Automated IDT xGen™ cfDNA & FFPE DNA Library Prep Kit and xGen NGS Hybridization Capture on Biomek™ i7 Hybrid Automation Workstation

INTRODUCTION

Next generation sequencing (NGS) has enabled collection of exome-wide information faster than any previous technology. Hybridization capture is an enrichment method that can provide disease-specific information tuned to user needs. In this flyer, we describe a workflow using the IDT xGen cfDNA & FFPE DNA Library Prep Kit followed by xGen NGS Hybridization Capture automated for a throughput of 96 samples on a Biomek i7 Hybrid Automation Workstation (Figure 1, 2).

The xGen cfDNA & FFPE DNA Library Prep 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 method of targeted next generation sequencing. IDT xGen NGS hybridization capture products include xGen NGS Hybridization Capture Core Reagents and a variety of predesigned and custom panels, available in a range of panel sizes.

The xGen cfDNA & FFPE DNA Library Prep Kit followed by xGen NGS Hybridization Capture automated on the Biomek i7 Automation Workstation provides:

- An end-to-end solution for NGS library preparation followed by enrichment on the same platform
- Improved efficiency and speed by minimizing hands-on time and increased throughput as compared to manual preparation
- Standardized workflow to help improve consistency by reducing system setup and potential pipetting errors
- Quick install with ready-to-implement methods
- Knowledgeable support from IDT and Beckman Coulter Life Sciences

Figure 1. Biomek i7 Hybrid Automation Workstation.

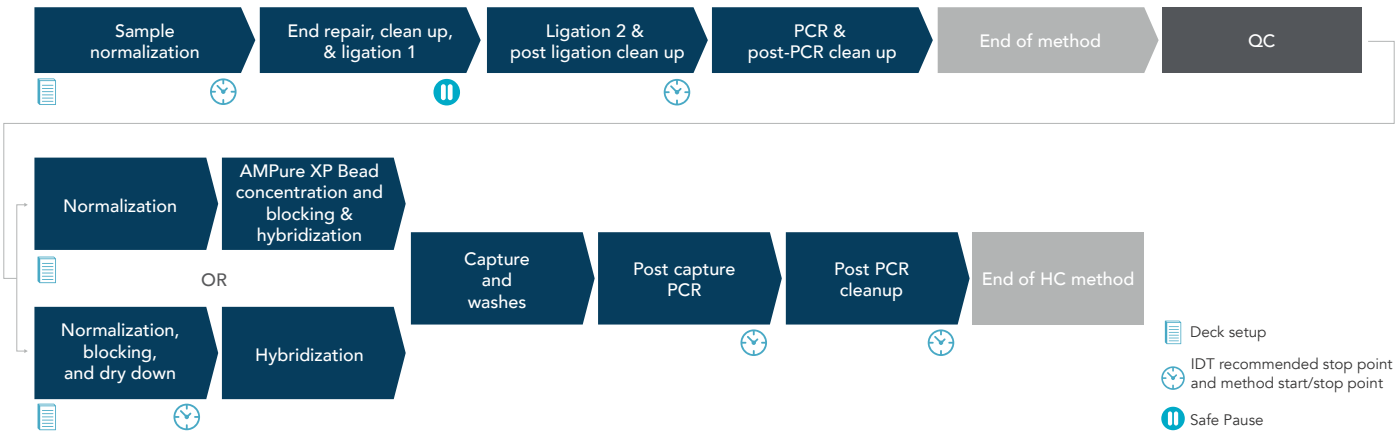

Figure 2. IDT xGen cfDNA & FFPE Library Prep followed by IDT xGen NGS Hybridization Capture automated workflow.

TIMING

The estimated time for processing 96 samples for library prep is 5 hours, and for the hybridization and capture is 9 hours. This estimate includes time for on deck automated thermocycler (ATC) incubations but does not include time for reagent thawing and QC.

DATA

All samples were prepared with 100 ng of Coriell NA24385 DNA and sheared to 150 bp (n = 96). Libraries were pooled and sequenced on an Illumina NextSeq™ 550 instrument for whole genome sequencing (WGS). Quality metrics can be found in Figure 3A, 3B. All samples, with one exclusion, met the 500 ng library yield input recommendation to proceed into hybridization capture (Figure 3C). Manual and automated hybridization capture workflows resulted in sequencing data of similar quality (Figure 4).

