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Life Sciences

Automation of IDT xGen™ cfDNA & FFPE DNA Library Prep MC kit on Beckman Coulter Biomek NGeniuS Next Generation Library Prep System

Abstract-Draft Info

As the costs of genome sequencing fall, more and more laboratories are exploring NGS. Laboratories are looking for highly reproducible NGS sample prep methods that limit potential for error. In this paper, we detail an automated process for the IDT xGen CcfDNA & FFPE DNA Library Prep MC kit that offers the laboratory optional settings to optimize a demonstrated application that will process between 4 and 24 samples from start to finish with minimal interaction from the user. Library preparation results using the IDT xGen cfDNA & FFPE DNA Library Prep MC kit on the Biomek NGeniuS Library Prep System indicate an average of 99.5% of reads align with reference genomes.

Introduction

Over the past 20 years, advances in DNA sequencing technology have changed the landscape of the field dramatically, lowering the cost to sequence human genomes. Methods have advanced from Frederick Sanger's discovery of the chain termination technique in the 1970s to the massively parallel sequencing techniques that were introduced in 2005. This new process involved making a library of DNA fragments that could be traced back to the original sample via "barcode." This unlocked the ability to track changes at the genetic level of tumor samples versus healthy samples in a cost-effective manner. Unfortunately, the creation of libraries for next generation sequencing (NGS) is a tedious process. The process can take anywhere from 2.5 hours to several days to complete depending on the type of library created. Great care must be taken to keep accurate records of which samples received which adapter. Pipetting each adapter by hand can lead to errors in creation of libraries with the correct adapters. Many of the processes require proper timing, and do not have safe stopping points, leading to a very long day. Due to these concerns, the automation of DNA library preparation kits using liquid handling systems like the Biomek NGeniuS is highly desirable (Figure 1). One such kit that is amenable to automation is the IDT xGen cfDNA & FFPE DNA Library Prep MC kit.

The IDT xGen cfDNA & FFPE DNA Library Prep MC kit provides a consistent library preparation method to increase sample conversion, especially from low-quality samples, such as cfDNA and FFPE. This allows researchers to identify and characterize rare single nucleotide variants occurring in malignant samples. The general workflow for the IDT xGen cfDNA & FFPE DNA Library Prep MC kit is outlined in Figure 1. Briefly, sample input amounts can range from 1 to 250 ng, and gDNA and FFPE DNA samples are mechanically sheared via Covaris, whereas cfDNA is not. DNA ends are then repaired and cleaned up. An innovative two-step ligation is performed, which reduces dimer-formation and increases sequencing coverage, allowing detection of rare mutational events. Adapter ligated samples are amplified according to manufacturer recommendations based on input amount and sample type. A final cleanup step

produces libraries suitable for hybridization capture methods or whole genome sequencing (reference 1). In this application note, we demonstrate that samples from the automated method on the Biomek NGeniuS system are comparable to manual DNA preparations for input quantities ranging from 1 to 250 ng. Through the use of automation, the hands-on time and likelihood of user-introduced errors are reduced, as compared to manual processing.

