

# Automated IDT xGen™ cfDNA & FFPE DNA Library Prep Kit on a Biomek i7 Hybrid Workstation

## Introduction

The IDT **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.

Since adapter-dimer formation is negligible, a fixed concentration of adapter can be used, and aggressive size-selection is no longer required post-ligation. Altogether, this strategy delivers higher library complexity than conventional TA ligation-based methods, enabling precise low-frequency variant identification.

The automated method has a throughput of 96 samples on a Biomek i7 Dual Hybrid workstation.

Here, we show a workflow of the xGen cfDNA & FFPE DNA Library Prep Kit automated on a Biomek i7 Hybrid Genomics Workstation.

The xGen cfDNA & FFPE DNA Library Prep Kit automated on the Biomek i7 platform provides:

- Reduced hands-on time
- 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

## Spotlight

The Biomek i7 Hybrid (Multichannel 96, Span-8) Genomics Workstation System features deliver reliability and efficiency to increase user confidence and walk-away time as compared to manual library preparation (**Figure 1, 2**).

- 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 Thermo Cyclers (ATC)]



**Figure 1. Biomek i7 Hybrid Genomics Workstation with optional enclosure on a Biomek Mobile Workstation.** Deck layout shown in right image.



**Figure 2. IDT xGen cfDNA & FFPE Library Prep Kit automated workflow.**

## 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 IDT xGen cfDNA & FFPE DNA Library Prep Kit to make libraries with 10 ng of gDNA on the Biomek i7 Hybrid Genomics Workstation.**

	IDT xGen cfDNA & FFPE DNA Library Prep Kit		
	Sample number 24	Sample number 48	Sample number 96
Instrument setup time	10 min	15 min	20 min
Sample normalization	3 min	5 min	9 min
End Repair, End Repair Cleanup, Ligation 1	45 min	48 min	55 min
Ligation 2 & Post Ligation 2 Cleanup	50 min	54 min	1 hr
PCR (10 cycles) & Post PCR Cleanup	46 min	51 min	52 min
Method run time	2 hr 34 min	2 hr 53 min	3 hr 15 min
<b>Total Time (with on-deck ATC)*</b>	<b>4 hr 21 min</b>	<b>4 hr 40 min</b>	<b>5 hr 3 min</b>

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

The method can be run using Method Options Selector, which is an interactive user interface that supports modular design and logical start and stop points based on IDT's 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 gives each laboratory the opportunity to address their individual requirements for sample processing and throughput. The instrument has a static Peltier for chilling the reagents, an orbital 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 provides the users with the opportunity to remotely track the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed (**Figure 3**).



**Figure 3. Biomek Method Launcher provides a straightforward interface to launch the method.**

## 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 4).

Figure 4. IDT xGen cfDNA & FFPE DNA Library Prep Method Option Selector.

## 3. Guided Labware Setup (GLS)

GLS is generated based on the options selected in the MOS and provides the user with specific graphical setup instructions with reagent volume calculations and step-by-step instructions to prepare reagents (Figure 5).

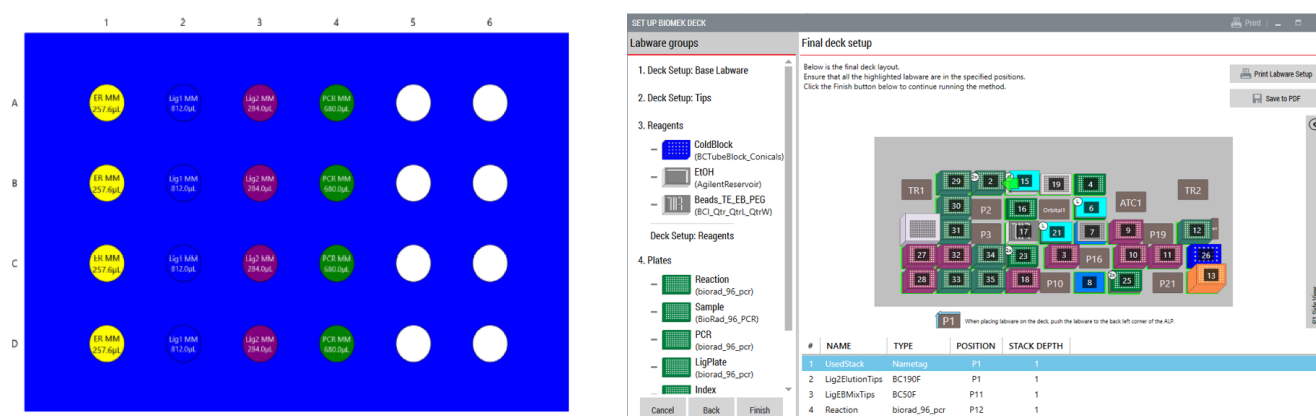


Figure 5. Guided Labware Setup provides recipe notes, indicates reagent volumes (left), and guides the user through correct deck setup (right).

