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Life Sciences

Automation of Roche KAPA HyperPlus Library Preparation Kit on the Beckman Coulter Biomek NGeniuS Next Generation Library Preparation System

Abstract

As next generation sequencing becomes more important for biomarker research and genome discovery, more laboratories are exploring bringing NGS Library Prep in-house as a tool to lower overhead and control turnaround time. These laboratories are looking for highly reproducible methods that limit potential for error. Here we detail a process for performing library construction with the Roche KAPA HyperPlus library kit that offers laboratory customizable settings in a demonstrated application that will process between 4 and 24 samples and a wide range of sample types and starting concentrations (1 ng -1000 ng) from start to finish, with minimal interaction from the user.

Introduction

Over the past 20 years, advances in DNA sequencing technology have changed the landscape of the scientific field dramatically, lowering the cost to sequence human genomes. The ability to quickly and cheaply sequence genomes has had a large impact on everything from applications in basic research to advancing the field of personalized medicine. Unfortunately, the creation of libraries for NGS is a tedious process that 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 sample-adapter sequence pairs and pipetting each adapter by hand can lead to user errors. Many of the processes require precise timing and do not have safe stopping points, leading to a very long workday for the user.

The Roche KAPA HyperPlus library construction kit allows for a large range of sample inputs (1 ng - 1 µg) and is suitable for whole genome sequencing or as a precursor to target capture methods in preparation for sequencing. The chemistry in this kit has been optimized to achieve higher conversion rates from DNA to adapter ligated library than standard library kits. This is especially helpful for samples where sample quantity may be limited, or where FFPE samples are outside of normal quality inputs1.

In this application note, we have demonstrated the automated preparation on the Biomek NGeniuS system at 1000 ng, 100 ng and 1 ng and have compared data obtained from processing using the Biomek NGeniuS system with the data that can be found in the manufacturer's instructions. The hands-on time required to run this assay is reduced, and the interactions with the system are limited.

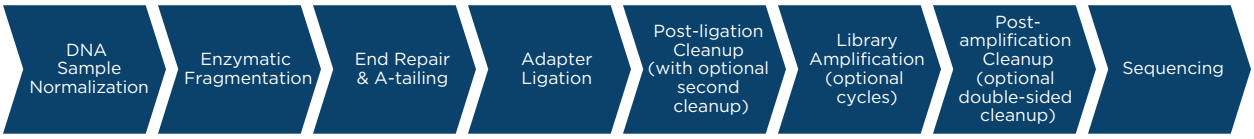


Figure 1. Workflow for Roche KAPA HyperPlus protocol.

Materials and Methods

1) Run Setup

Genomic DNA samples (*Homo sapiens* NA12878 gDNA from the Coriell Institute) and formalin-compromised DNA reference standards (HD798 fcDNA samples from Horizon Discovery) (Table 1) were quantified using the Qubit DNA BR kit (Thermo Fisher Scientific) and diluted to an initial starting concentration appropriate for the Biomek NGeniuS system. Normalization of input nucleic acid is performed on the Biomek NGeniuS system by diluting an aliquot of the sample to the input volume required by the library preparation kit to arrive at the correct starting concentration. In order to reduce manual pipetting errors, the concentration input nucleic acid must be within 100X of the concentration required by the library preparation kit so that the operator is not attempting to manually pipette small volumes of highly concentrated input nucleic acid.

Sample	Vendor	Part Number
Homo sapiens gDNA – CEPH/Utah pedigree NA12878	Coriell Institute	NA12878
Quantitative Multiplex Reference Standard fcDNA (mild)	Horizon Discovery	HD798

Table 1. Sample types and inputs used in preparations of samples for Roche HyperPlus kit.

When the samples were ready to run, they were set up in the Biomek NGeniuS customer portal. The first step was to select the **+create** button to create a batch to be run on the system (Figure 2). Next, the Roche KAPA HyperPlus App was selected to process samples. The setup is broken up 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 may be locked by the lab administrator. The Batch name is a unique run name for the samples being processed. Number of samples is any number between 4-24 for this application, as indicated by the light grey numbers below the input box. Table 2 lists the app settings and descriptions of each setting.