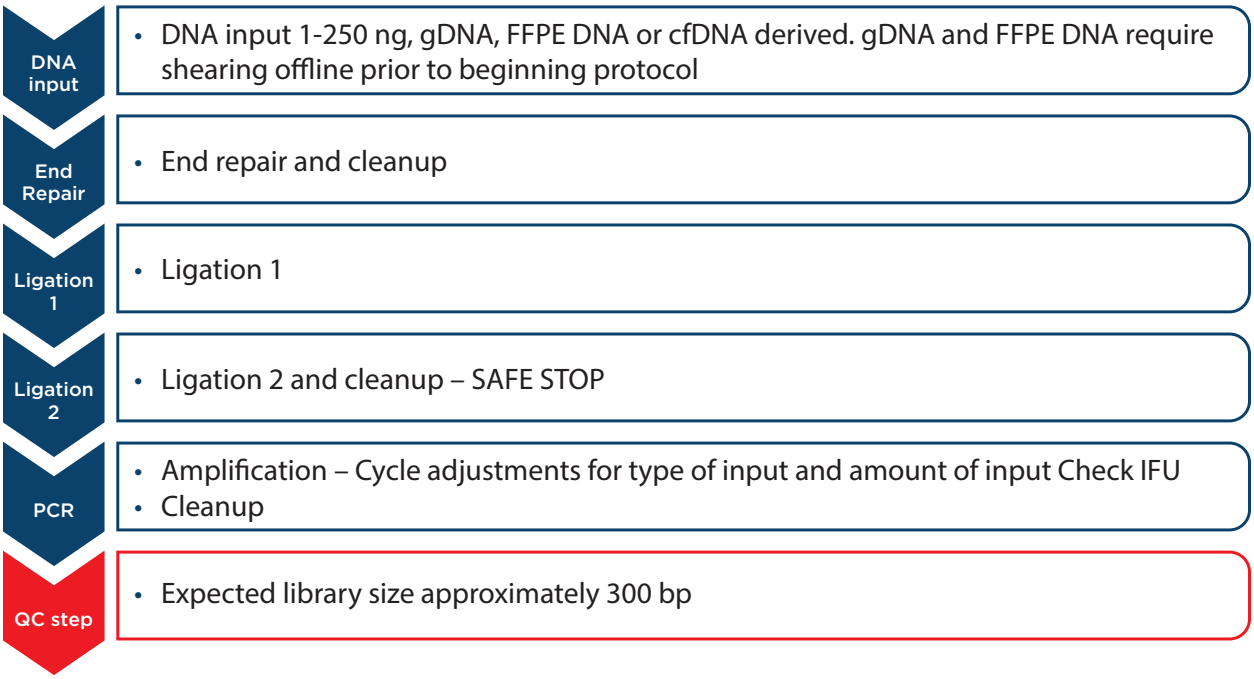


Figure 1. Workflow for IDT xGen cfDNA & FFPE DNA Library Prep protocol. (Blue: On system, Red: Off system)

Materials and Methods

1. Run Setup

DNA samples (Table 2) were diluted to 1-250 ng concentrations appropriate for sample type according to library prep instructions. Once samples were ready for library preparation, the Biomek NGeniuS system's customer portal was used to prepare the instrument. The first step was to select the **+create** button to create a batch to be run on the system (Figure 2). Next, 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** (Figure 3). **App Settings** contains variables specific to the library kit that may be changed between runs or locked by an administrator. 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 light grey numbers below the input box. Table 1 lists the app settings and descriptions of each setting.

Figure 2. The **+create** button is used to begin a new batch setup.

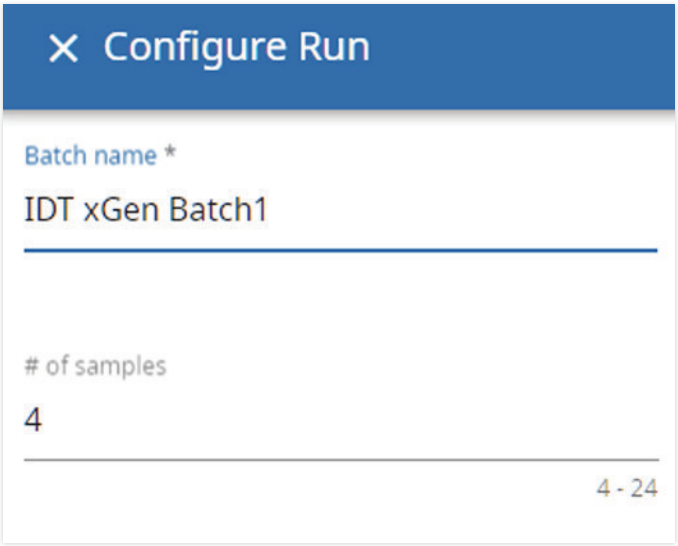
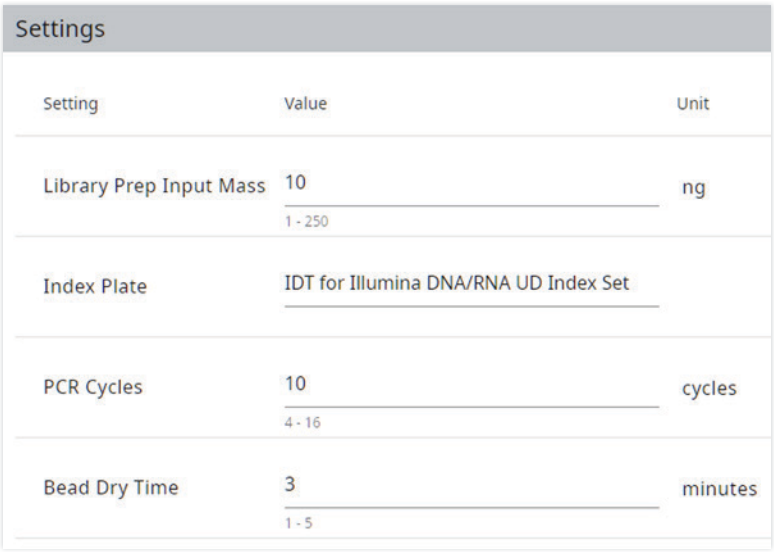


