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Life Sciences

Automation of Illumina DNA Prep Kit on Beckman Coulter Biomek NGeniuS Next Generation Library Prep System

Introduction

Over the past several decades, DNA sequencing 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. These modern sequencing techniques have become common laboratory practices and dubbed next generation sequencing (NGS). One key step in NGS workflows involves preparation of a sequencing-ready library of DNA fragments, but unfortunately this process has been a bottleneck in NGS workflows. The creation of libraries for NGS is a tedious process, which 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 pairing. Pipetting each adapter by hand can lead to errors in creation of libraries with the correct adapters. Many of the processes are timed, and do not have safe stopping points, leading to a very long day. Due to these factors, many labs have found automation of these critical NGS steps to be highly desirable.[1]

Illumina's DNA Prep Library kit is a library preparation method that takes approximately 3.5 hours. Unlike Nextera XT by Illumina, DNA prep is suitable for both small and large genomes. The kit supports a range from 1-500 ng input. During the tagmentation step, a transposon cuts the DNA and inserts a nucleotide fragment on the end of each piece. The piece of DNA added is complementary to a portion of the index adapters that will be added during amplification. This technique is different in Illumina's Nextera XT, in which the tagmentation as well as the amplification processes are done on a bead. The bead-based tagmentation selects fragments that are approximately 300-350 base pairs by limiting the size of fragments that can bind onto the bead. A double-sided cleanup is done post PCR to remove any unwanted fragments. A QC step is performed by running 1 µL on an Agilent Bioanalyzer to determine size distribution. The average size of the library at the end of the protocol should be ~600 bp.[2,3]

In this application note, we have demonstrated the automated preparation on the Biomek NGeniuS system at 10 ng and 500 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 Illumina DNA Prep protocol on Beckman NGeniuS. Red box is the part of the process not done on the NGeniuS system. All blue boxes are done on the Biomek NGeniuS system.

Methods

1. Run Setup

Coriell NA12878 DNA 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 amount (in ng) and dividing by the input volume in µL. This concentration is what the Normalization section (dilute ds DNA sample, in figure 1) diluted sample to. There was a cap of max dilution ratio of 1:100.

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 Illumina DNA Prep 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, 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 1 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.

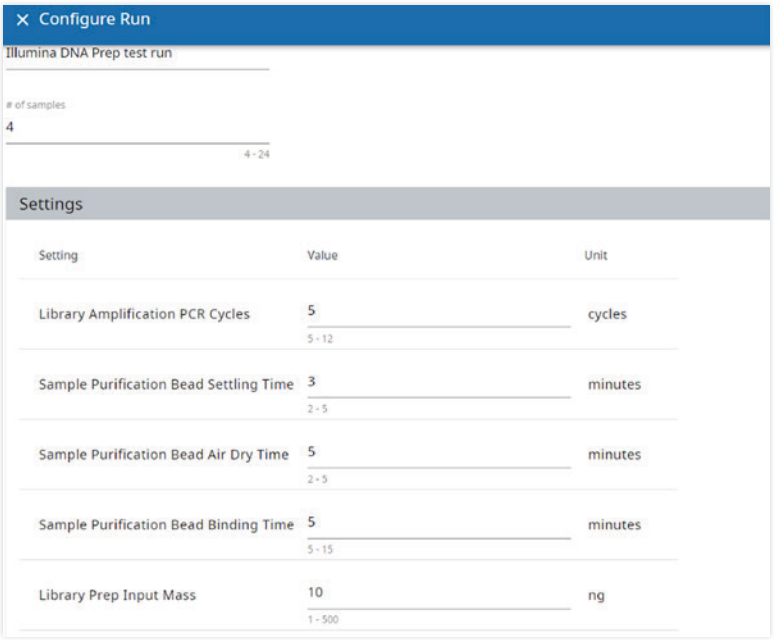

Figure 3. Batch info and app settings for batch run.

Setting	Description
Library Amplification Cycles	The number of cycles of PCR amplification to perform. In Illumina DNA Prep instructions, the number of PCR cycles depends on the amount of input DNA.
Sample Purification Bead Settling Time	The amount of time that the beads are expected to take to settle. The units (minutes) are indicated on the right side. The time to settle may change based on the temperature in the lab. Labs that are very cold may take longer to settle.
Sample Purification Bead Air Dry Time	The default is 5 minutes. This may also change based on environmental conditions like humidity.
Sample Purification Bead Binding Time	Default is set to 5 minutes. It may vary based on environmental conditions.
Library Prep Input Mass	The total number of nanograms to be used for each sample in library preparation.

