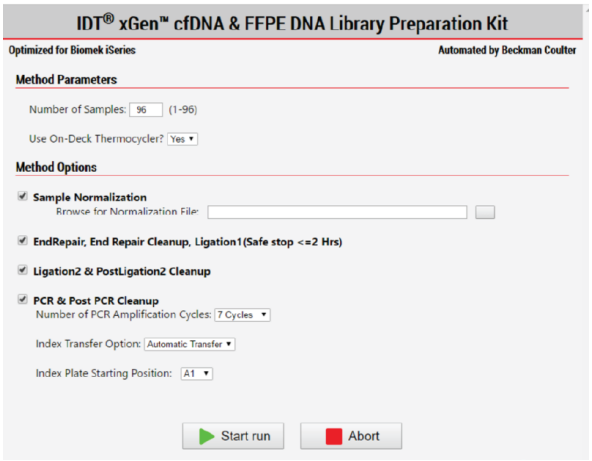

Figure 3. Example for IDT xGen cfDNA & FFPE DNA Library Prep MOS.

3. Guided Labware Setup (GLS)

   GLS is generated based on options selected in the MOS and provides the user specific graphical setup instructions with reagent volume calculation and step-by-step instructions to prepare reagents.

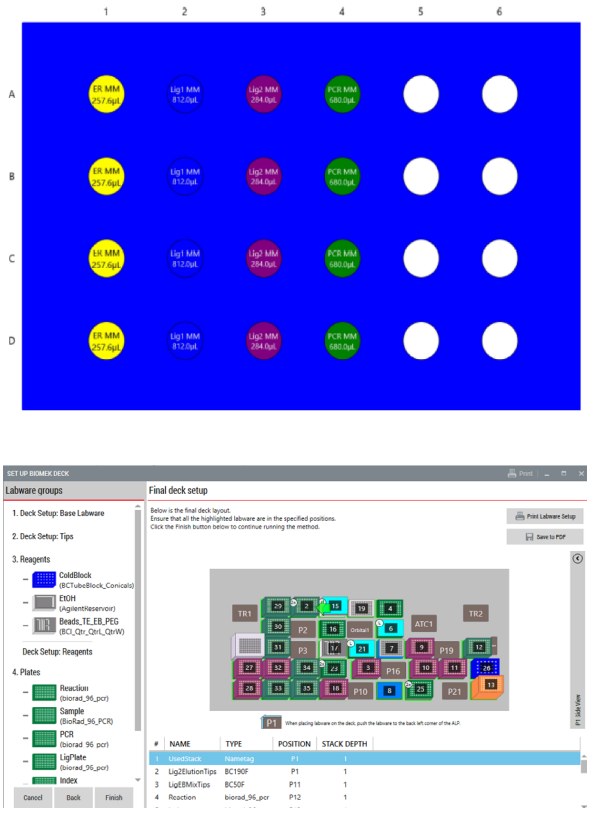

Figure 4. Guided Labware Setup provides recipe notes, indicates reagent volumes (upper image), and guides the user for correct deck setup (lower image).

4. DeckOptix Final Check (DFC)

   Prior to starting a run, DFC (included with BML) analyzes the deck to reduce setup errors and prevent a failed experiment because of missing or misplaced labware or using the wrong tip or plate type.

Experimental design

A 24-sample automated run for the Apostle MiniMax kit was carried out using cancer plasma samples. All samples used in this study were stored at –80°C for greater than six months. Before extraction, the plasma samples were thawed at room temperature. The plasma samples were centrifuged on a Beckman Coulter Life Sciences Avanti J-26 XPI centrifuge equipped with a JA-14.50 rotor set to 9500 RPM for 10 minutes. 3–4 mL plasma was used as starting material for the automated extraction. The quality of the extracted cfDNA was checked with Agilent TapeStation High Sensitivity D1000 tape (Figure 5). The extraction was carried out in a pre-PCR space on a Biomek i7 DH Workstation as described previously [1].

10 ng of cfDNA (1 sample was split into multiple wells depending on the yield) from the above prep was used to prepare libraries. Coriell NA12878 was sheared to 150 bp size and 10 ng input of the sheared gDNA was used as a positive control (PC, at least one in each quadrant) and a no template control (NTC) was used in random wells to cover each quadrant.