Figure 3. Batch information and default applications settings for batch run.



Setting	Description
Library Prep Input Mass	The starting mass of DNA in a sample for library preparation. In this application the weight is recommended to be between 1-250ng of DNA.
Index Plate	The plate (containing flow cell binding sequences and sequencing primers) used for creating libraries with input DNA. In this application the recommended plate is obtained from IDT.
PCR Cycles	The number of PCR amplification cycles. In this application the number can be between 4 and 16 cycles, depending on sample input mass and type.
Bead Dry Time	The amount of time beads with attached libraries dry after a post-PCR cleanup ethanol wash. In this application the dry time is between 1-5 minutes, never exceeding 5 minutes.

Table 1. App Settings for the IDT xGen CfDNA & FFPE Library Preparation kit and descriptions for each setting.

The next section of data to be filled out is **Sections** (Figure 4). IDT xGen cfDNA & FFPE App has 3 potential sections. Users can select where to start in the process just below the Sections marker in Figure 4. Some users may prefer to do the first section, **Normalize Samples**, by hand. If they do that, they can elect to utilize the **Start at section** dropdown to select **End Repair and Ligation**. A drop-down menu allows the user to select any other section as a starting point. The starting points are determined by safe stops defined in the instructions for use of the library prep kit, which are also suitable for a safe stop on the Biomek NGeniuS system. The blue slider to the left of the sections allows the user to select a safe stop to end processing of the samples. The instrument is designed to be run unattended, but users can elect to stop processing and store samples safely before resuming the run at the next shift.

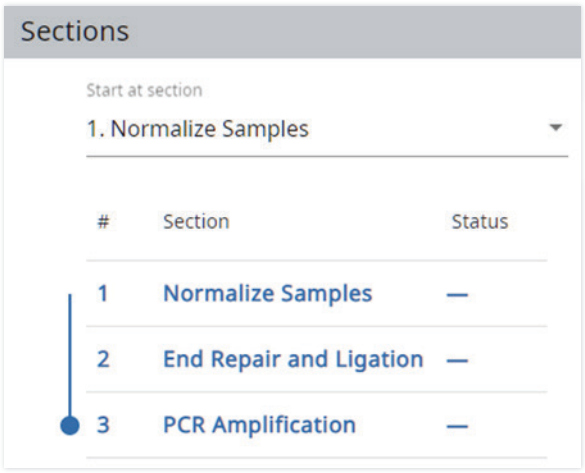


Figure 4. Sections for IDT xGen cfDNA & FFPE DNA Application.

The final step in setting up a batch run is to input the sample data (Figure 5.) In the sample data section, users can click the **DOWNLOAD SAMPLE DATA TEMPLATE** and fill in the appropriate information. This is a .csv file that is filled out and uploaded into the sample data by clicking the **Upload** button. Users can utilize tool tips to determine what information goes into each column by hovering over the header of each column in the NGeniuS Portal. Illumina DNA Prep has 4 different data pieces that are required for tube-based index processing. The IDT xGen CfDNA & FFPE DNA Library Preparation Kit requires the user to define three data pieces for each sample. The first user defined column is the **Sample_ID** of each sample. The second item, **Index1**, is the index plate's well to be associated with the sample. The final column, **initialConcentration**, is the concentration of DNA that will be placed into each well for dilution and processing for library preparation. Once data is entered in the template and saved, the user clicks the **Upload** button. If there are any errors in the sample data file, a red box will appear indicating the source of the problem. Users can fix the data file and upload again if needed. The final step is to click the **READY TO RUN** button in the top right of the screen. The batch can be initiated at any Biomek NGeniuS system within the same tenant.