Table 1. App setting and descriptions of each setting.

The next section of data to be filled out is **Sections** (Figure 4). Illumina DNA Prep has 4 potential sections. Users can select where to start in the process just below the Sections marker in Figure 3. 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** to select Tagment gDNA. A drop-down menu allows the user to select 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.

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. Illumina DNA Prep 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, **Index1**, is the H5xx index to be used. The third column, **Index2**, is the H7xx index to be used. 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 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.

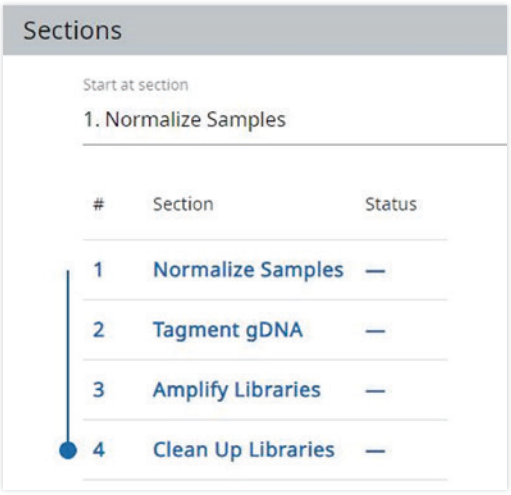

Figure 4. Sections for Illumina DNA Prep Application.

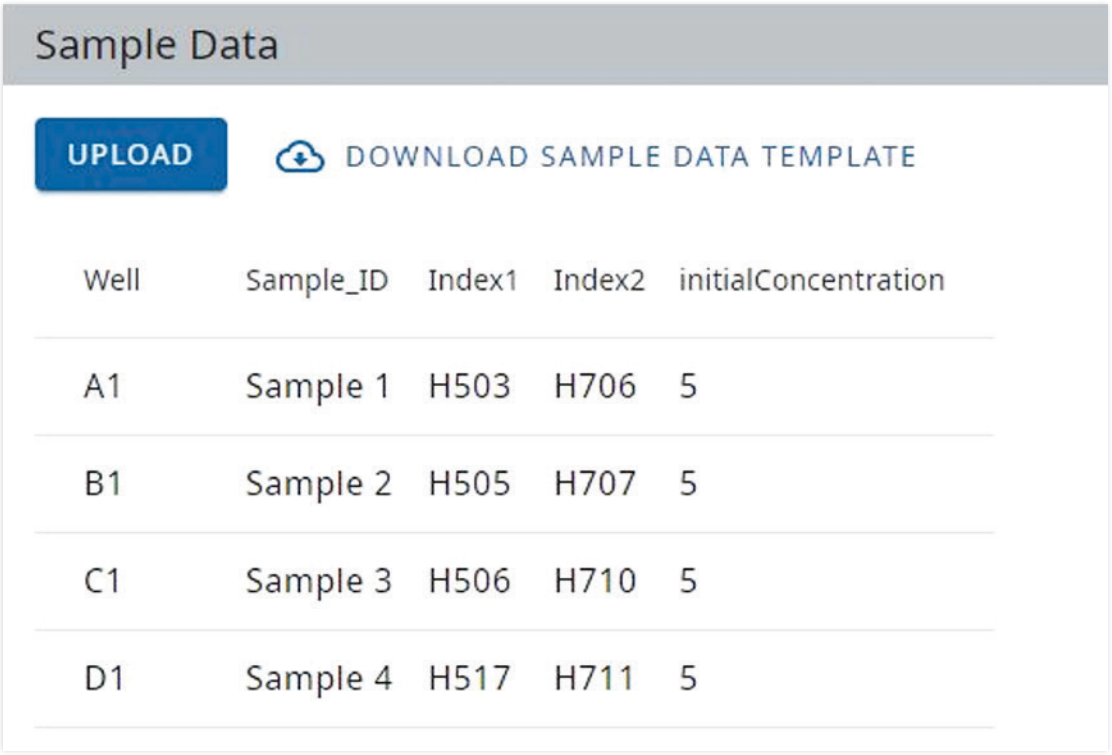

Figure 5. Sample Data information.