The quality of these libraries was checked with Agilent TapeStation High Sensitivity D1000 tape and samples were quantified using Quant-iT™ PicoGreen dsDNA Quantitation Kit. We did the quantification manually in this experimental setup; however, these have been automated previously and can be easily mapped to a Biomek i7 Workstation [4,5].

500 ng of the prepared 96 libraries were then individually enriched using the hybridization capture automated protocol. For NTCs, 3 µL of elute was used. The remaining libraries were pooled and sequenced on an Illumina NextSeq™ 550 instrument for whole genome sequencing (WGS) (Figure 6).

For hybrid capture, a 2 Mb xGen Custom Hyb Panel designed against mutated gene targets was used for target pull down. The xGen Human Identification (HuID) hybridization panel was also used as a spike in to enable identification of individual samples. Captured libraries were quantified using AccuClear and analyzed on TapeStation HS D1000 tape. 24 capture samples were selected for sequencing on a NextSeq 2000 instrument (Illumina) in order to achieve deeper coverage depth (positive control Coriell NA12878 $n = 4$, colorectal $n = 4$, breast $n = 3$, pancreatic $n = 7$, and unknown $n = 5$).

Results

Apostle MiniMax High Efficiency cfDNA Isolation Kit generated high-quality cfDNA from colorectal, breast, pancreatic, and unknown tissue samples. The combination of IDT's xGen cfDNA & FFPE DNA 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. Regardless of cfDNA type, results from an end-to-end workflow achieved high on-target percentage and uniform coverage against the Custom Pan-Cancer Hyb Panel design.

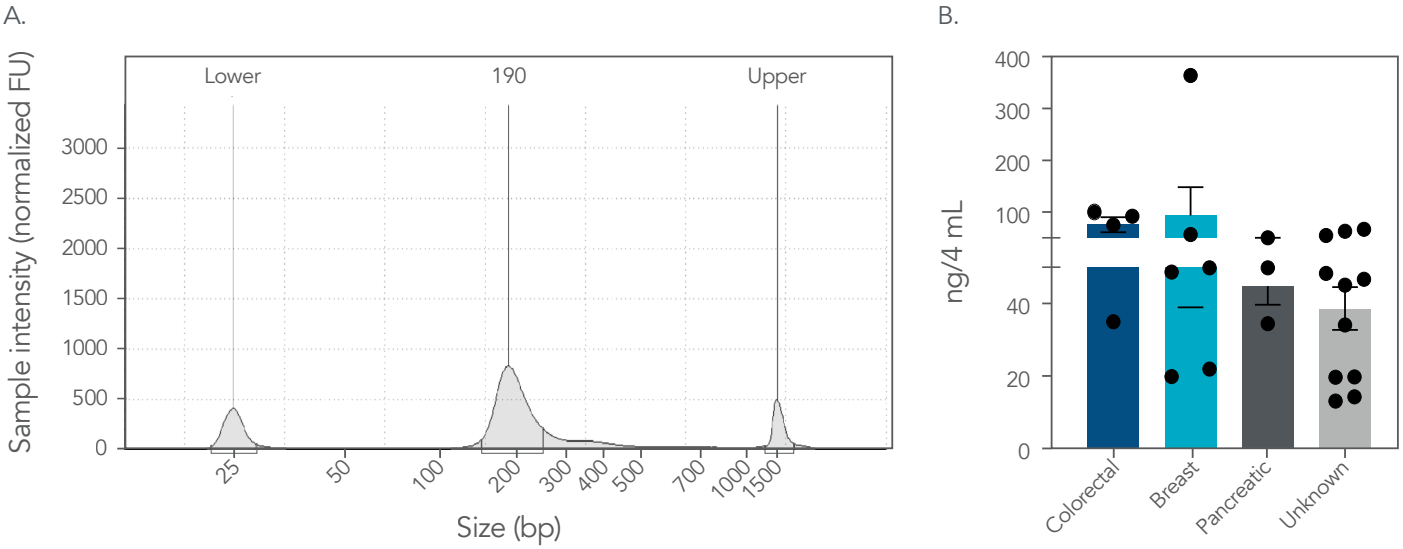

Figure 5. Quantification of cfDNA after recovery using Apostle cfDNA extraction kits. (**A**) Representative trace from cfDNA on TapeStation prepared using automated Apostle MiniMax method. (**B**) Total yields in ng/4mL plasma from different cancer types.Error bars reflect the standard deviation of replicates.