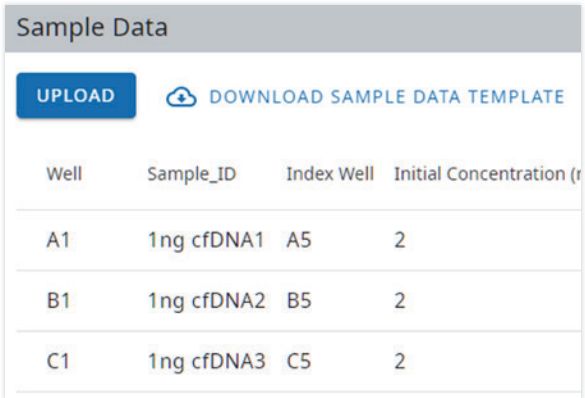


Figure 5. Simulated Sample Data Information. The three columns starting with Sample_ID must be user-defined in the sample-data-template.csv file and uploaded to the Biomek NGeniuS Portal.

Sample	Vendor	Part Number
Quantitative Multiplex Reference Standard fcDNA (mild) [FFPE]	Horizon Discovery	HD798
Multiplex I cfDNA Reference Standard Set	Horizon Discovery	HD780
Homo sapiens gDNA NA12878	Coriell Institute	NA12878

Table 2. App Settings for the IDT xGen CfDNA & FFPE Library Preparation kit and descriptions for each setting.

Equipment	Manufacturer
Biomek NGeniuS Next Generation Library Prep System	Beckman Coulter
NextSeq 550 Sequencer	Illumina
Allegra X-14 Centrifuge	Beckman Coulter
Qubit Fluorometric Quantification system	Thermo Fisher Scientific
Agilent 4200 TapeStation	Agilent

Table 3. App Settings for the IDT xGen CfDNA & FFPE Library Preparation kit and descriptions for each setting.

Reagents	Manufacturer	Part Number	Lot Number
xGen CfDNA & FFPE DNA Library Kit, 96 rxn	IDT	10006203	0006493031
TE Buffer pH 8.0, 300ml	Thermo Fisher	12090015	2417499
xGen UDI Indexing Primers, Index 1-96	IDT	10005975	0000471069
UltraPure Water (Invitrogen)	Invitrogen	10977015	2360360
100% Ethanol (AmericanBio)	AmericanBio	64175	19216600
AMPure XP (Beckman)	Beckman Coulter	A63882	19006800
Elution Buffer, EB (Qiagen)	Qiagen	19086	169027940
KAPA HotStart ReadyMix (2X)	Invitrogen-Life Technologies	KK 2601	0000125324

Table 4. Reagents used in preparation of libraries with IDT xGen CfDNA & FFPE DNA Library Prep and sequencing on Illumina sequencer.

Library Prep Input Mass (ng)	Sample Type	Index Plate	PCR Cycles	Bead Dry TIme (min)
1ng	cfDNA	IDT for Illumina DNA/ RNA UD Index Set	12	3
25ng	FFPE	IDT for Illumina DNA/ RNA UD Index Set	10	3
250ng	gDNA	IDT for Illumina DNA/ RNA UD Index Set	5	3

Table 5. Method variables and selections for IDT xGen CfDNA & FFPE DNA Library Prep. Parameters outlined in this table correspond to selections made in "Settings" when initiating a run (Figure 3).

Consumable	Manufacturer	Part Number
1025 µL Filter Tips	Beckman Coulter	C59585
70 µL Filter Tips	Beckman Coulter	C62712
NGeniuS 24 well plates/lids	Beckman Coulter	C62706
NGeniuS Bulk reservoirs	Beckman Coulter	C62707
NGeniuS seal pads	Beckman Coulter	C70665
NGeniuS, reagent plugs	Beckman Coulter	C51978
Qubit Assay Tubes	ThermoFisher Scientific	Q32856
Covaris AFA microtube	Covaris	520045

Table 6. Consumables required for sample processing and library preparation.

