Automated DNA Library Construction Using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® on the Beckman Coulter Biomek FX^P Automated Liquid Handler

Abstract/Introduction

The ability to construct DNA sequencing (DNASeq) libraries from a wide range of starting input masses is essential for many next generation sequencing (NGS) applications. The New England Biolabs® NEBNext Ultra DNA Library Preparation Kit for Illumina (Catalog # E3730) provides users with the ability to construct indexed DNASeq libraries from inputs ranging from 5 ng to 1 ug of starting DNA. In this technical note, we describe the automation of the New England Biolabs NEBNext Ultra DNA Library Preparation Kit for Illumina on the Beckman Coulter Biomek FX^P Dual-Arm Multichannel 96 and Span-8 automated liquid handler (Biomek FX^P). The automation method allows the user to prepare up to 96 individually indexed DNASeq libraries in approximately 4-1/2 hours. An intuitive HTML-driven user interface allows the user to specify the number of samples to process (up to 96 samples), and which size selection option to be used. The user interface also provides users with the option to utilize either off-deck incubations using an external thermocycler, or to perform incubations on-deck with a Biometra T-Robot thermocycler integrated to the Biomek FX^P liquid handler. To help simplify and reduce errors during system setup, an HTML-driven reagent calculator is presented to the user with information on the required reagents, their respective volumes, and their location on the instrument deck based on the user's input regarding number of samples and steps to be run. The method also incorporates all recommended stop points described in the NEBNext Ultra DNA Library Preparation Kit for Illumina protocol, allowing users the maximum amount of flexibility in planning their experiments.

Method Overview and Hardware Description

This automation method was developed on the Biomek FX^P liquid handler, equipped with low volume tubing and 1 ml syringes. The deck configuration of the instrument

Biomek FX^P Workstation

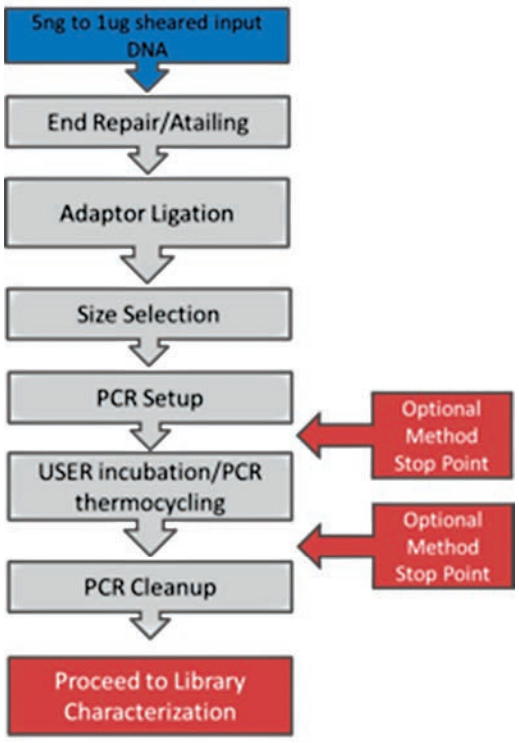

Fig. 1. NEBNext Ultra DNA automated method workflow.

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Method Overview and Hardware Description

This automation method was developed on the Biomek FX^{P} liquid handler, equipped with low volume tubing and 1 ml syringes. The deck configuration of the instrument is displayed in Figure 2. A list of automated labware position (ALP) hardware is presented in Table 1. The method employs individual Span-8 probes to deliver enzyme and reagent transfers while DNA cleanup, wash, and elution transfers are performed using the multichannel 96 pipetting head. A static Peltier unit ensures that enzyme master mixes are kept cool during the course of the method. For on-deck incubations, a Biometra T-Robot integration is required, otherwise the method prompts

Fig. 2. NEBNext Ultra DNA automated deck layout with integrated Biometra T-Robot.

the user to remove the plate from the deck and perform the incubation in an off-deck thermocycler. Filter tips are employed throughout the method to reduce the possibility of instrument or sample contamination. A list of automation consumables and user-supplied reagents can be found in Tables 2 and 3, respectively.