2. Library preparation

Several runs were performed on the Biomek NGeniuS system with inputs of 10 ng and 500 ng. Input samples are required to be within 1:100 dilution of the sample input by the system prior to processing. The system processed dilution of samples to a standard concentration to be used for library concentration based on the total ng of input DNA. A variable number of samples were prepared to test various input samples for processing. The variables selected and environmental conditions for processing were as seen in Table 2.

After all reagents had been aliquoted to proper storage locations, the user was instructed to remove reagents, and notified of an estimated time of completion for the library prep based on selections the user inputs at the start of the protocol. The only deviation from the written protocol while processing samples was to use 50 µL of ethanol to wash the beads instead of a full 200 µL per protocol to reduce the time needed to wash beads. After completion of the runs, automated samples were analyzed on an Agilent Bioanalyzer. Thermo Qubit High Sensitivity assay was used to determine sample yields (Table 3). Sequencing was performed on an Illumina MiSeq 2x 151 cycle v2 chemistry kit with a 10 pM load of multiplexed samples (10ng run). Data was analyzed on Illumina BaseSpace. Libraries were analyzed using the BWA Aligner App on BaseSpace (v1.1.4) aligning the reads to the human reference genome (UCSC HG19) default settings.

Sample Input (ng)	Sample Type	PCR cycles	Well	I5 Index	I7 Index	Temp (˚C)	Humidity (%)
500	Coriell NA12878-210 ng/µL	5	A1	H506	H705	23.1	46
500	Coriell NA12878-210 ng/µL	5	B1	H506	H706	23.1	46
500	Coriell NA12878-210 ng/µL	5	C1	H517	H707	23.1	46
500	Coriell NA12878-210 ng/µL	5	D1	H517	H705	23.1	46
500	Coriell NA12878-210 ng/µL	5	E1	H517	H706	23.1	46
10	Coriell NA 12878-5 ng/µL	8	A1	H503	H710	23	49
10	Coriell NA 12878-5 ng/µL	8	B1	H503	H711	23	49
10	Coriell NA 12878-5 ng/µL	8	C1	H503	H714	23	49
10	Coriell NA 12878-5 ng/µL	8	D1	H505	H711	23	49
10	Coriell NA 12878-5 ng/µL	8	E1	H505	H714	23	49
10	Coriell NA 12878-5 ng/µL	8	F1	H506	H710	23	49
10	Coriell NA 12878-5 ng/µL	8	G1	H506	H711	23	49
10	Coriell NA 12878-5 ng/µL	8	H1	H506	H714	23	49
10	Coriell NA 12878-5 ng/µL	8	A2	H517	H710	23	49
10	Coriell NA 12878-5 ng/µL	8	B2	H517	H711	23	49
10	Coriell NA 12878-5 ng/µL	8	C2	H517	H714	23	49

Table 2. Sample information, processing information and environmental conditions for samples processed on the Biomek NGeniuS system.

Results & Discussion

Agilent Bioanalyzer traces were compared to results demonstrated in the manufacturer's instructions.

The Biomek NGeniuS system prepared libraries were in the range of manufacturer's recommendations, indicating successful sample preparation (Figures 6 & 7 are from the 2 different runs on the Biomek NGeniuS system; Typical average library size in the Illumina DNA Prep kit is 600 base pairs using an Agilent Bioanalyzer,[3]). Table 3 indicates the results from the Thermo Qubit High Sensitivity assay.

