



Automating IDT xGen™ Hybridization Capture of DNA libraries on Biomek i7 Dual Hybrid 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 the user needs. From small target spaces used to detect rare variants to exome-wide data, the IDT hybridization system can confidently provide the flexibility needed by researchers. This workflow is compatible with NGS libraries prepared using ligation-based techniques, such as TruSeq® library kits, KAPA library kits and tagmentation-based library kits such as Nextera® XT DNA Library Preparation Kit. Users can achieve uniform coverage and robust capture performance across a broad range of xGen hybridization probe panels. The automated method has a throughput of 96 samples and supports concentration of DNA from Dry Down or AMPure XP Bead for Hybridization. It can generate highly reproducible targeted NGS libraries using a 1200 µL MC head and a deck layout that supports maximum efficiency and minimum hands-on time.

In this flyer, we demonstrate performance of the IDT xGen Hyb Capture of DNA libraries automated on the Biomek i7 Hybrid Genomics Workstation.

In comparison to the use of manual pipetting, the IDT xGen Hyb Capture of DNA libraries automated on Biomek platform provides:

- Reduced hands-on time and increased throughput
- Reduction in system setup and potential pipetting errors
- Standardized workflow for improved results
- Quick install with ready-to-implement methods
- Knowledgeable support from IDT and Beckman Coulter Life Sciences

Spotlight

Biomek i7 (Multichannel 96, Span-8) Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time

- 300 µL or 1200 µL Multichannel head with 1-300 µL and 1-1200 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with 45 positions
- Orbital Shakers, Peltiers, Span-8 and 96 channel Tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)



Figure 1. Biomek i7 Hybrid Genomics Workstation with optional Enclosure on a Biomek Mobile Workstation. Deck layout in the lower image.

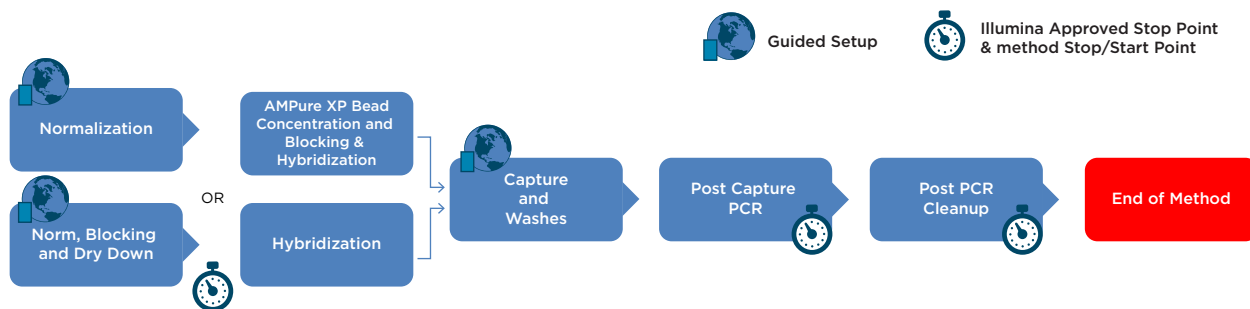


Figure 2. Automated workflow of IDT xGen Hybridization Capture of DNA libraries.

Automated Method

Automation provides increased efficiency, with minimal hands-on time (**Table 1**). The method can be run with user interactive Method Options Selector with modular design and supports logical start and stop points based on IDT’s recommendations. Guided Labware Setup helps with ease of deck setup and DeckOptix Final Check software minimizes costly setup errors. Automated method provides flexibility to the users in scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput. The instrument has 2 Static Peltiers and 1 Shaking Peltier for chilling the reagents and uniformly heating buffers and an Orbital Shaker as well as on-deck thermocycling capability.

Major Process Description

	24 Samples	48 Samples	96 Samples
Instrument Setup Time	10 min	15 min	30 min
Norm, and Blocking and Hyb Setup			
Method Run Time (Dry Down/Bead Concentration)	9 min/48 min	18 min/56 min	32 min/ 1 hr 3 min
Capture, Washes and PCR Setup			
Method Run Time	2 hr 16 min	2 hr 23 min	2 hr 36 min
Post PCR Cleanup			
Method Run Time	35 min	38 min	45 min
Total (4hr Hyb and 20 min PCR)	7 hrs 30 min/ 8 hrs 9 min	7 hrs 54 min/ 8 hrs 32 min	8 hrs 43 min/ 9 hrs 14 min

* Timing estimates include Thermocycling, 4 hr Hyb

*Timing does not include reagent thawing or dry down in speedvac

Table 1. Estimated run times for IDT xGen Hyb Capture of DNA libraries on the Biomek i7 Hybrid Genomics Workstation.

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. It also features the new DeckOptix™ Final Check Software to reduce deck setup errors and prevent a failed experiment because of missing or misplaced labware, wrong tip or plate type. The manual control options provide the opportunity to interact with the instrument, if needed.