The automation method follows NEBNext Ultra DNA Library Preparation Kit for Illumina protocol with a few modifications. Sheared gDNA is added to a 96-well plate by the user, along with the master mixes and reagents as directed by the reagent calculator. The NEBNext adapter is kept separate from the Ligation Master Mix to inhibit the formation of adapter dimers prior to the adapter ligation step. Another important modification to the NEBNext Ultra DNA Library Preparation Kit for Illumina protocol involves the USER enzyme. The USER enzyme has been integrated into the PCR[1] master mix, requiring a 15-minute, 37°C incubation added to the beginning of the PCR program outlined in the NEBNext Ultra DNA Library Preparation Kit for Illumina protocol. This reduces the number of tips required by the protocol in addition to offering time savings overall. Lastly, the automation method employs Beckman Coulter's SPRIselect reagent in place of AMPure XP to provide for more consistent size selection. The automation workflow is presented in Figure 1.

The automated NEBNext DNA Ultra method utilizes an HTML-driven User Interface (UI) that allows the user to customize their workflow by offering a number of different options. Some major options offered by the UI include the following:

1. Selecting any number of samples to process, between 1 and 96.
2. Selecting which procedures of the library construction workflow to run.
3. Selecting from an extensive list of size selection options to be used in the library construction

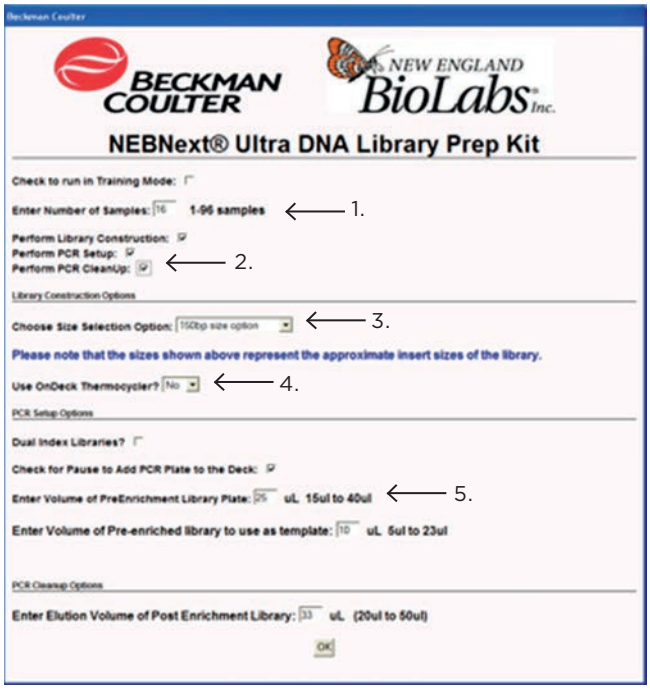

Fig. 3. NEBNext Ultra DNA user interface.

procedure (150 bp, 200 bp, 250 bp, 300–400 bp, 400–500 bp, 500–700 bp inserts or no size selection at all).

4. Choosing to conduct on-deck or off-deck incubations.
5. User-defined transfer volumes for various steps to further increase the flexibility of the method.

An image of the user interface is presented in Figure 3.

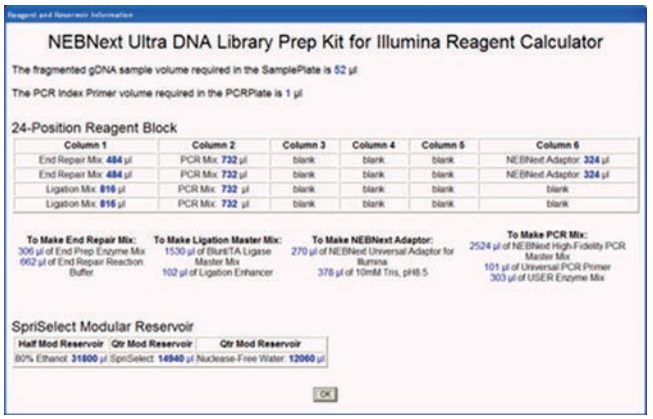

Fig. 4. NEBNext Ultra DNA reagent calculator.