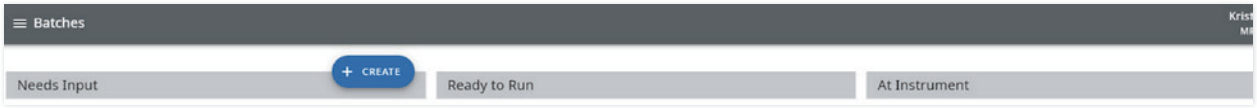
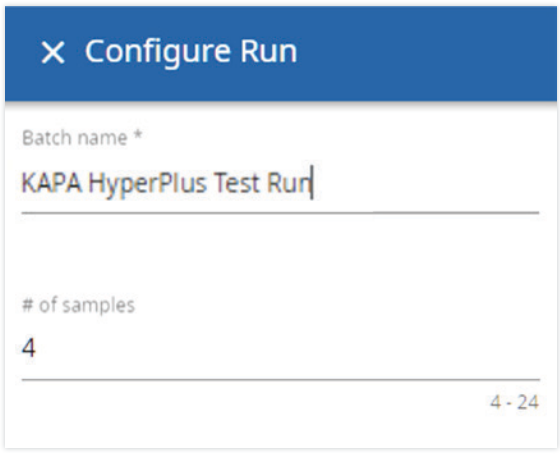


Figure 2. The **+create** button in the above figure is used to begin a new batch setup.



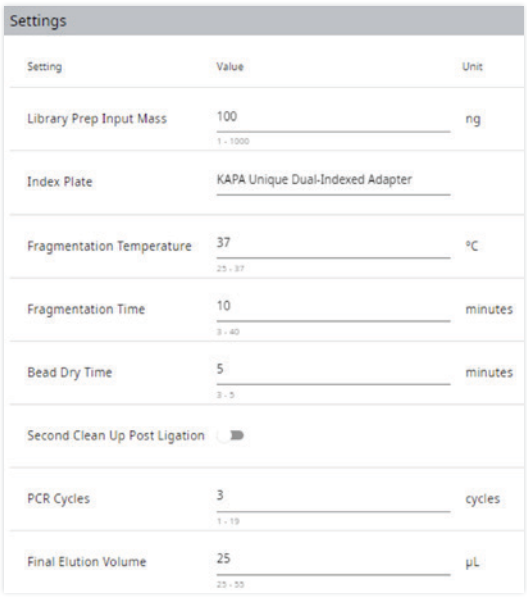


Figure 3. Batch information and application settings for batch run.

Setting	Description
Library Prep Input Mass	The total number of nanograms of DNA to be used for each sample in the library preparation.
Index Plate	Details on the specific Index Plate used in the batch.
Fragmentation Temperature	The temperature (º C) at which enzymatic fragmentation is performed. Fragmentation temperature can be adjusted to influence final library fragment size.
Fragmentation Time	The number of minutes used for the enzymatic fragmentation reaction. Fragmentation time can be adjusted to influence final library fragment size.
Bead Dry Time	The number of minutes the cleanup beads will be left to air dry during all DNA cleanup steps. In this application this time is selectable between 2-5 minutes.
Second Cleanup Post-Ligation	This toggle switch is used to perform an additional cleanup after ligation steps.
PCR Cycles	The number of PCR amplification cycles. In this application the number can be 0-20 cycles.
Final Elution Volume	The final volume of eluate used when recovering the final DNA libraries. In this application the volume can be between 15-30 µL.

Table 2. Application settings and descriptions of each setting.

The next section of data to be filled out is **Sections** (Figure 4). Roche KAPA HyperPlus 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 so, they can elect to utilize the **Start at section** drop-down menu to select another point of the library preparation protocol. 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 samples. The instrument is designed to 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 Roche KAPA HyperPrep Application.

The final step in setting up a batch to 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. KAPA HyperPlus has 4 different data pieces that are required for tube-based index processing. The first column is the **Sample_ID** of each sample. The second item, **IndexWell**, corresponds to the indexing primer to be used for each 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 the data is entered in the template and saved, the user clicks the **Upload** button. If there are any unexpected values detected by the system 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, or grouping of instruments, activated within the lab.