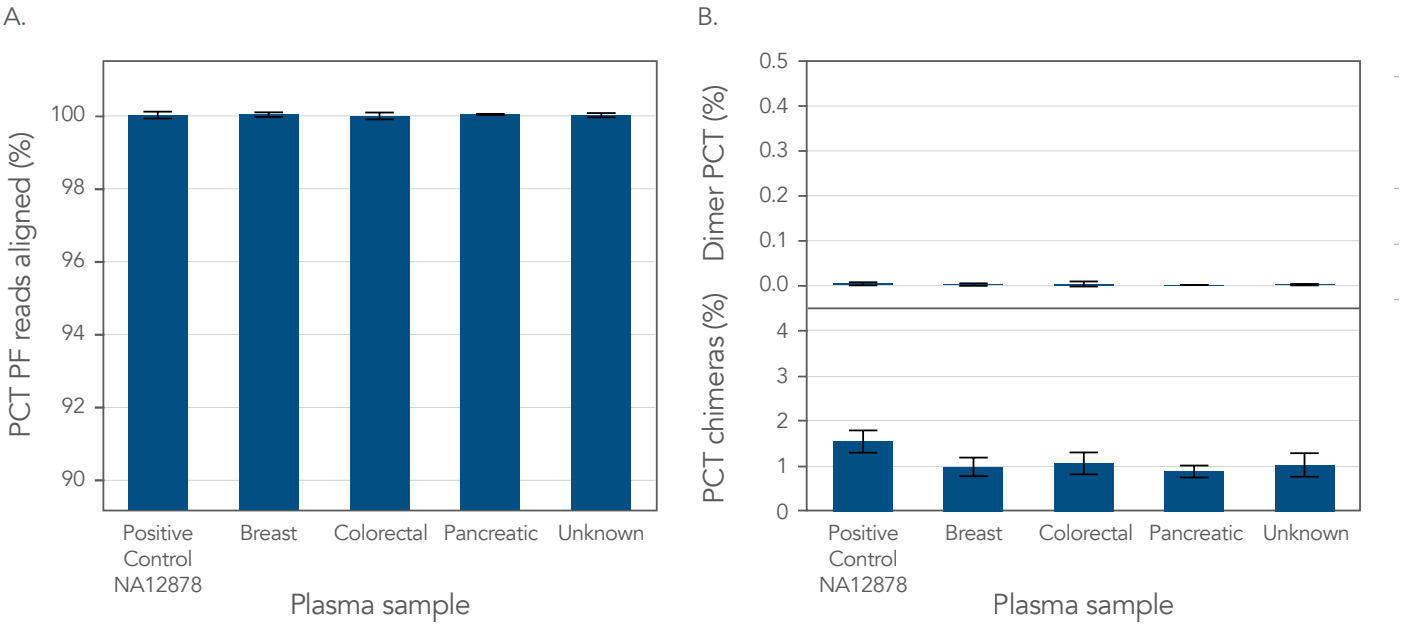

Figure 6. High-quality libraires generated from 10 ng plasma samples. All plasma extraction types had 10 ng input for library prep and subsampled to 3M reads (n = 96). Positive controls using Coriell gDNA NA12878 was arrayed throughout the plate (n = 5). The xGen cfDNA & FFPE DNA Library Prep Kit demonstrated high mapping rates of ≥99.2% (**A**) Almost zero dimer percentage at < 0.2% and low chimera molecules at < 2% (**B**) All metrics were calculated using the Broad Institute's Picard HsMetrics. Error bars reflect the standard deviation of replicates.