2) Library Preparation

Samples of various type and input (Table 2) were processed on the Biomek NGeniuS system using instruments and equipment detailed in Table 3. Input samples were diluted to starting concentrations appropriate for a max of 1:100 dilution of sample prior to preparing the library. This was determined by taking the library prep input mass (in ng) and dividing by the input volume in µL. System requested reagents (contained in the IDT xGen CfDNA & FFPE kit, consumables from IDT, and bulk reagents [Table 4]) and Biomek NGeniuS consumables (Table 6) were loaded onto the system for processing. The variables selected for processing for both manual and automated processing were as seen in Table 5. After all reagents and consumables had been allocated to proper indicated storage locations, the user was instructed to remove reagents and notified of an estimated time of completion for the library prep based off selections the user input at the start of the protocol. The system processed dilution of samples. Then, samples were processed and libraries were constructed on the system. After completion of the runs, the constructed libraries were analyzed by traces on an Agilent 4200 TapeStation system (Figure 6), DNA fluorometric yields (Table 7), and Illumina sequencing (Table 8).

Library Starting Material	Average Qubit Yield (ng/µl)	# Libraries Prepared
cfDNA	16.67	24
FFPE	48.11	10
gDNA	17.83	4

Table 7. Qubit Yields for library preparations for IDT xGen CfDNA & FFPE DNA Library Prep. Qubit yields are averages taken across the total number of libraries prepared from each sample.

Results and Discussion

After completion of the runs by the Biomek NGeniuS NGS Library Prep System, libraries were sequenced using an Illumina NextSeq 550 instrument using High output v3 Reagent Kits (Illumina Inc.). Fragment size was measured with an Agilent 4200 TapeStation system (Agilent Technologies, Inc.), and library prep yield masses were measured with Qubit fluorometric quantification (Thermo Fisher). Sequencing results returned 874M reads, with cluster densities of 208.75 K/mm2, in line with Illumina's optimal raw cluster density range of 170-220 K/mm2. Sequencing results had scores of Q30 or greater for 86.98% of bases.

24 libraries (one negative control) were prepared from cfDNA samples (Horizon Discovery). Libraries returned average masses of 16.67 ng/ul. Post-ligation and amplification fragments in the cfDNA samples displayed a consistent and normal distribution of lengths, averaging 362 bp (Figure 6a). Of this sample, 99.49% of reads were aligned to the reference genome, with 99.84% of reads pairing to the reference genome. An average of 0.46% of reads across 23 samples were duplicates (Table 8).

Libraries prepared from FFPE samples displayed a normal distribution of lengths, averaging 378 bp (Figure 6b). Of this sample, 99.8% of reads were aligned to the reference genome, with 99.84% of reads pairing to the reference genome. Across 10 samples, an average of 0.37% of reads were duplicates (Table 8)

Libraries prepared from gDNA samples displayed a distribution of lengths consistent with the expectations of the IDT xGen CfDNA & FFPE NGS Library Prep kit, averaging 391 bp (Figure 6c). Of this sample, 99.2% of reads were aligned to the reference genome, with 99.3% of reads pairing to the reference genome. An average of 0.56% of reads across three samples were duplicates (Table 8)