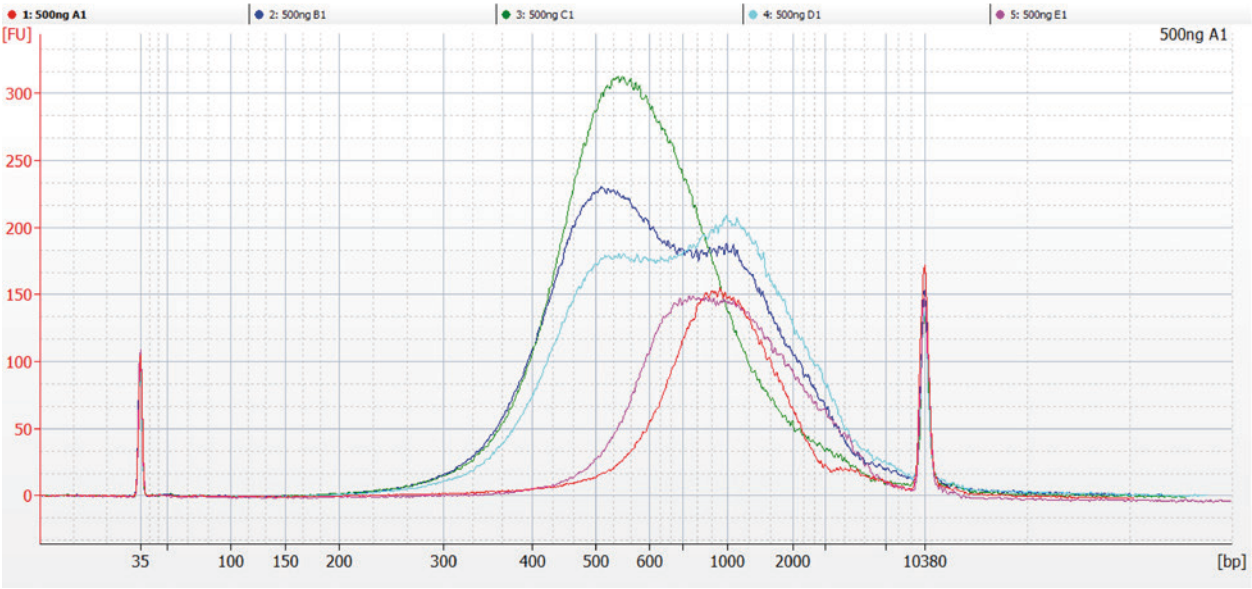

Figure 6. Bioanalyzer traces from Biomek NGeniuS system libraries run post PCR- 500 ng Library Prep Run.

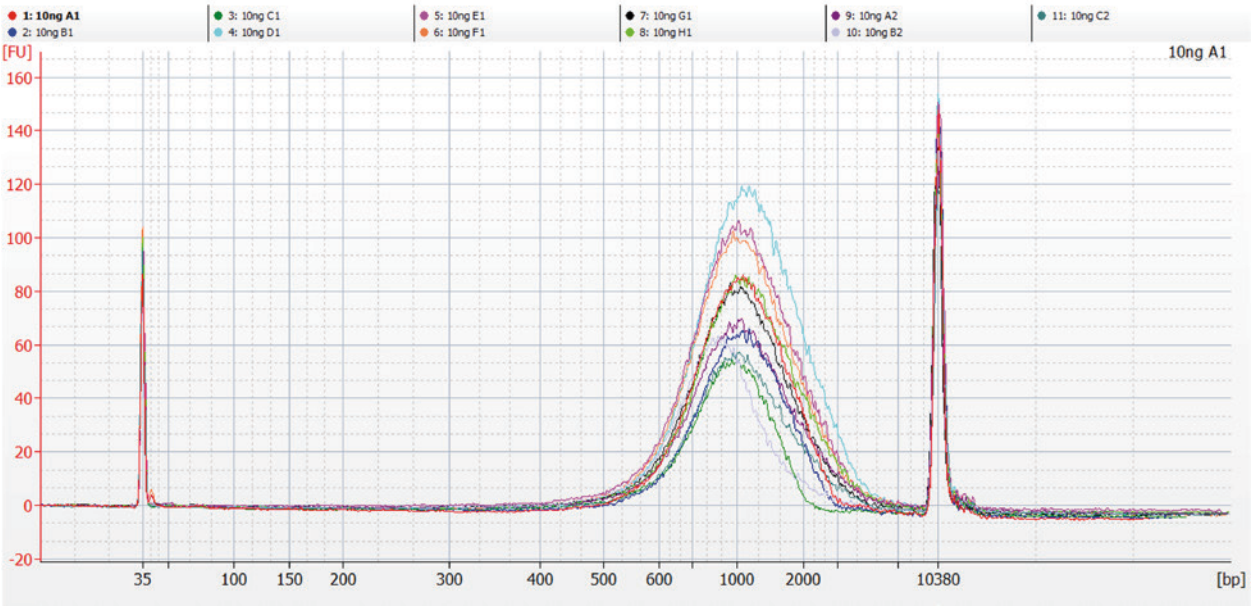

Figure 7. Bioanalyzer traces from Biomek NGeniuS system sample libraries run post PCR 10 ng Library Prep Run.