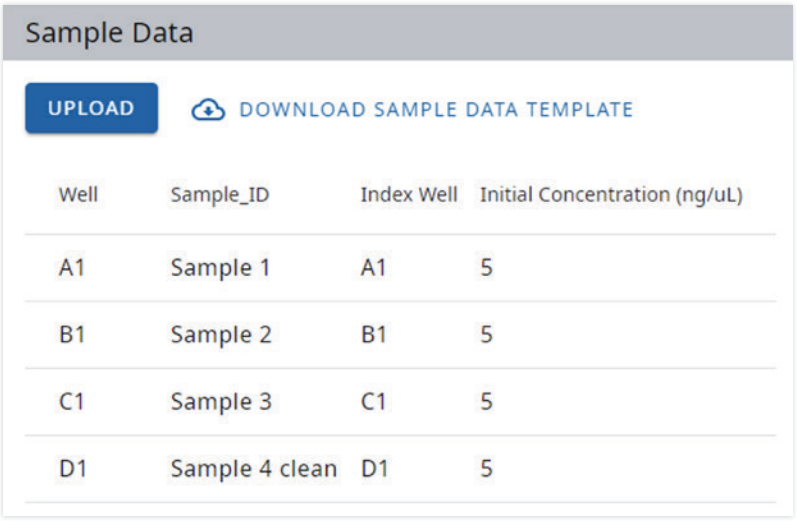


Figure 5. Sample data information.

Sample Input (ng)	Sample Number	Sample Type	Index Plate	Fragmentation Temp	Fragmentation Time	Bead Dry Time (minutes)	PCR Cycles	Cleanup Post Ligation	Cleanup Post PCR	Elution Volume	Index Adapter Dilution
1000	4	NA12878 gDNA and FFPE	KAPA Unique Dual-Indexed Adapter	37	35	5	3	No Size Selection, Standard Cleanup and Optional Second Cleanup	Standard	25	15 µM
100	13	NA12878 gDNA and FFPE	KAPA Unique Dual-Indexed Adapter	37	30	5	4	No Size Selection, Standard Cleanup and Optional Second Cleanup	Standard	25	15 µM
1		NA12878 gDNA and FFPE	KAPA Unique Dual-Indexed Adapter	37	30	5	11	No Size Selection, Standard Cleanup and Optional Second Cleanup	Standard	25	300 nM
100		NA12878 gDNA and FFPE	KAPA Unique Dual-Indexed Adapter	37	30	5	4	No Size Selection, Standard Cleanup	Standard	25	15 µM

Table 3. Sample information and processing information for samples processed on the Biomek NGeniuS system.

Equipment	Manufacturer
Biomek NGeniuS system	Beckman Coulter Life Sciences
NextSeq 1000 Sequencer	Illumina Inc.
Allegra X-14 Centrifuge	Beckman Coulter Life Sciences
Qubit Fluorometer	Thermo Fisher Scientific
4200 TapeStation System	Agilent
S220 Focused-ultrasonicator	Covaris

Table 4. Equipment used in sample preparation and processing of KAPA HyperPlus.

Reagents	Manufacturer	Part Number
KAPA HyperPlus Kit, 96 reactions	Roche	07962428001
KAPA Dual-Indexed Adapter Kit, (15 µM, 20 µL each)	Roche	08861919702
Qubit High Sensitivity Kit	Thermo Fisher Scientific	Q32854
Bioanalyzer High Sensitivity Kit	Agilent	5067-4627
NextSeq 1000/2000 300 cycle kit	Illumina	20044466
AMPure XP Beads	Beckman Coulter Life Sciences	A63882
PCR grade Water	Invitrogen-Life Technologies	10977-015
Ethanol	American Bio	AB00515-00500

Table 5. Reagents used in preparation of libraries with KAPA HyperPlus kit and sequencing on Illumina sequencer.