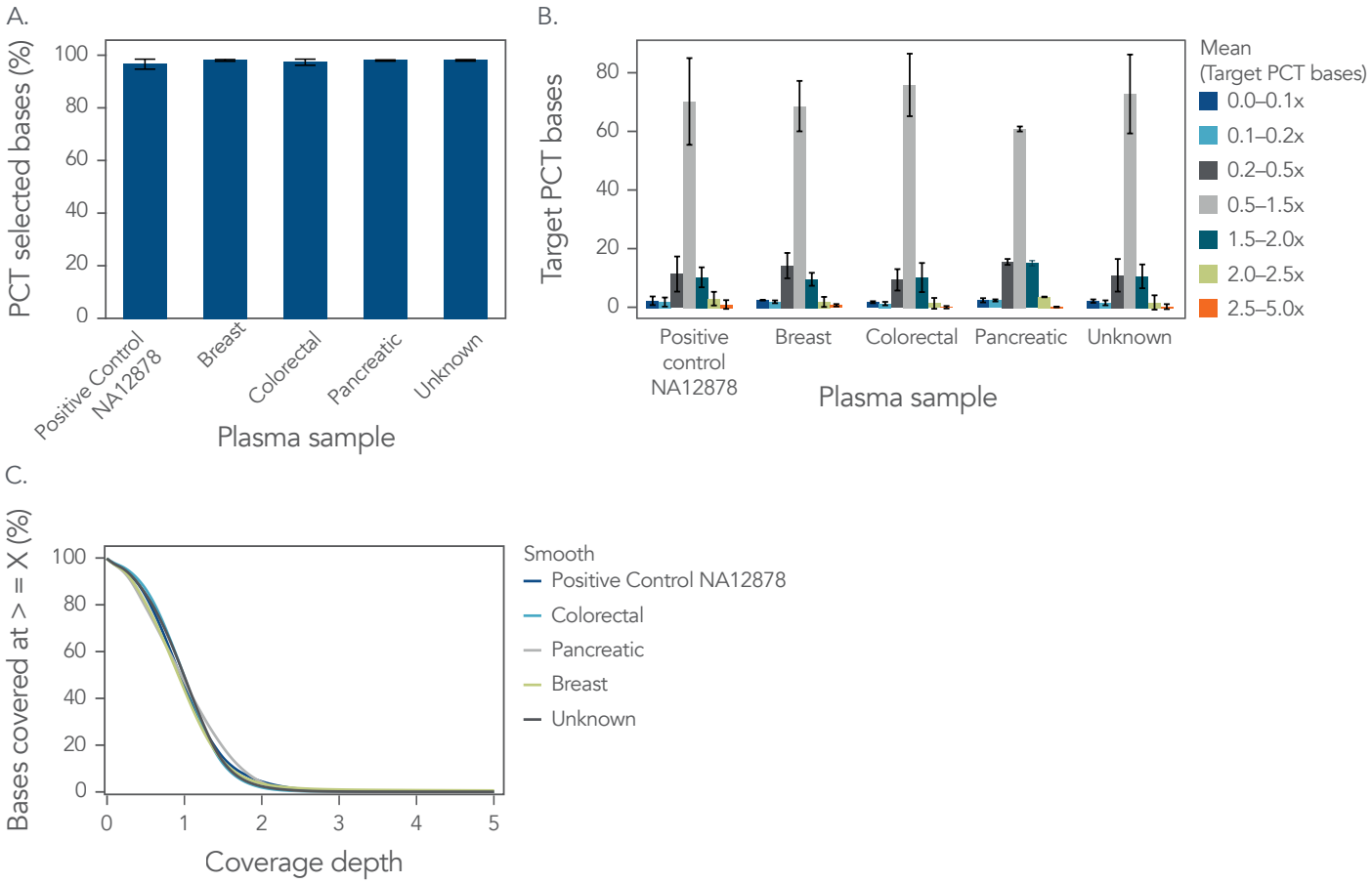

Figure 7. High on-target percentage, uniformity across targets, and uniform coverage depth are important components for low-frequency variant detection. (**A**) High on-target percentage (Picard HsMetrics) was obtained for all plasma extractions (n = 20) using a custom xGen Hyb Panel with a 2 Mb design 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. Error bars reflect the standard deviation of replicates.

Summary

We demonstrated the extraction of cfDNA and preparation of libraries with IDT's xGen cfDNA & FFPE DNA Library Prep Kit followed by hybridization capture on the Biomek i7 DH Workstation. This automated solution provides an efficient, flexible, and scalable solution for any size lab.

References

1. Biomek Automated Genomic Sample Prep Accelerates Research. Biomek i-Series Automation of the Apostle MiniMax™ High Efficiency cfDNA isolation kit. *Beckman Coulter Life Sciences (AAG-5653FLY07.19)*
2. Automated IDT xGen cfDNA & FFPE DNA Library Prep Kit on a Biomek i7 Hybrid Workstation. *IDT and Beckman Coulter Life Sciences (2023-GBL-EN-100829-v1)*
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