Library Concentration	Qubit (ng/ul)	Average Size (bp)
10 ng sample A1	0.916	600
10 ng sample B1	0.556	600
10 ng sample C1	0.552	600
10 ng sample D1	1.51	600
10 ng sample E1	1.01	600
10 ng sample F1	1.15	600
10 ng sample G1	0.634	600
10 ng sample H1	0.855	600
10 ng sample A2	0.586	600
10 ng sample B2	0.551	600
10 ng sample C3	0.565	600

Table 3. Qubit yields for library preparations and average size for 10 ng sample input run.

The sequencing run from the Biomek NGeniuS system preparation generated 11,718,608 raw reads with 6,148,202 reads passing filter (52.4%). Q30 scores were greater than 90.12% for a total yield of 965.27 Mb. 94% of pass filter reads were successfully identified, with all 11 libraries represented (Table 4). Successful sequencing is indicated by high percentage alignment and read pairing and low percentage of duplicates (Illumina.com). All libraries had >99% percent aligned reads, >75% properly paired reads and <6% duplicates indicating successful sample prep and sequencing.

Library	Reads	Percent Aligned (%)	Percent Properly Paired (%)	Percent Duplicates (%)
A1	678,258	99.26	83.35	4.98
B1	456,315	99.33	81.97	4.11
C1	413,732	99.21	87.02	4.67
D1	289,839	99.38	76.68	3.77
E1	812,586	99.39	79.55	4.48
F1	779,685	99.41	79.95	5.66
G1	458,716	99.42	81.15	4.95
H1	614,675	99.49	78.57	5.18
A2	526,341	99.38	82.41	4.2
B2	614,967	99.47	83.15	4.02
C2	388,375	99.36	85.67	3.66

Table 4. Sequencing reads, alignment, duplicates, and properly paired % results of BaseSpace analysis of 10 ng library prep.

Summary

Yields, sizes and sequencing data demonstrate that the automation of Illumina DNA Prep library kit using a manufacturing prototype unit of the Biomek NGeniuS system will produce libraries with >99% alignment, low duplicates and >75% properly paired fragments. Further optimizations are taking place to get better mixing in fragmentation to get more consistent fragmentation sizes and to decrease the sample-to-sample variation to improve the consistency of the library preparation.

Materials

Sample	Vendor	Part Number
Human gDNA- NA12878	Coriell	RM8398

Table 6. Sample types and inputs used in preparations of samples for Illumina DNA Prep.

Reagents	Manufacturer	Part Number
Illumina DNA Prep – (M) Tagmentation (96 Samples)	Illumina	20018705
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	Illumina	20027213
Qubit High Sensitivity Kit	Thermo	Q32854
Bioanalyzer High Sensitivity Kit	Agilent	5067-4627
MiSeq 300 cycle v2 sequencing kit	Illumina	MS-102-2002
AMPure XP Beads	Beckman Coulter	A63882
PCR grade Water	Invitrogen-Life Technology	10977-015
Ethanol	American Bio	AB00515-00500

Table 7. Reagents used in preparation of libraries with Illumina DNA Prep kit and sequencing on Illumina sequencer.

Equipment	Manufacturer
NGeniuS Sample Prep	Beckman Coulter Life Sciences
Next Seq Sequencer	Illumina
Allegra X-14 Centrifuge	Beckman Coulter Life Sciences
Qubit	Thermo
BioAnalyzer	Agilent

Table 8. Equipment used in sample preparation and processing of Illumina DNA Prep.

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

Table 9. Consumables required for sample processing.

References

1. Bruinsma S, Burgess J, Schlingman D, Czyz A, Morrell N, Ballenger C, Meinholz H, Brady L, Khanna A, Freeberg L, Jackson RG, Mathonet P, Verity SC, Slatter AF, Golshani R, Grunenwald H, Schroth GP, Gormley NA. Bead-linked transposomes enable a normalization-free workflow for NGS library preparation. BMC Genomics. 2018 Oct 1;19(1):722. doi: 10.1186/s12864-018-5096-9. PMID: 30285621; PMCID: PMC6167868.
2. Illumina DNA Prep Reference Guide Document 10000000025416 V09, June 2020, **https://sapac.support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/illumina_prep/illumina-dna-prep-reference-guide-1000000025416-09.pdf**
3. Illumina Nextera DNA Flex Library Preparation Kit Technical Data Sheet, **https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/nextera-dna-flex-data-sheet-770-2017-011.pdf**

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