Consumable	Manufacturer	Part Number
Qubit Tubes	Thermo Fisher Scientific	Q32851
Foil Plate Seals	Beckman Coulter Life Sciences	538619
Biomek NGeniuS Instrument Reaction Vessel, 24 well	Beckman Coulter Life Sciences	C62705
Biomek NGeniuS Instrument Lid, 24 well	Beckman Coulter Life Sciences	C62706
Biomek NGeniuS Bulk reservoirs	Beckman Coulter Life Sciences	C62707
Biomek NGeniuS seal pads	Beckman Coulter Life Sciences	C70665
Biomek NGeniuS reagent plugs	Beckman Coulter Life Sciences	C62706
1025 µL Conductive Filtered Tips, Case	Beckman Coulter Life Sciences	C59585
70 µL Conductive Filtered Tips, Case	Beckman Coulter Life Sciences	C62712
Empty Tip box 1025 µL, Case	Beckman Coulter Life Sciences	C70672
Empty Tip box 70 µL, Case	Beckman Coulter Life Sciences	C70673

Table 6. Consumables required for sample processing.

2) Library Preparation

Samples of various types and input (Table 3) were processed both manually and on the Biomek NGeniuS system using instruments, reagents, and consumables detailed in Tables 4, 5 and 6. System requested reagents from the Roche KAPA HyperPlus kit and bulk reagents as well as consumables (Table 6) were loaded onto the Biomek NGeniuS system for processing. The variables selected for processing for both manual and automated processing were as seen in Table 2. After all reagents and consumables used had been allocated to proper storage locations, the user was instructed to remove excess reagents and notified of an estimated time of completion for the library prep based off selections chosen at the start of the protocol. The system processed dilution of samples. Then, Roche KAPA HyperPlus libraries were constructed on the system. After completion of the runs, the resulting libraries were analyzed using a 4200 TapeStation with D1000 High Sensitivity ScreenTape (Agilent) to determine library size and the Qubit dsDNA HS assay (Thermo Fisher) to determine library concentration. Libraries were then sequenced on a NextSeq 1000 utilizing NextSeq 1000/2000 300 cycle kits. Data was analyzed using the DRAGEN Germline App (version 4.0.3) on Illumina BaseSpace. The Human HG38 Alt-Masked v2 reference genome provided by BaseSpace was used for library alignment.

Results and Discussion

After completion of the runs by the Biomek NGeniuS NGS Library Prep System, libraries were sequenced using an Illumina NextSeq 500 instrument. (Illumina Inc.). Fragment sizes were measured with a 4200 TapeStation system for all samples (Agilent Technologies, Inc.). Prepared library yield masses were measured with Qubit fluorometric quantification (Thermo Fisher). Sequencing results from the two NextSeq 500 runs returned 227 M and 275 M reads, with a total of 95.99 Gbp and 116 Gbp respectively of sequencing data generated between all runs. Sequencing results had scores of Q30 or greater for 91.2% and 87.9% of bases for the two runs respectively. (Table 7).

Sample Input DNA	Average Library Size (bp)	Qubit Conc. (ng/µL)	Average % >Q30	Average % of Reads Mapped to Reference Genome	Average % of Reads Paired to Reference Genome	Average % Duplicates	Average Total Reads
1000 ng NA12878 gDNA and HD798 cfDNA	449 bp	70 ng/µL	91.47%	99.62%	97.5%	1.18%	16,727,559
100 ng HD798 cfDNA	363 bp	24.3 ng/µL	91.2%	99.7%	97.8%	1.9%	35,190,978
1 ng NA12878 gDNA	372 bp	35 ng/µL	91.47%	99.02%	97.3%	0.68%	35,085,137
100 ng NA 12878 gDNA and HD798 cfDNA	344 bp	4.1 ng/µL	87.9%	99.29%	97.49%	1.43%	49,265,139

Table 7. Average sequencing depth and region coverage information.

Figure 6. Percent Mapped Reads, Percent Properly paired, and Percent duplicates across all the samples of all 4 runs.