In addition to the user interface, the automated NEBNext Ultra DNA method provides the user with an HTML-driven reagent calculator that provides the user with the final volumes of all of the reagents and master mixes required on the deck, as well as instructions on how to generate the various master mixes—based upon the number of samples to be processed and the amount of the workflow that the user wishes to pursue. An image of the reagent calculator is presented in Figure 4.

Results

Row/Column	1	2	3
A	*E.coli (S1)*	*H. sapiens*	bacteria
B	*A. thaliana*	*E.coli (S10)*	bacteria
C	*A. thaliana*	*A. thaliana*	bacteria
D	*A. thaliana*	*H. sapiens ChIP*	*E.coli (S20)*
E	*A. thaliana*	*H. sapiens*	bacteria
F	*E.coli (S6)*	*H. sapiens*	*C.elegans*
G	*A. thaliana*	*E.coli (S15)*	*C.elegans*
H	*A. thaliana*	*H. sapiens ChIP*	*E.coli (S24)*

Fig. 5. Automated library construction plate layout.

Sixteen genomic DNA (gDNA) samples from *A. thaliana* and *H. sapiens*, 2 *C. elegans* amplicon pools, 2 *H. sapiens* ChIP DNA, and 4 bacterial gDNA samples were supplied by various laboratories at Indiana University, Bloomington, for Biomek automated library construction at Indiana University. In addition, *E. coli* K12 gDNA was supplied by New England Biolabs as a positive control. The 200 ng aliquots for each of the gDNA samples were sheared to an average size of 400 bp (data not shown) and arrayed in the sample plate as shown in Figure 5.

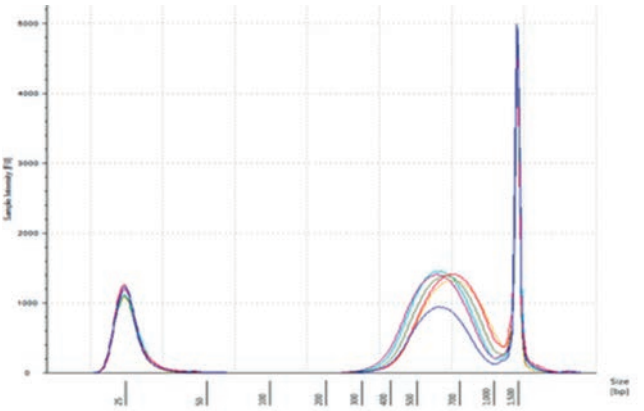

Fig. 6. *E. coli* K12 gDNA control libraries created with the NEBNext Ultra DNA automated method.

Libraries were constructed using the NEBNext Ultra DNA automation method utilizing the 400–500 bp insert size selection option. Eight cycles of PCR enrichment were used to amplify the libraries using an off-deck thermocycler. Following analysis on the Agilent 2200 TapeStation, all of the libraries processed were then sequenced on an Illumina MiSeq® using a 2×300 cycle paired end run. For the purposes of this document, we concentrated our analysis on the six *E. coli* K12 control libraries, which were assayed on the Agilent 2200 TapeStation using D1000 ScreenTape (PN# 5067-5582). The electropherograms for all 6 *E. coli* K12 control libraries are presented in Figure 6.

