Automating cell-free DNA library preparation and hybridization capture for breast cancer panel

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Introduction

Automation is often counted on in laboratories to generate reproducible next generation sequencing (NGS) sample libraries. Automated workflows need to work well and consistently for cancer research utilizing low input cell-free DNA (cfDNA) from liquid extractions. The Biomek NGeniuS Next Generation Library Prep System provides a flexible sample batch size in a closed and controlled instrument while also allowing technicians to walk away, reduce hands on time, errors, and re-work. Integrated DNA Technologies' (IDT) xGen™ cfDNA & FFPE DNA Library Prep kit utilizes novel chemistry to maximize sample input conversion, suppress adapter-dimer formation, and facilitate consensus analysis, allowing researchers to identify and characterize variants occurring in malignant samples. 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.

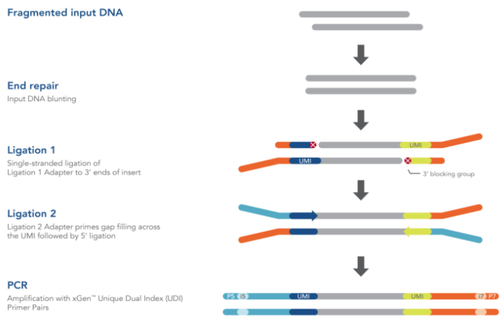

Figure 1. IDT xGen cfDNA & FFPE DNA Library Prep workflow. In an initial step, end repair enzymes convert cfDNA into bunt-ended DNA ready for ligation. A Ligation 1 Enzyme catalyzes the single-stranded addition of a Ligation 1 Adapter to the 3' end of the insert. This novel enzyme is unable to ligate inserts together which minimizes chimera formation. The 3' end of the Ligation 1 Adapter also contains a blocking group to prevent adapter-dimer formation. The Ligation 2 Adapter acts as a primer to gap fill bases complementary to the Ligation 1 Adapter, followed by ligation to the 5' end of the DNA insert which creates a double-stranded product. Finally, PCR with IDT 2X HiFi PCR MM incorporates sample index sequences for sequencing on Illumina platforms.

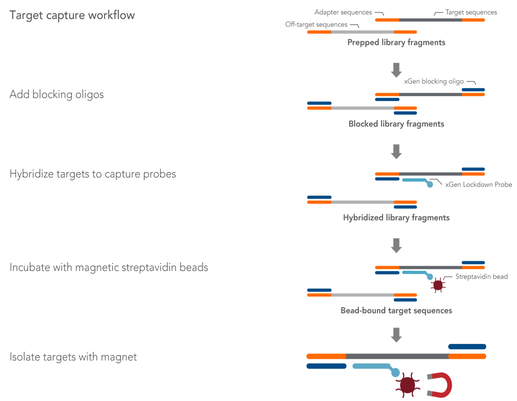

Figure 2. IDT xGen Hybrid Capture Core Reagent workflow. Desired prepared library fragments are separated from off-target fragments using hybridization capture. First, xGen Universal Blockers are mixed with prepared cfDNA library fragments to prevent adapter to adapter hybridization. Library fragments are then annealed to the 5' biotinylated oligonucleotides probes from an xGen Breast Cancer Panel. The probe and fragment duplexes are then separated from the unbound fragments by streptavidin-coated magnetic bead purification. The final cfDNA library sample contains just the DNA fragments of interest.

Materials and Methods

Apostle MiniMax™ High Efficiency cfDNA Isolation Kit was used to isolate cfDNA from plasma from unhealthy individuals who had varying cancer diagnoses. IDT's xGen™ cfDNA & FFPE DNA Library Prep kit was run on Beckman Coulter's Biomek NGeniuS Next Generation Library Prep System to prepare cfDNA libraries from 10ng of plasma extraction, then whole genome sequencing (WGS) was performed on an Illumina NextSeq 550 instrument (2x150 PE). Qubit fluorometric and Agilent 4200 TapeStation were used for quantification.

Figure 4. Reagent Management System. Instrument combines an innovative input carousel with temperature-controlled reagent storage zones minimizing unnecessary pipetting steps while limiting reagent exposure to the external environment. Reagent Identification System uses advanced optical character recognition technology for reagent confirmation and error detection before one drop of your valuable reagent is used.

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. After batch creation, a work aid is generated for reagent and deck setup.

Figure 5. NGeniuS Next Generation Library Prep System Run Setup for xGen cfDNA & FFPE Library Prep Kit. **(A)** Default applications settings for a batch run are selected. **(B)** Sections for IDT xGen cfDNA & FFPE DNA Application.

The App for the IDT xGen cfDNA & FFPE DNA Library Prep Kit App was selected to process samples. The setup is broken into 4 sections: Batch Info (name of batch and number of samples to be run), App Settings, Sections and Sample Data. The App Settings screen contains variables specific to the library kit that may be changed between runs. The Batch name is a unique run name for the samples being processed. Number of samples is any number between 4 and 24 for this application, as indicated by the input box.

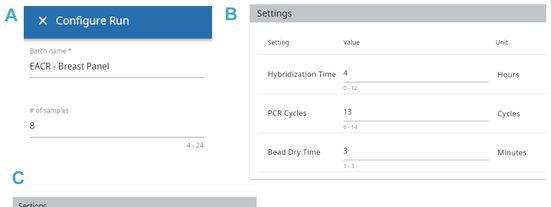

Figure 6. NGeniuS Next Generation Library Prep System Run Setup for xGen Hybridization and Wash Kit. (A & B) Batch information and default applications settings for batch run. **(C)** Sections for IDT xGen Hybridization and Wash Application.