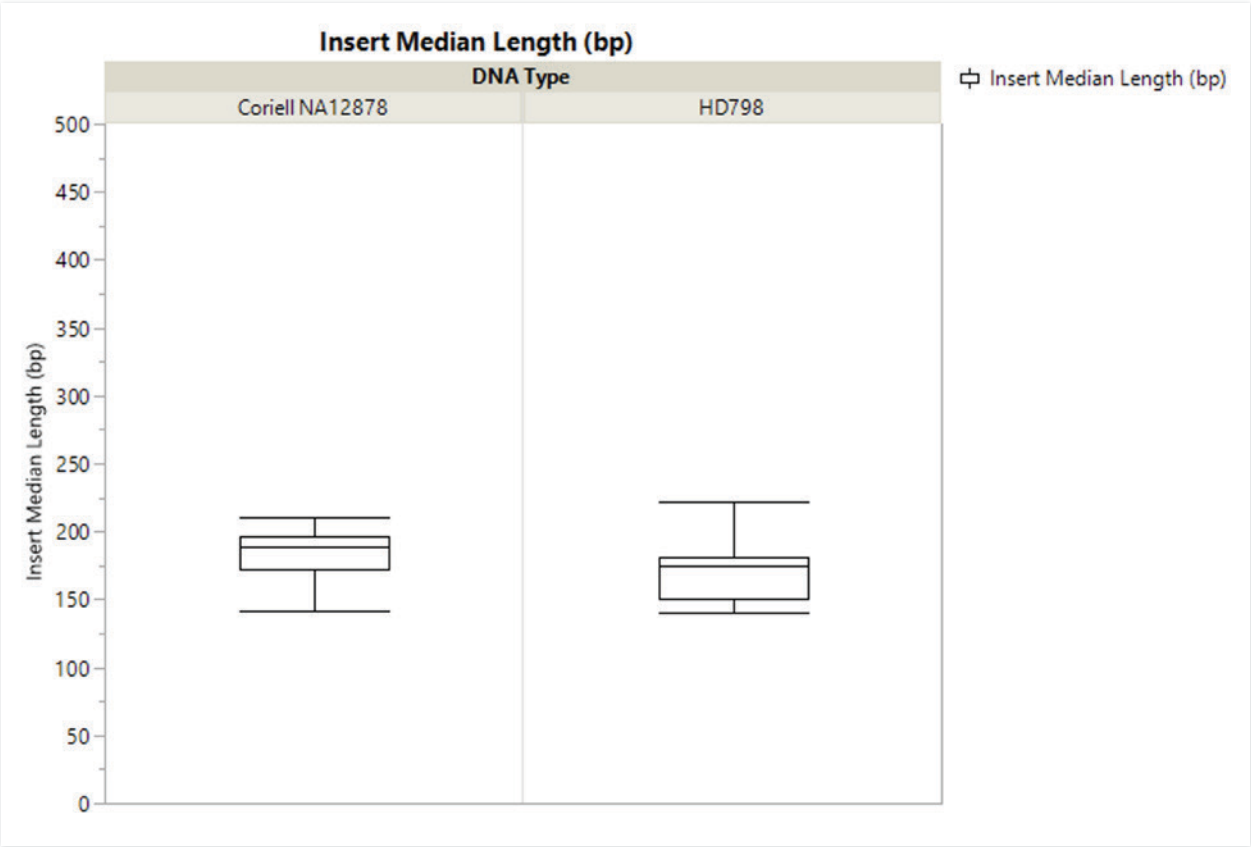


Figure 7. Insert median length for two different types of samples.