Data analysis was performed at New England Biolabs using a local instance of Galaxy. For each of the 6 *E. coli* K12 control libraries, over 1.4 million pass filter reads for each library were generated by the MiSeq run. Pass filter read counts are presented in Figure 7. Reads were then trimmed using SeqPrep prior to mapping back to

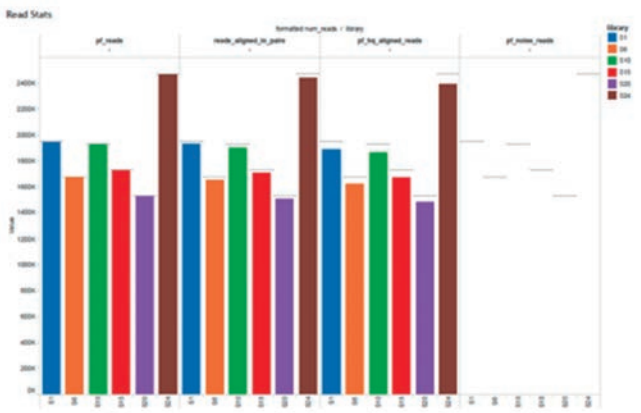

Fig. 7. *E. coli* K12 gDNA control library pass filter read counts.

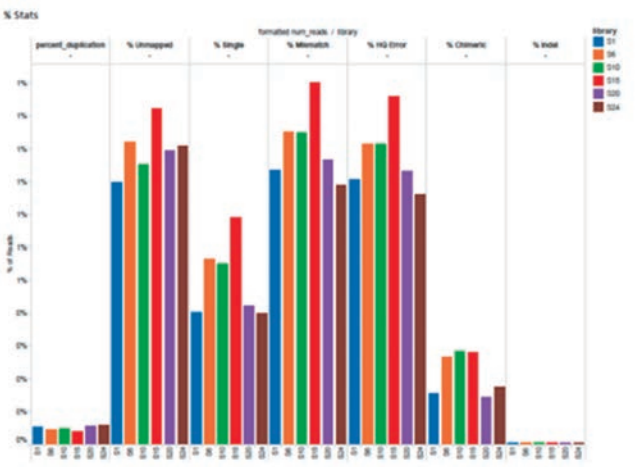

Fig. 8. *E. coli* K12 gDNA control library quality metrics.

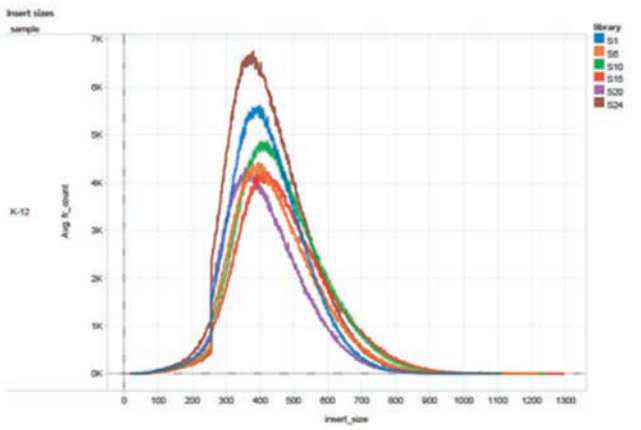

Fig. 9. *E. coli* K12 gDNA control library insert size distributions.

the *E. coli* K12 MG1655 reference genome using Bowtie (version 2.1.10). Less than 1% of the reads from the *E. coli* K12 control libraries failed to map back to the *E. coli* K12 MG1655 reference genome. We also observed low percentages of chimeric reads, PCR duplicates, and mismatched reads. These metrics indicate that the libraries were high-quality libraries. Data on basic library quality metrics are presented in Figure 8. Additionally, we investigated the average size of the library inserts of the *E. coli* K12 control libraries utilizing the library mapping data. As shown in Figure 9, the average size inserts of the *E. coli* K12 control libraries was approximately 400 bp.

	E.coli Control Libraries					
Organism	S1	S6	S10	S15	S20	S24
A. thaliana	0.03%	0.07%	0.04%	0.00%	0.00%	0.00%
C.elegans	0.03%	0.01%	0.01%	0.01%	0.09%	0.01%

Fig. 10. Cross-contamination analysis of *E. coli* K12 gDNA control libraries.

E. coli K12 control library reads were also mapped back to the *A. thaliana* (TAIR10) and *C. elegans* (ce10) reference genomes using Bowtie to check for the presence of contaminating sequences. As shown in Figure 10, the percentages of reads in the *E. coli* K12 control libraries that mapped back to the *A. thaliana* and *C. elegans* reference genomes were generally quite low, and reveal no particular pattern when the physical layout of the samples is considered. These results show that the libraries derived from the automated NEBNext Ultra DNA method on the Biomek FX^P show no discernible evidence of cross-contamination during library construction.

