BECKMAN COULTER
Life Sciences

Biomek i-Series Automated Illumina® Nextera DNA Flex Library Prep Kit

Introduction

The Illumina Nextera DNA Flex Library Prep Kit is used to prepare up to 96 indexed paired-end libraries for sequencing on Illumina platforms. Nextera DNA Flex accommodates various sample types and has an integrated extraction protocol for whole blood and saliva and also allows for variable DNA input quantities. The simplified workflow utilizes On-Bead Tagmentation and upstream normalization, resulting in reduced operator touch points and faster turnaround time for Whole Genome library preparation.

In this flyer, we demonstrate the automated performance of Illumina Nextera DNA Flex Library Prep Kit on the Biomek i7 Hybrid Genomics Workstation.

In comparison to the manual pipetting, the Illumina Nextera DNA Flex Library Prep Kit automated on Biomek platform provides:

- Reduced hands-on time and increased throughput

- Reduction in potential pipetting errors

- Standardized workflow for improved results

- Quick implementation with ready-to-implement methods

- Knowledgeable support

Spotlight: Biomek i7 Dual Hybrid Genomics Workstation

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

- 300uL or 1200uL Multichannel head with 1-300uL and 1-1200uL 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)
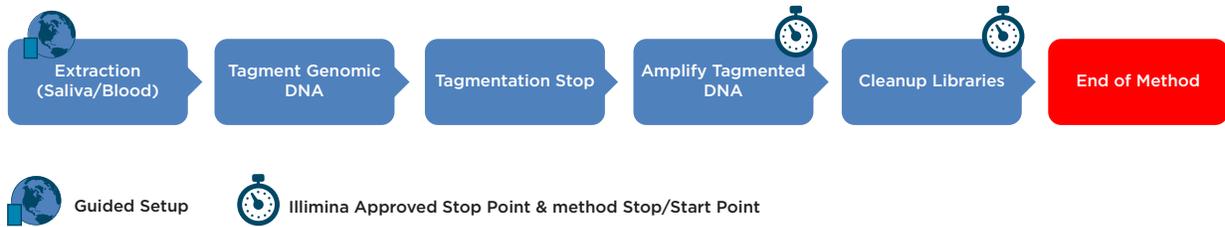
Figure 1. Illumina Nextera DNA Flex Library Prep Kit workflow.

Automated Method

Automation provides increased efficiency, reducing the hands-on time (Table 1). The complete method can be run with full walk away capability but does include logical start and stop points assigned based on Illumina's recommendations, providing flexibility to the users in scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput.

Major Process Description	Time		
	24 Samples	48 Samples	96 Samples
INSTRUMENT SETUP TIME	10 min	15 min	30 min
EXTRACTION (BLOOD OR SALIVA)			
Method Run Time	19 min	21 min	25 min
PRE -PCR			
Method Run Time (Automatic Index transfer from Plate)	44 min	55 min	1 hr 16 min
POST -PCR			
Method Run Time	43 min	45 min	50 min
Total	2 hrs 56 min	3 hrs 16 min	4 hrs 1 min

*Timings estimates include Thermocycling but do not include reagent thawing
*Method can be run with full walk-away capability after initial deck setup

Table 1. Estimated run times for Illumina Nextera DNA Flex Library Prep Kit on the Biomek i7 Hybrid Genomics Workstation for plate based index transfer. Application is designed to also perform extraction from blood or saliva samples.

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. The manual control options provide the opportunity to interact with the instrument, if needed.

Figure 2. Biomek Method Launcher provides an easy 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.
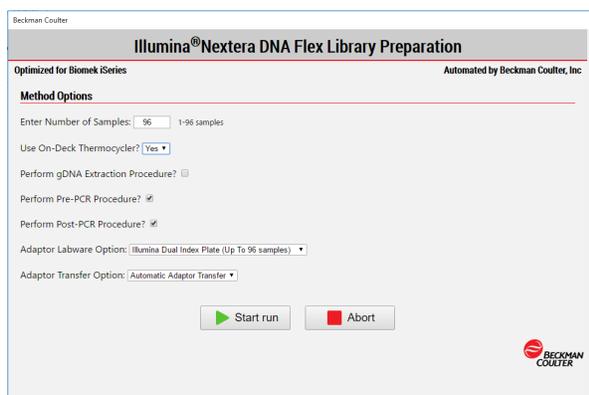

Figure 3. Biomek Method Options Selector enables us to select sample number and processing options. Extraction can be done on deck, if desired.

Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

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.

Experimental Design

Eight technical replicates of 200ng of Coriell NA12878 DNA were used to generate libraries with Nextera DNA Flex Library preparation kit. The quantity of the original sample was assessed with Qubit ds HS Kit. Fragments distribution was assessed with Agilent Technologies 2100 Bioanalyzer. Equal volumes (5 µl per sample) of 8 Nextera DNA Flex libraries were pooled together without additional normalization. The library pool was sequenced on HiSeqX sequencing platform. After paired end 2x151 bp run, sequencing reads were trimmed to 380 million. Data analysis was performed using the BaseSpace Sequence Hub Whole Genome Sequencing v5.0 App.

Results

A robust and reliable automation solution for NGS library prep is essential to take full advantage of Illumina's sequencing technology. Automation saves up valuable time and money and helps the researchers to obtain an efficient process with more walk-away time.
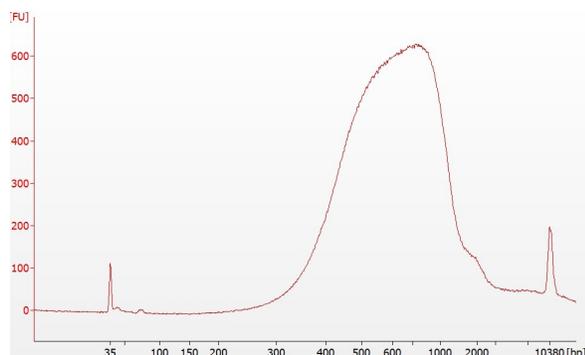

Figure 5. Example of Nextera DNA Flex library distribution on Agilent Technologies 2100 Bioanalyzer High Sensitivity DNA Chip. The 8-plex library median is within expected 650bp +/-50bp range. The library pool yielded 13.8 ng/uL as measured by Qubit dsDNA HS Assay Kit.
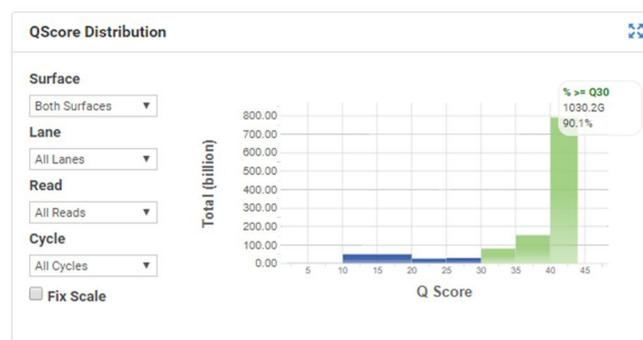

Figure 6. Q Score distribution for the Nextera DNA Flex HiSeqX run.

METRICS		MANUAL PREPARATION NA12878	BIOMEK I7 SALIVA	BIOMEK I7 NA12878
Yield (Qubit), ng/µl	Average	13.2	14.2	13.8
	CV	5.1%	4.2%	3.9%
Index CV		10.8%	8.13%	13.6%
Median insert size	Average	352bp	373bp	363bp
	CV	2%	2.3%	1.6%
Autosome mean coverage	Average	31.5x	30.7x	31.5x
	CV	1.0%	2.1%	1.2%
Coverage across the human genome at 15X	Average	97.6%	97.6%	97.7%
	CV	0.0%	0.1%	0.3%
Coverage across exonic regions at 10X	Average	99.6%	99.7%	99.7%
	CV	0.0%	0.0%	0.0%
Autosome callability	Average	95.0%	95.3%	95.2%
	CV	0.1%	0.1%	0.1%
Duplicate aligned reads	Average	8.4%	8.4%	8.1%
	CV	5.0%	6.0%	7.0%
Aligned reads		95.0%	92%	95.4%

Figure 7. Comparison of manual and automated Nextera DNA Flex performance. CV- coefficient of variation. Lower percentage of aligned reads for saliva sample is due to presence of bacterial reads in tested material.

Summary

We've demonstrated automation of Nextera DNA Flex Library preparation kit on the Biomek i7 Hybrid Genomics Workstation delivers libraries that yield quality results. The solution is fully walk-away and scalable thus providing fast and efficient library construction. In addition to the library prep, the optional extraction from saliva or blood is also part of the protocol.