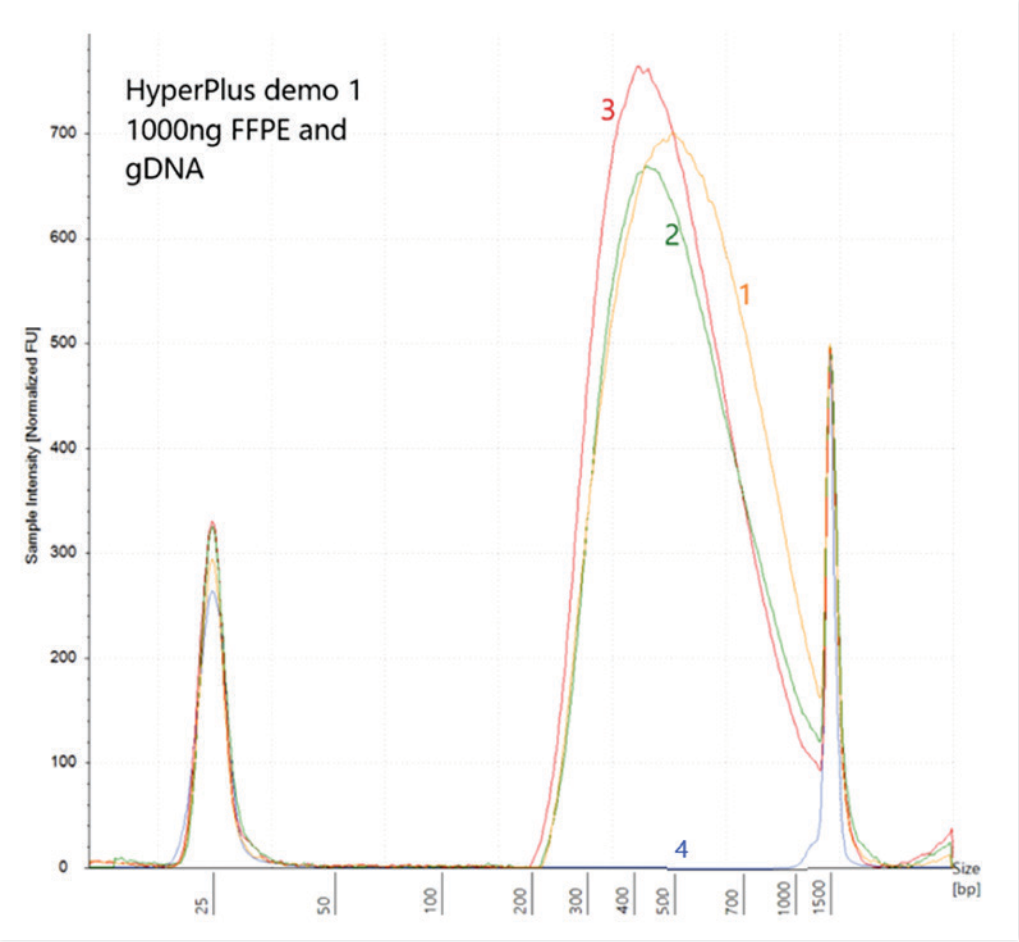


Figure 8. Agilent TapeStation trace results from NGS libraries created on the Biomek NGeniuS system and from 2 NA12878 gDNA (sample 2 and 3), 1 HD 798 fcDNA (sample 1) and one negative control (sample 4) using the Roche KAPA HyperPlus kit. Libraries have an average fragment size of 449 bp, averaged across all libraries created from these samples.