Table 1. Biomek FX^P Dual-Arm Multichannel 96 and Span-8 Configuration[2]

Part Number	Qty.	Manufacturer	Description
719948	1	Beckman Coulter, Inc.	ALP, High-Density, 12-Position, 4 x 3
379448	1	Beckman Coulter, Inc.	ALP, Shaking, Orbital, Single-Position
719357	3	Beckman Coulter, Inc.	ALP, Standard Single-Position
719361	1	Beckman Coulter, Inc.	ALP, Cooling/Heating, Single-Position
719590	1	Beckman Coulter, Inc.	Waste, Span-8, ALP
719356	1	Beckman Coulter, Inc.	Disposable Tip Loader ALP
719654	1	Beckman Coulter, Inc.	ALP, Tip Wash, 8-Channel
719363	1	Beckman Coulter, Inc.	Wash Station including Pump and Tubes
719366	1	Beckman Coulter, Inc.	Biomek FX^P Device Controller
Contact Beckman Coulter, Inc.	1	Biometra	(*Optional*) Biometra T-Robot for On-Deck Incubations

Table 2. Automation Consumables Required.

Part Number	Qty.	Manufacturer	Description
B01124	1	Beckman Coulter, Inc.	Biomek Span-8 P1000 Tips, Pre-Sterile with Barrier
379503	1	Beckman Coulter, Inc.	Biomek Span-8 P250 Tips, Pre-Sterile with Barrier
A21586	3	Beckman Coulter, Inc.	Biomek P50 Tips, Pre-Sterile with Barrier
717253	1	Beckman Coulter, Inc.	Biomek AP96 P250 Tips, Pre-Sterile with Barrier
372790	2	Beckman Coulter, Inc.	Quarter Reservoir
534681	1	Beckman Coulter, Inc.	Half Reservoir, Nonpyrogenic
C5064	1	Acme-Automation	Reactor Adapter 96 Flat*
372795	1	Beckman Coulter, Inc.	Frame for Reservoirs*
A32782	1	Beckman Coulter, Inc.	Agencourt SPRIPlate 96R—Ring Super Magnet Plate*
A83054	1	Beckman Coulter, Inc.	Blue Heater/Chiller 24-Well Block*
AB-1127	3	Thermo Scientific	ABgene 96-Well Storage Plate, Square Well, 1.2 mL
16466-042	10	VWR	2 mL SuperClear™ Screw Cap Microcentrifuge Tubes—Conical Bottom
HSP-9641	5	Bio-Rad	Hard-Shell® Thin-Wall 96-Well Skirted PCR Plates
MSL-2022	1	Bio-Rad	Arched Auto-Sealing Lids**

* One-time purchase.
** For on-deck thermocycling only.

Table 3. User-Supplied Reagents.

Part Number	Manufacturer	Description
N/A	User-Preferred	Elution Buffer Nuclease-Free Water, TE, 10 mM Tris, pH 8.5
B23318	Beckman Coulter, Inc.	SPRIselect Reagent Kit
AB00138-01000	American Bioanalytical	Ethanol
E7370S (24 rxns) or E7420L (96 rxns)	New England Biolabs	NEBNext Ultra DNA Library Prep Kit for Illumina
E7335L (Set 1, 96 rxns) or E7500L (Set 2, 96 rxns) or E7600	New England Biolabs	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1 or 2) or NEBNext Multiplex Oligos for Illumina Dual-Index Primers Set 1

Software used in QC Testing

Bowtie (version 2.1.10) installed on New England Biolabs Galaxy instance.

SeqPrep (version 1.0) installed on New England Biolabs Galaxy instance.

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References

1. The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.

2. Contact a Beckman Coulter sales consultant for a system quotation at www.beckmancoulter.com.

Galaxy Citations

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