Figure 3. Biomek Method Launcher provides a straight-forward interface to launch the method.

2. Method Options Selector (MOS)

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

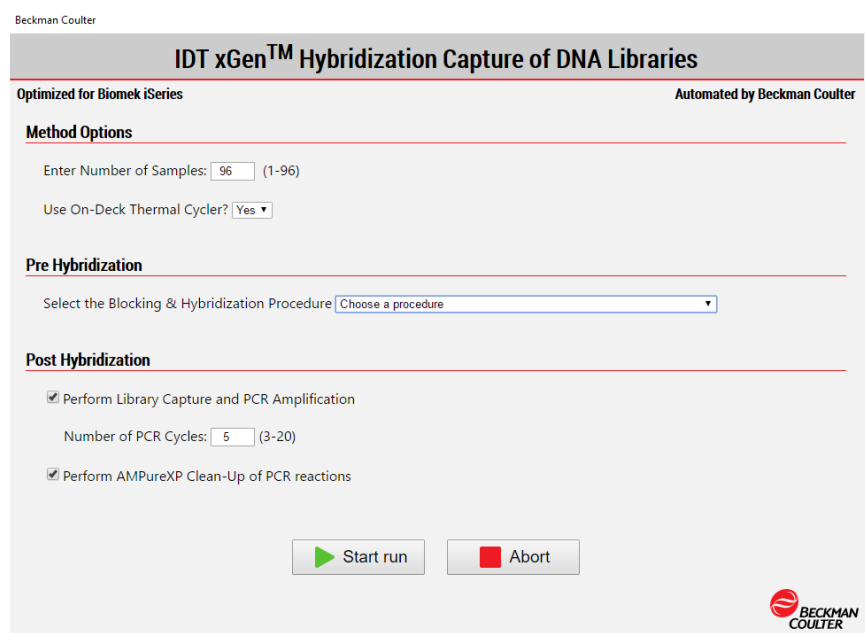


Figure 4. IDT xGen Hyb Capture of DNA libraries Method Options Selector.

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.

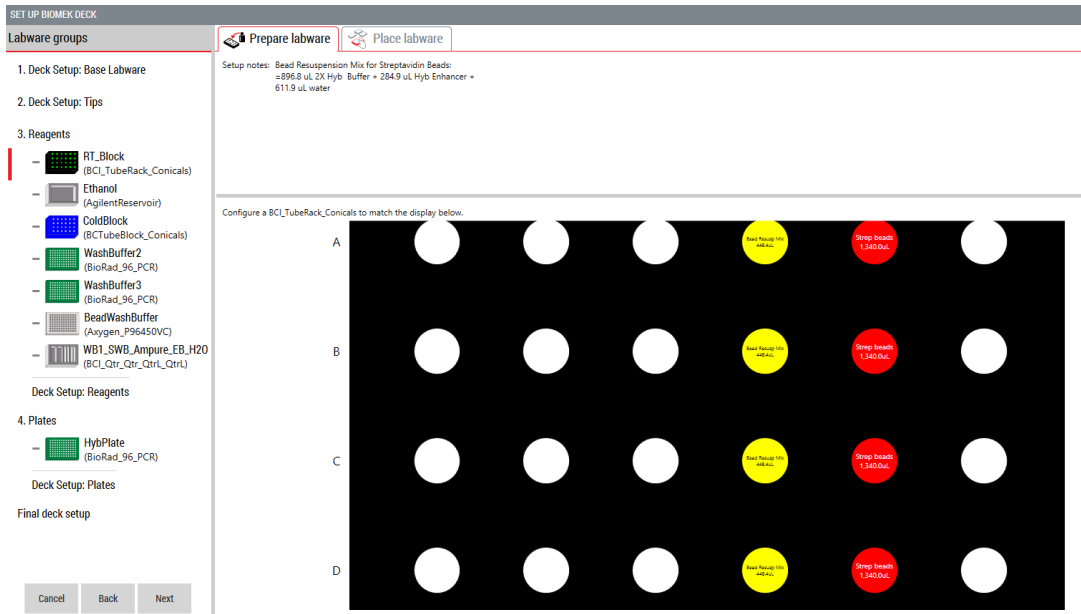


Figure 5. Guided Labware Setup indicates reagent volumes, recipe notes and guides the user for correct deck setup.

Experimental Design

Libraries were prepared with 1µg Coriell NA12878 input into KAPA Hyperprep. Eight libraries were captured using IDT's xGen Hybridization and Wash Kit and xGen Exome Research Panel. 500ng of each library was dried down per xGen Hybridization Capture of DNA Libraries for NGS Target Enrichment with an overnight hybridization. Captured libraries were quantified with Qubit™ ds HS DNA Kit, analyzed on TapeStation HS D1000 tape, and sequenced by IDT on a NextSeq 500 (Illumina). 2x150bp, paired end reads were generated.