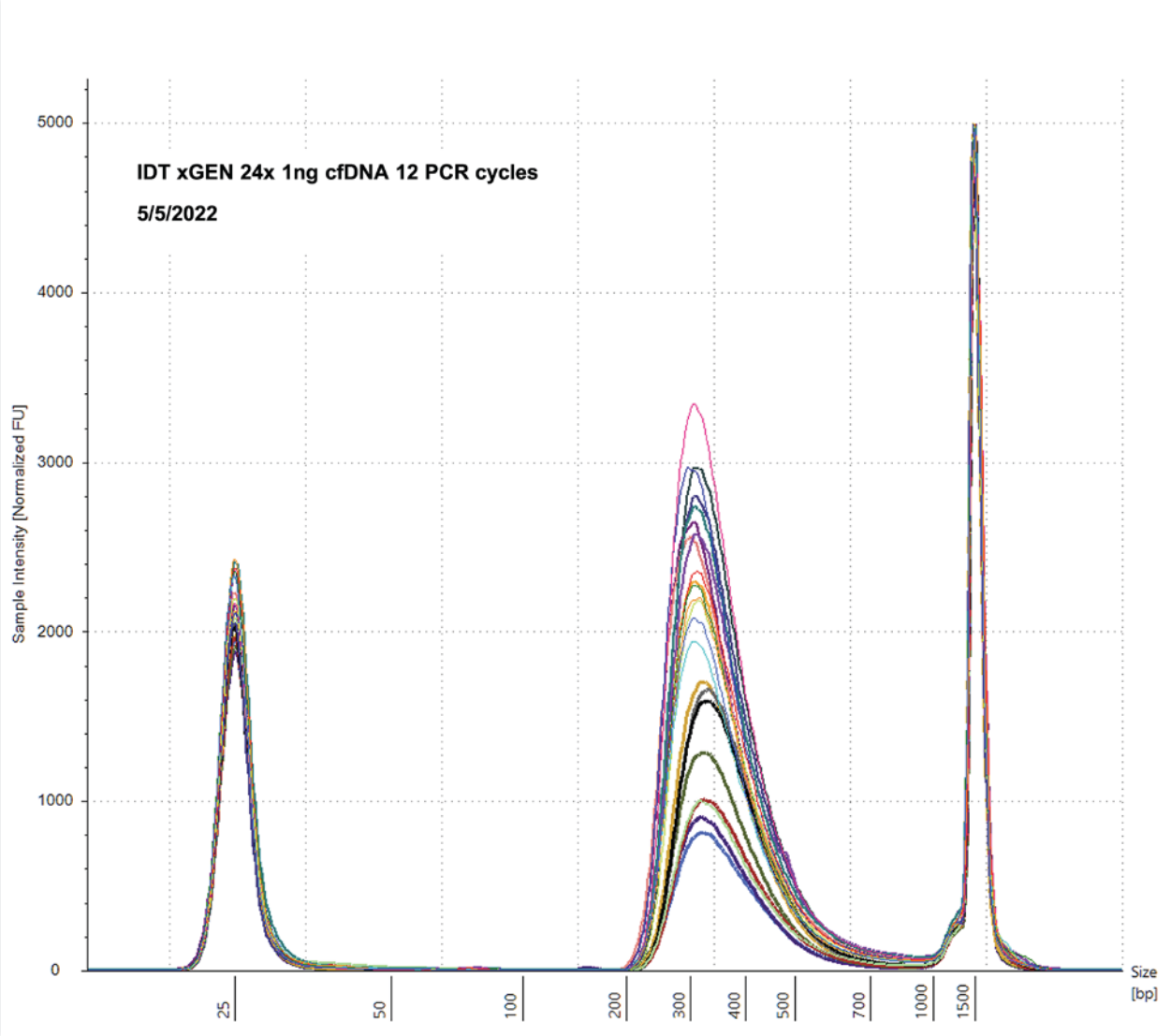


Figure 6a. Agilent TapeStation trace results from libraries created on the Biomek NGeniuS next generation library prep system from 23 cfDNA samples using the IDT xGen CfDNA & FFPE NGS library prep kit. Libraries have an average fragment size of 362 bp, averaged across all libraries created from these samples.