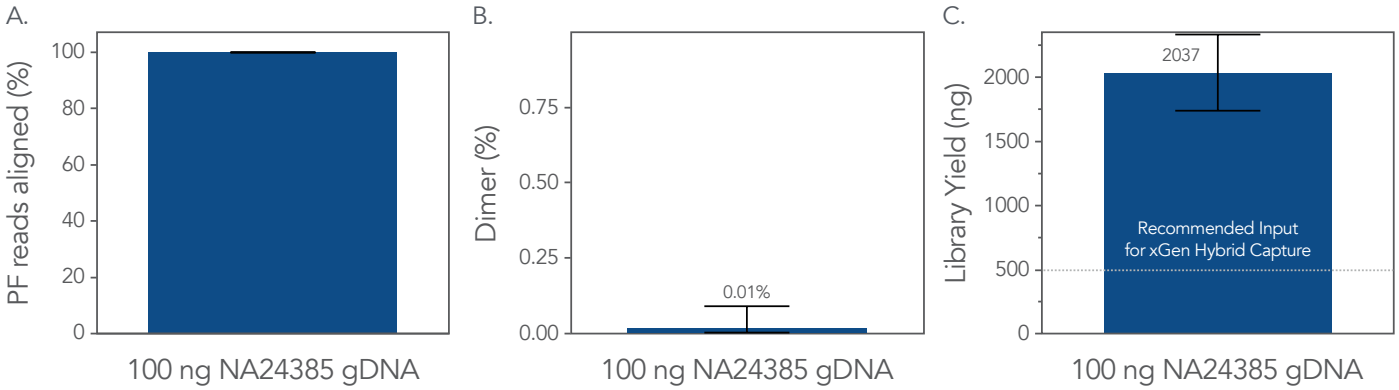

Figure 3. Average library quality metrics and yield resulting from samples prepped on the Biomek i7 Hybrid Automation Workstation using the IDT xGen cfDNA & FFPE DNA Library Prep Kit. (**A-B**) Percent passing filter (PF) reads aligned and dimer percentage were checked to assess library quality. (**C**) Mean library yield was 2037 ng, n = 96.

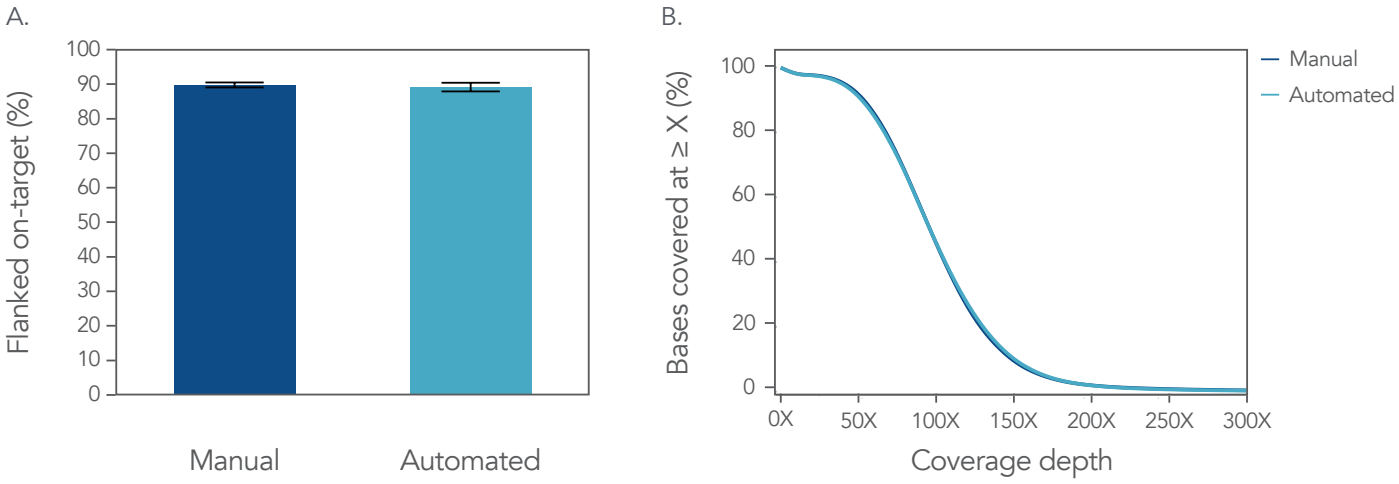

Figure 4. Sequencing quality metrics after manual and automated hybridization capture workflows. All sequencing libraries with an average insert size of ~150 bp were prepared on the Biomek i7 Hybrid Automation Workstation using the IDT xGen cfDNA & FFPE DNA Library Prep Kit and amplified with xGen 2X HiFi PCR Master Mix. DNA libraries were enriched using the xGen Exome Hyb Panel v2 and the indicated combinations of methods [manual or automated (i7)]. In single-plex captures, n = 16 for manual plate, and n = 44 for automation. The xGen Hybridization and Wash Kit was used according to the IDT protocol, xGen hybridization capture of DNA libraries. Sequencing was performed on a NextSeq 2000 instrument to generate 2 x 150 bp, paired-end reads. (**A**) Flanked on-target rates were calculated using Picard HS Metrics (Broad Institute). (**B**) Coverage depth and bases covered at ≥ X (%) were similar between the manual and automation preparations.

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/NGS**