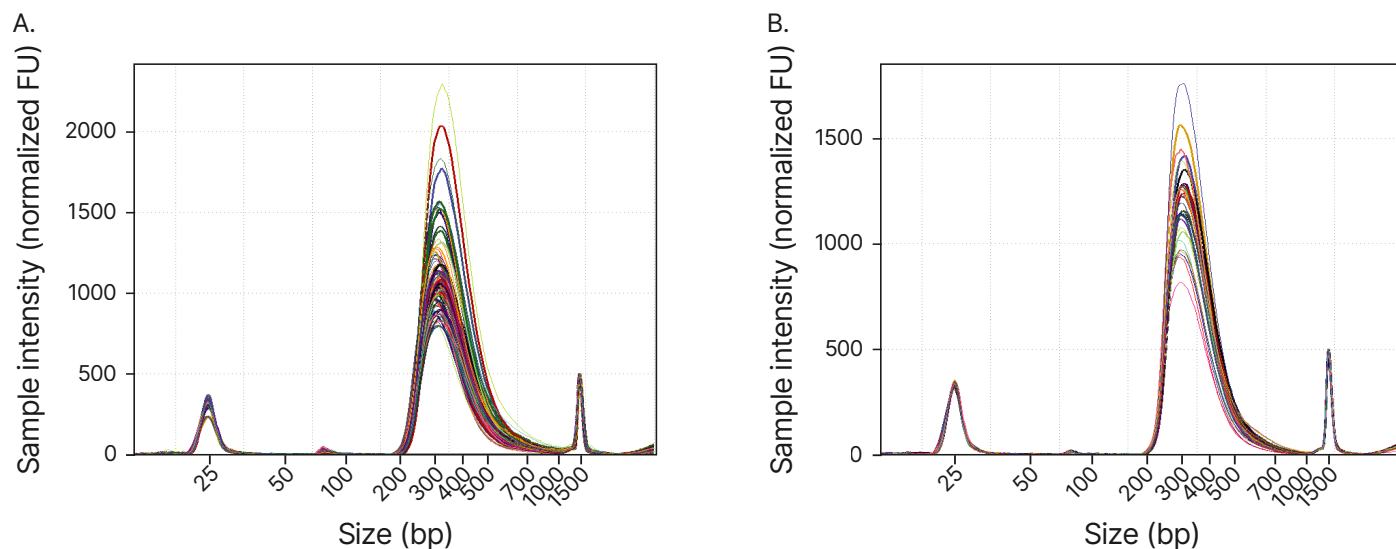
## 4. DeckOptix Final Check (DFC)

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.