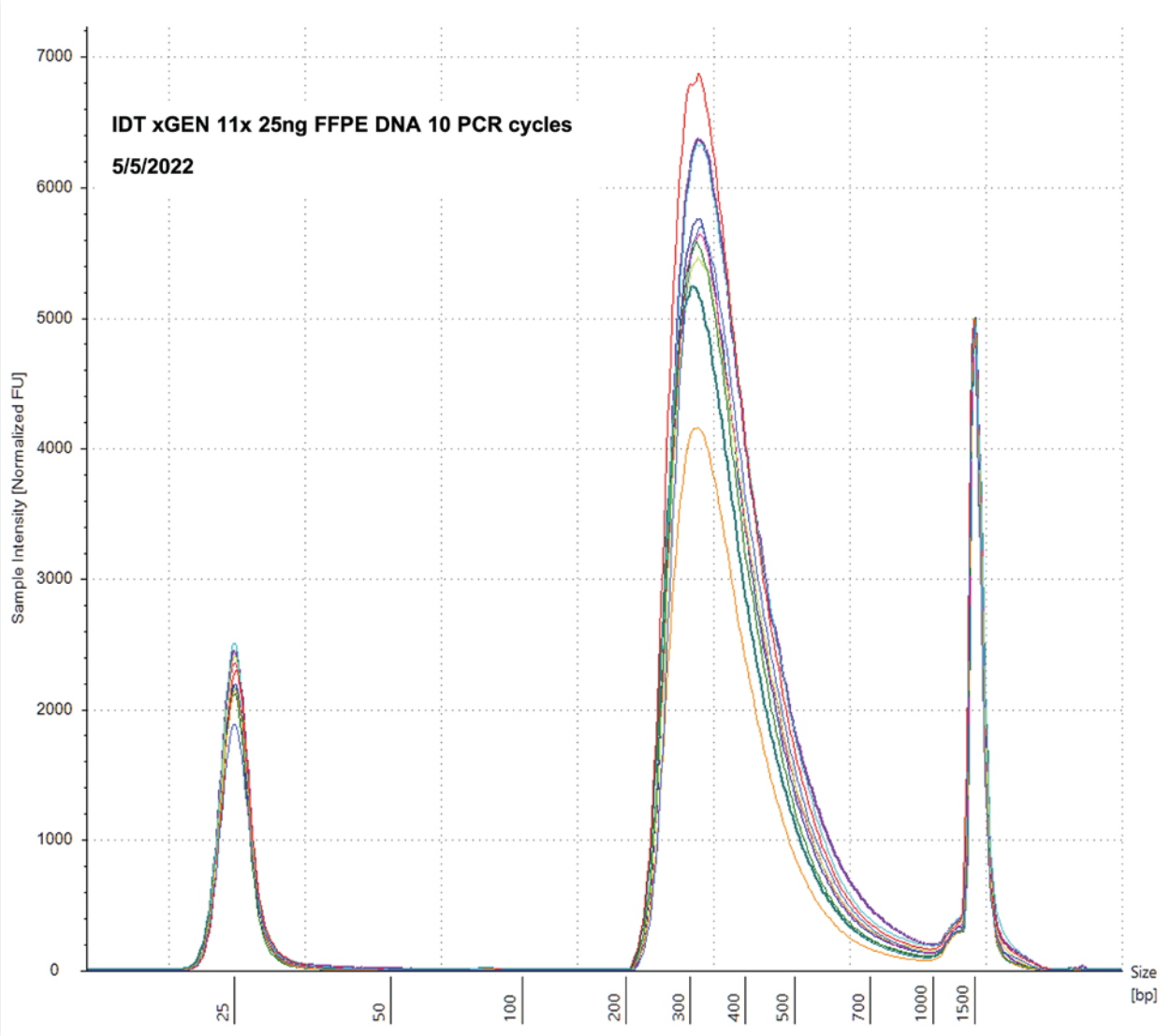


Figure 6b. Agilent TapeStation trace results from libraries created on the Biomek NGeniuS next generation library prep system from 10 FFPE samples using the xGen CfDNA & FFPE NGS library prep kit.. Libraries have an average of 378 bp per fragment, averaged across all libraries created from these samples.