Results

The flanked-on target percentage was 94 % with a mean insert size of 295 bp. The duplicate percentage was <3% and GC skew 60/40 was 1.2.

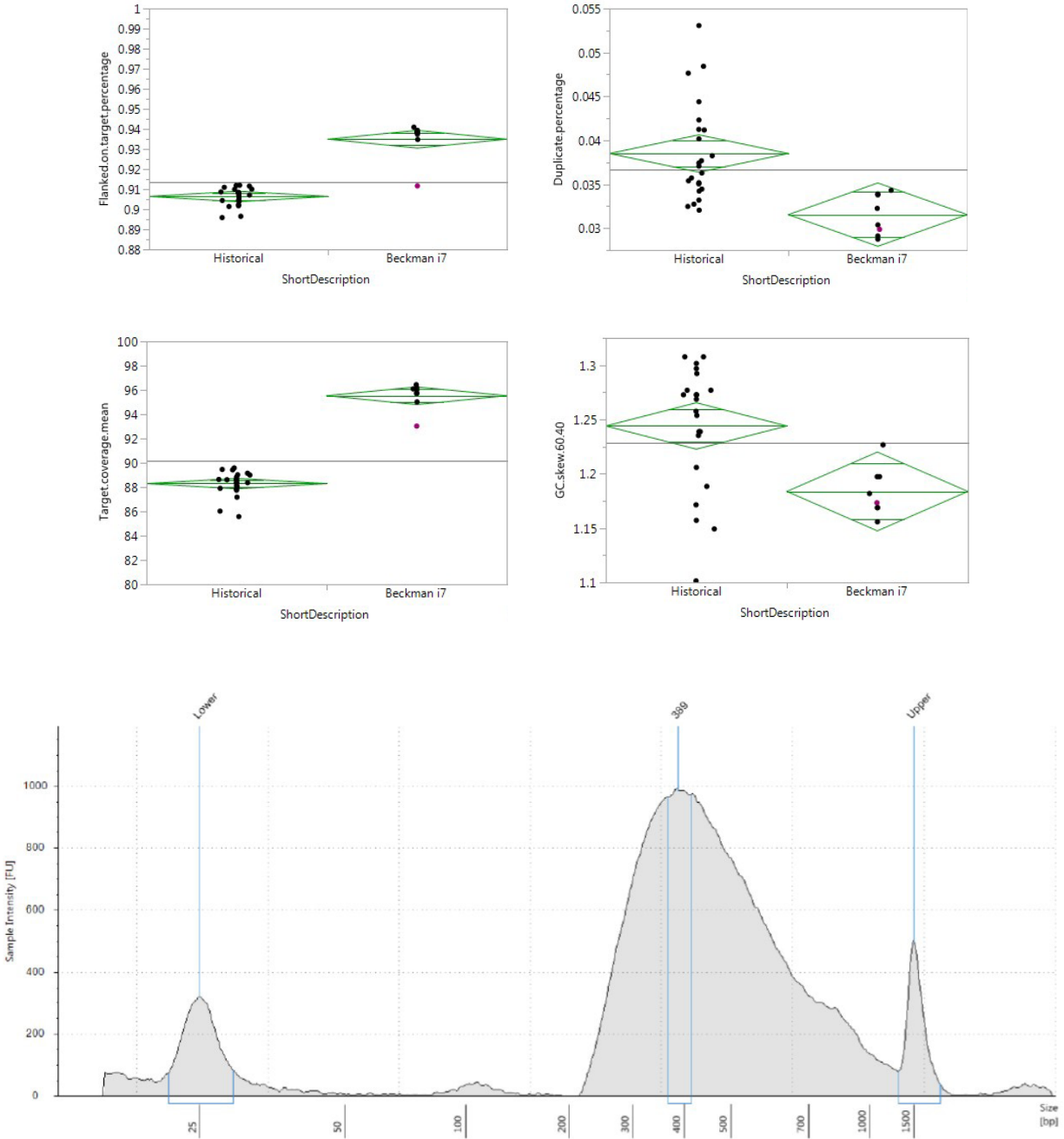


Figure 6. KAPA library target capture distribution on HS D1000 tape run on Agilent TapeStation 2100. The yield was 94 ng.

Summary

A robust and reliable automated solution with accurate heating and cooling solutions for target capture is essential to take full advantage of IDT's protocol. The automated method on the Biomek i7 hybrid workstation saves valuable time and money and helps researchers obtain an efficient process with more walk-away time.

We've demonstrated that automation of IDT xGen Hybridization Capture of DNA libraries for NGS Target Enrichment on the Biomek i7 Hybrid Genomics Workstation is a fast and efficient process that delivers quality results and provides a flexible and scalable solution for any size lab.

Biomek Method Launcher software package has to be purchased separately.

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