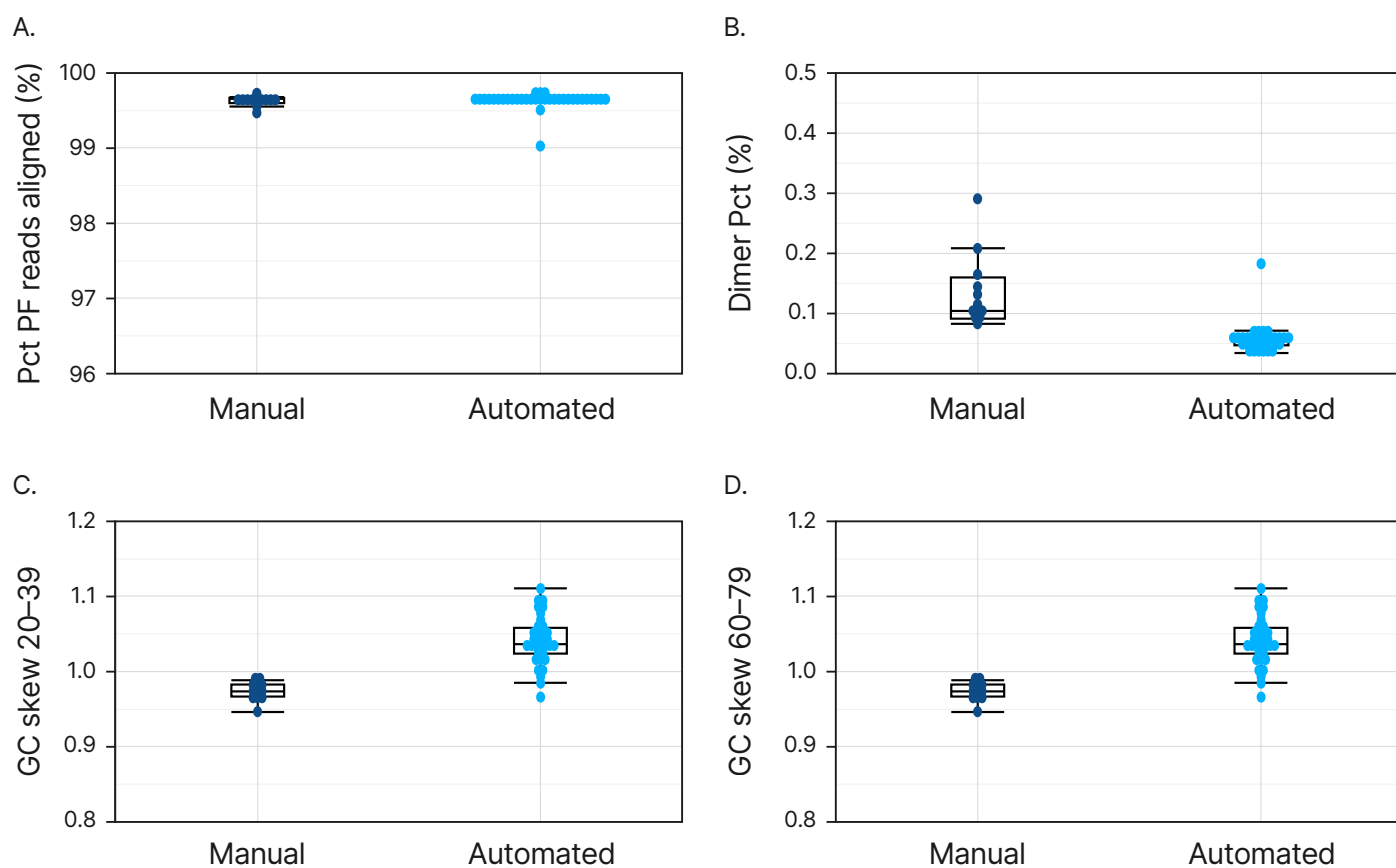
# Experimental design:

Coriell NA12878 genomic DNA (gDNA) was sheared to 150 bp size and 10 ng of the sheared gDNA was used for xGen cfDNA & FFPE DNA Library Prep across 96 technical replicates. Simultaneously, 20 libraries were prepared manually. The quality of these libraries was checked with Agilent TapeStation High Sensitivity D1000 reagents (Figure 6), and samples were quantified using AccuClear® dsDNA Quantitation Kit. The prepared libraries were pooled and sequenced on an Illumina® NextSeq 550 instrument for whole genome sequencing (WGS) (Figure 7). Next, we performed manual and automated library preps in batch sizes of 20 samples to test lower DNA inputs (2, 3, and 4 ng). These libraries were pooled, sequenced, and analyzed the same way as in the 10 ng input experiment (Figure 8).