The App for the IDT xGen Hybridization and Wash Kit App was selected to process samples. The setup is broken into 5 sections: Batch Info (name of batch and number of samples to be run), App Settings, Sections and Sample Data. The App Settings screen contains variables specific to the hybrid capture kit that may be changed between runs. Number of samples is any number between 4 and 24 for this application, as indicated by the input box. PCR cycle number is tunable based on panel size and multi-plexing. User mixes blockers and library off-system before sample dry-down section (also off-system).

Results and Discussion

Figure 7. Apostle MiniMax™ High Efficiency cfDNA Isolation Kit workflow. The Apostle Minimax isolation kit was used to isolate cfDNA from plasma collected from blood collection tubes. Blood samples were drawn from donors who had breast cancer, colorectal, or an unknown diagnosis.

Extraction yields dinucleosome and trinucleosome peaks

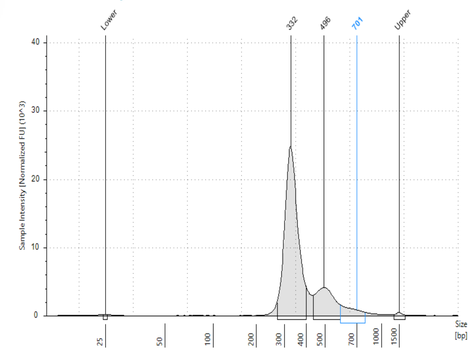

Figure 8. Library size distribution. The cfDNA library size distribution was analyzed by measuring fluorescence units (FU) on Agilent TapeStation High Sensitivity D1000 ScreenTape. Dinucleosome peak at 496bp, and trinucleosome peak at 701bp.

High quality libraries evaluated by WGS

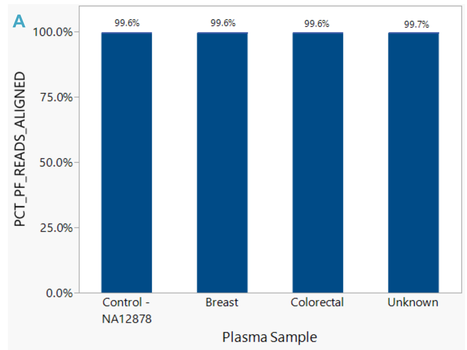

Figure 9. High-quality libraries generated from cfDNA. Libraries were prepared using 10ng input from plasma extractions. Libraries were sequenced (Illumina NextSeq 550, 2X150 PE) and subsampled to 10M reads (n = 23). A positive control using Coriell gDNA NA12878 was also prepared (n= 1). The libraries prepared using the xGen cfDNA & FFPE Library Prep kit **(A)** demonstrated high mapping rates of ≥99.6% **(B)** almost zero dimer percentage at ≤0.11%. All metrics were calculated using the Broad Institute's Picard HsMetrics

xGen™ cfDNA & FFPE DNA Library Prep kit was used to prepare cfDNA libraries from 10ng input of plasma extracted material on the Biomek NGeniuS Next Generation Library Prep system (n=16). Hybridization capture was then run with an xGen™ breast cancer hyb panel and xGen™ Hybridization Capture Core Reagents (n=7). xGen™ breast cancer hyb panel targets approximately 80 mutated genes for target pull down. A positive control using Coriell gDNA NA12878 was also prepared (n=1). The combination of this workflow within a closed automation instrument, provides a reliable and consistent solution for analysis of low input cancer research samples

Hybrid capture target coverage of AKT1, CCND1, and FOXA1

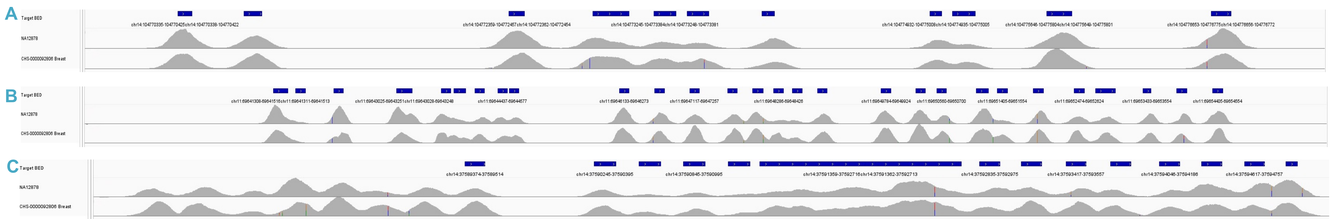

Figure 10. Hybrid capture target coverage from cfDNA mutated breast samples. Captures were prepared using 500ng input from cfDNA libraries. Libraries were sequenced on an Illumina NextSeq 2000, 2X150 PE, P3 flowcell and subsampled to at least 50M reads (n = 6). A Positive control using Coriell gDNA NA12878 was also prepared (n= 1). IGV Target coverage is shown for **(A)** AKT1 target region **(B)** CCND1 target region and **(C)** FOXA1 target region.

Conclusions

The combination of IDT's xGen library preparation and hybridization capture on the Biomek NGeniuS Next Generation Library Prep System has enabled variant identification across a range of variant allele frequencies from plasma extracted cfDNA, providing a walk away solution for researchers.

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