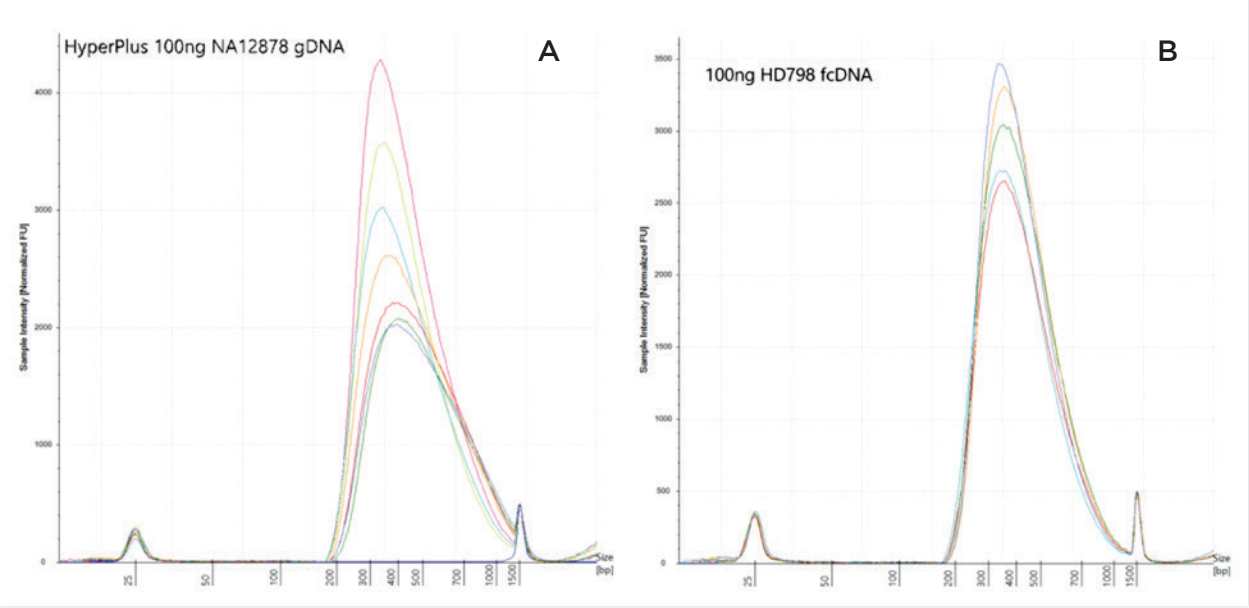


Figure 9a and b. Agilent TapeStation trace results from DNA NGS libraries created on the Biomek NGeniuS system and from 7 NA12878 gDNA, 5 HD798 fcDNA samples and one negative control using the Roche KAPA HyperPlus kit. Libraries have an average fragment size of 372 bp for gDNA and 350 bp for HD798 fcDNA averaged across all libraries created from these samples.

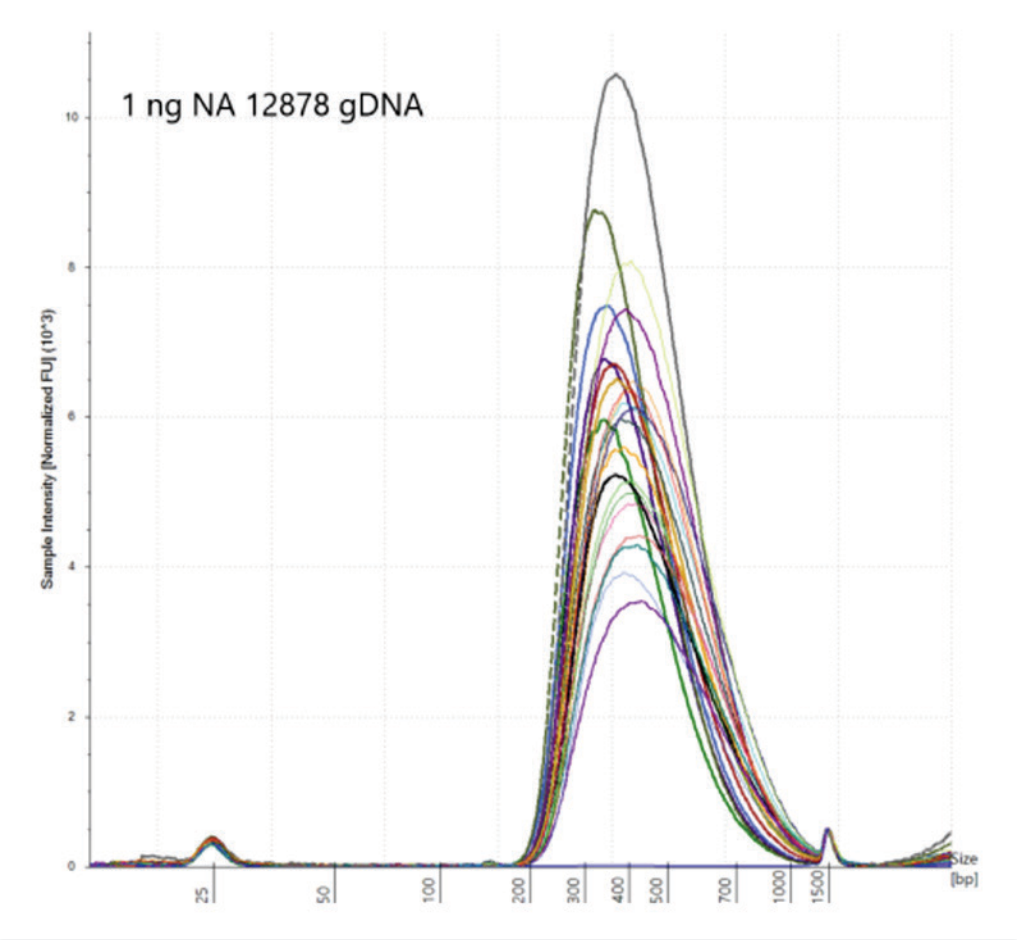


Figure 10. Agilent TapeStation trace results from DNA NGS libraries created on the Biomek NGeniuS system and from 23 NA12878 gDNA samples and one negative control using the Roche KAPA HyperPlus kit. Libraries have an average fragment size of 388 bp, averaged across all libraries created from these samples.