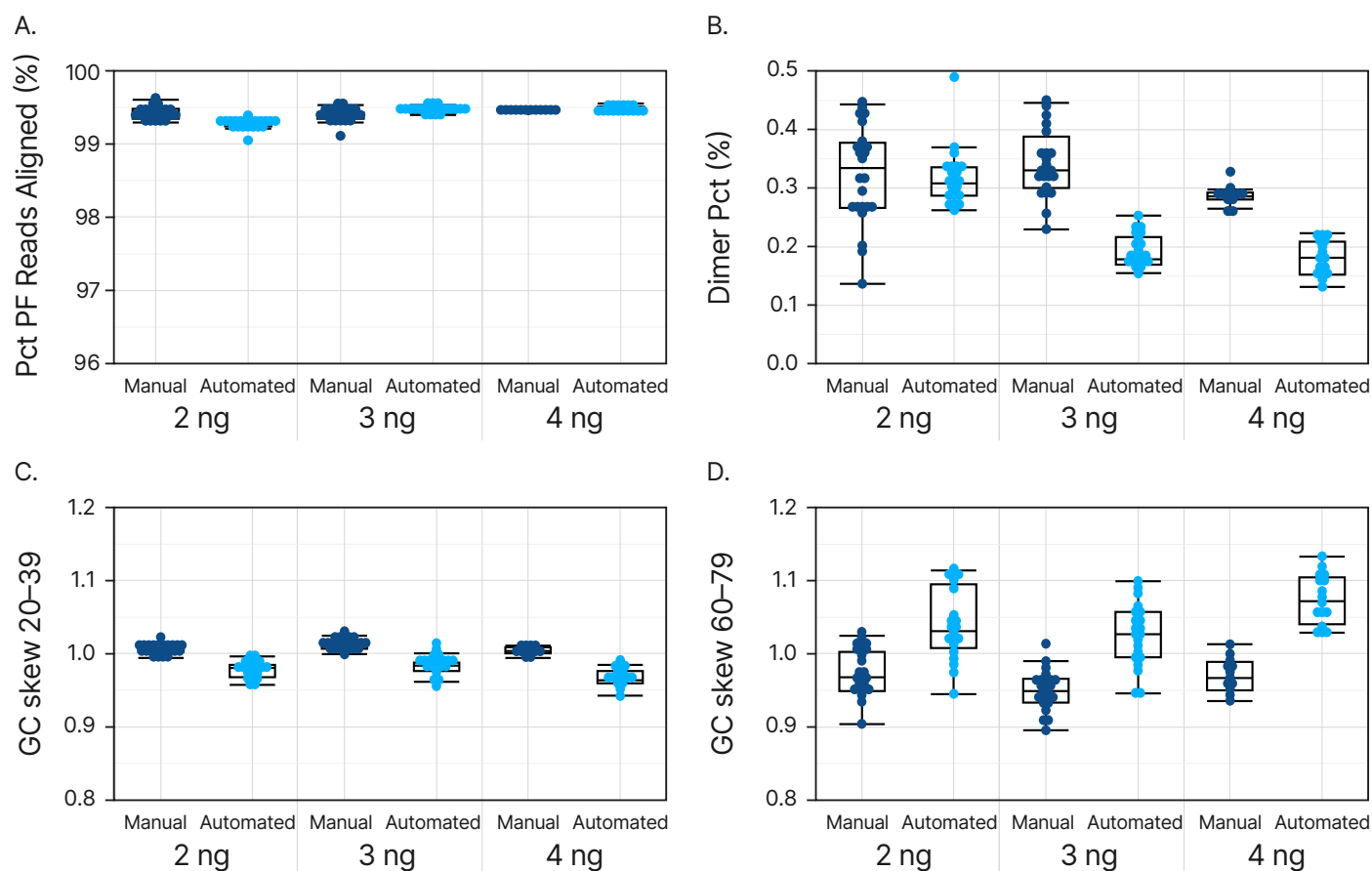
## Results



**Figure 6. 1:20 dilution traces for libraries on Agilent TapeStation High Sensitivity D1000 ScreenTape.** Libraries were prepared from 10 ng of Coriell NA12878 gDNA: (A) 12 libraries prepared manually; (B) 96 libraries prepared using automated method.



**Figure 7. Libraries prepared manually and using an automated library prep from 10 ng of gDNA resulted in sequencing data of similar quality.** To assess the difference in library quality between manual ( $n = 12$ ) and automated library prep ( $n = 96$ ) from 10 ng of Coriell NA12878 gDNA sheared to 150 bp, a comparison analysis was completed to estimate (A) percent passing filter reads aligned, (B) dimer percentage, and normalized coverage over GC content ranging (C) from 20 to 39 and (D) from 60 to 79. Picard Alignment Summary Metrics and GC Bias Summary Metrics calculations were used for analysis (Broad Institute).



**Figure 8. Libraries prepared manually and using an automated library prep from 2, 3, and 4 ng of gDNA resulted in sequencing data of similar quality.** Comparison of Coriell NA12878 gDNA sheared to 150 bp with inputs of 2 ng (manual:  $n = 20$ ; automated:  $n = 20$ ), 3 ng (manual:  $n = 20$ ; automated:  $n = 20$ ), and 4 ng (manual:  $n = 20$ ; automated:  $n = 20$ ). Manual and automation libraries prepped on the i7 workstation show equivalent (A) percent passing filter reads aligned, (B) dimer percentage, and normalized coverage over GC content ranging (C) from 20 to 39 and (D) from 60 to 79. Picard Alignment Summary Metrics and GC Bias Summary Metrics calculations were used for analysis (Broad Institute).

## Summary

We show that automation of the **xGen cfDNA & FFPE DNA Library Prep Kit** on the Biomek i7 Hybrid Genomics Workstation is a fully walk-away workflow that provides an efficient, flexible, and scalable solution for any size lab. The automation solution delivers reliable libraries that yield quality results in downstream workflows and saves valuable time.

## Automated xGen™ cfDNA & FFPE DNA Library Prep Kit on a Biomek™ i7 Hybrid Workstation

For more information, go to: [idtdna.com/ContactUs](https://idtdna.com/ContactUs)

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