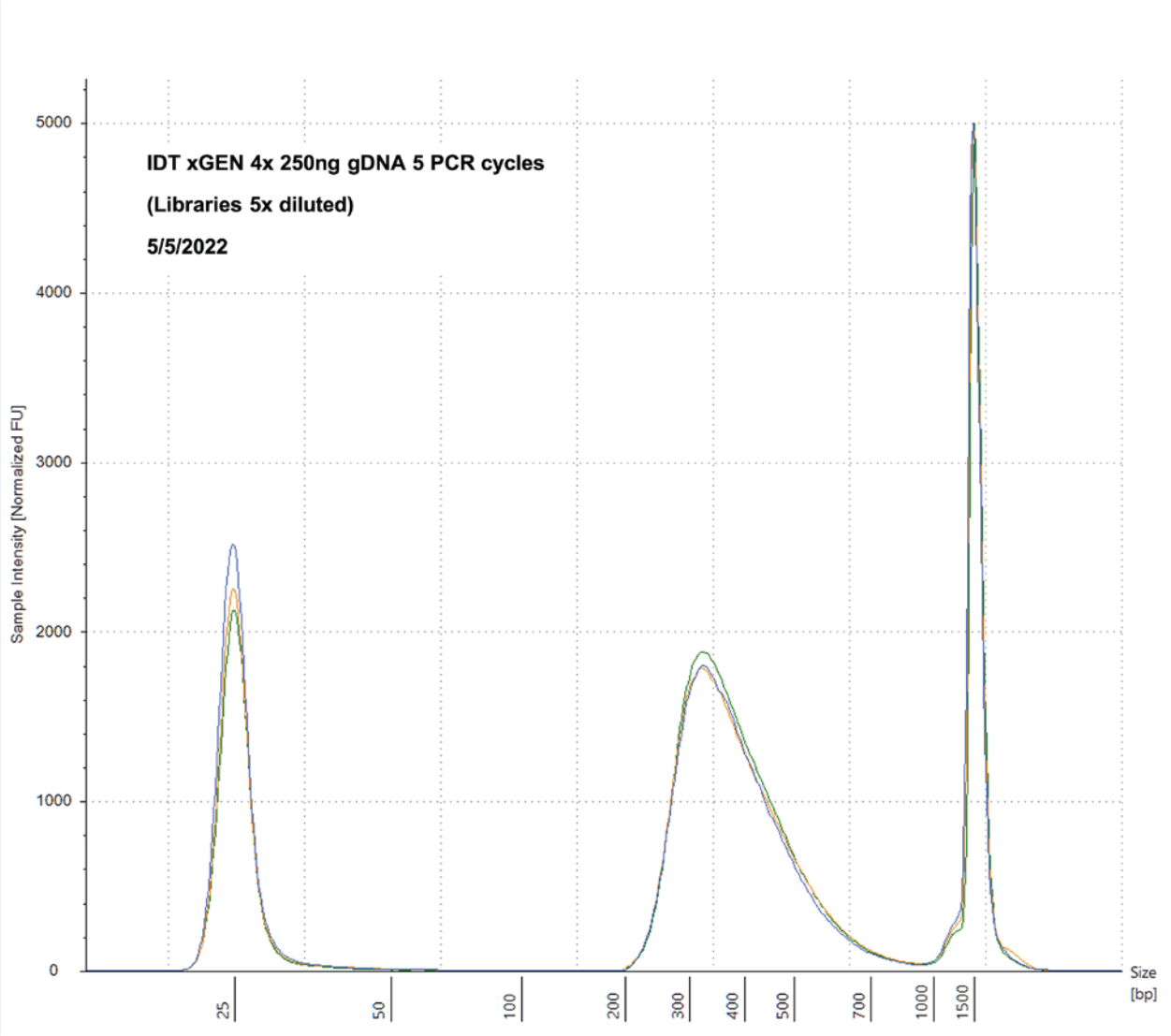


Figure 6c. Agilent TapeStation trace results from libraries created on the Biomek NGeniuS next generation library prep system from 3 gDNA samples using the IDT xGen CfDNA & FFPE NGS library prep kit. Libraries have an average fragment size of 391 bp, averaged across all libraries created from these samples.

Sample Input DNA	Average Library Size (bp)	Qubit concentration	% >Q30	Average % reads aligned to reference genome	Average % of reads paired to reference genome	Average % duplicates
cfDNA	362	16.67	86.98	99.49%	99.84%	0.46%
FFPE	378	48.11	86.98	99.8%	99.84%	0.37%
gDNA	391	17.83	86.98	99.2%	99.3%	0.56%
cfDNA Neg. Ctrl	472	0.0675	86.98	10.1%	96.9%	38.4%
FFPE Neg. Ctrl	438	0.0938	86.98	4.0%	96.4%	36.2%
gDNA Neg. Ctrl	N/A	0.063	86.98	14.9%	98.5%	9.5%

Table 8. Sequencing results from the IDT xGen Library Preparation kit on the Biomek NGeniuS Library Prep System. Results fall within library preparation parameters defined by IDT.[1]

Summary

Libraries generated using the IDT xGen CfDNA & FFPE DNA Library Prep kit on the Biomek NGeniuS Next Generation Library Prep System show uniform size distribution on the Agilent 4200 TapeStation system (Figure 6) and fall within the recommended library size range of the IDT xGen CfDNA & FFPE DNA Library Prep kit. Sequencing of the replicates of samples for 3 different concentrations produced an average library sizes in a range between 362-391. Over 99.2% of reads were aligned to the reference genome, 99.3% of reads were properly paired and less than 0.56% of reads were duplicates (Table 8).

We demonstrated the Biomek NGeniuS next generation library prep system can successfully produce high-quality whole genome sequencing libraries suitable for sequencing on the Illumina platforms using the IDT xGen DNA Library Prep Kit.

References

1. IDT xGen cfDNA & FFPE DNA Library Prep MC Kit Reference Manual Version 2.

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