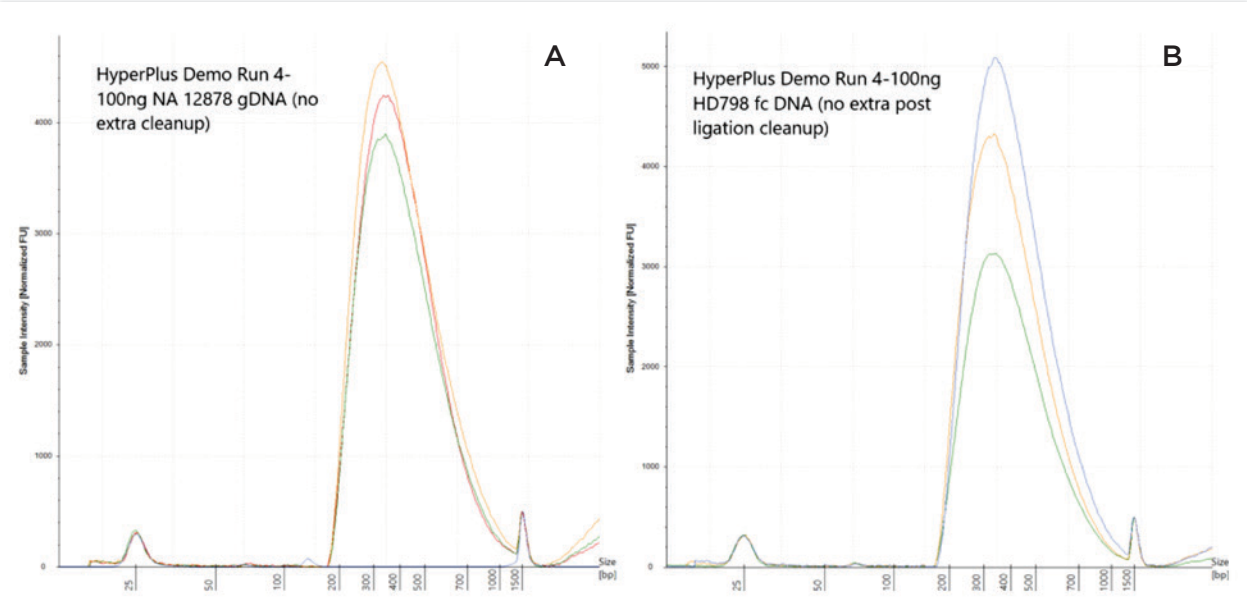


Figure 11a and b. Agilent TapeStation trace results from DNA NGS libraries created on the Biomek NGeniuS system and from 3 NA12878 gDNA, 3 HD798 fcDNA samples and one negative control using the Roche KAPA HyperPlus kit with no extra post-ligation cleanup. Libraries have an average fragment size of 345 bp for gDNA and 342 bp for HD798 fcDNA averaged across all libraries created from these samples.averaged across all libraries created from these samples.

Summary

Libraries generated using the Roche KAPA HyperPlus kit on the Biomek NGeniuS Next Generation Library Prep System show uniform size distribution on the Agilent TapeStation (Figures 8, 9a, 9b, and 10) and fall within the recommended library size range of the Roche KAPA HyperPlus kit. Sequencing of replicates of samples for three different concentration ranges produced over 970 million pass filter reads per library. Over 99% of reads were aligned to the reference genome, >97% of reads were properly paired and less than 2% of reads were PCR duplicates (Table 7).

We demonstrated that the Biomek NGeniuS system can successfully produce high-quality whole genome sequencing libraries suitable for sequencing on Illumina platforms using the Roche KAPA HyperPlus kit.

References

1. KAPA HyperPlus Kit Technical Data Sheet KR1145 – v8.21. KAPA Biosystems.
2. Illumina NextSeq 1000/2000 System Guide Document # 200027171 v01.

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