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Life Sciences

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

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Introduction and Method Description

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 II DNA Library Preparation Kit for Illumina (Catalog Number E7645) provides users with the ability to construct high quality indexed DNASeq libraries from inputs ranging from 500 pg to 1 ug of starting DNA from sample inputs that include genomic DNA (gDNA), formalin fixed, paraffin embedded DNA (FFPE DNA) and chromatin immunoprecipitated DNA (ChIP DNA).

In this technical note, we describe the automation of the New England Biolabs NEBNext Ultra II DNA Library Preparation Kit for Illumina on the Beckman Coulter Biomek FX^P Dual Arm Multi-channel 96 and Span-8 automated liquid handler (Biomek FX^P) (Figure 1). The automation method utilizes an intuitive HTML-driven user interface (UI) which allows the user to specify the number of samples to be processed (1-96). The UI allows the user to select between seven size selection options and different indexing strategies including single and dual index systems. To increase the flexibility of the method, the user may enter a dilution factor for the NEBNext adaptor as appropriate for the input concentration of the user's samples. In addition to these and other smaller features, the UI also allows the user to choose between off-deck incubations using an external thermocycler or to perform incubations on-deck with a Biometra TRobot thermocycler integrated to the Biomek FX^P liquid handler for maximized walk-away time (Figure 2). The method also incorporates numerous stopping points through the workflow offering flexibility in the experimental planning (Figure 3). 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 on number of samples and steps to be run. The automation method allows the user to prepare up to 96 individually indexed DNASeq libraries in approximately four hours (Figure 4).

Figure 1. Beckman Coulter Biomek FX^P

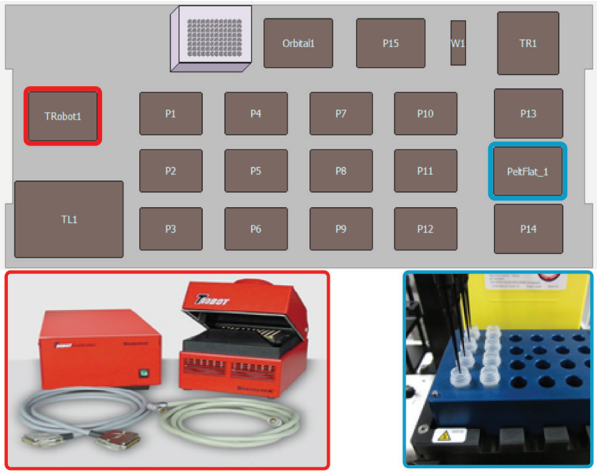

Figure 2. Biomek FX^P deck configuration (top). Biometra TRobot (bottom left) offers fully automated enzymatic incubations and thermocycling while the Static Peltier unit (bottom right) provides chilled enzyme master mix storage during the course of the run.

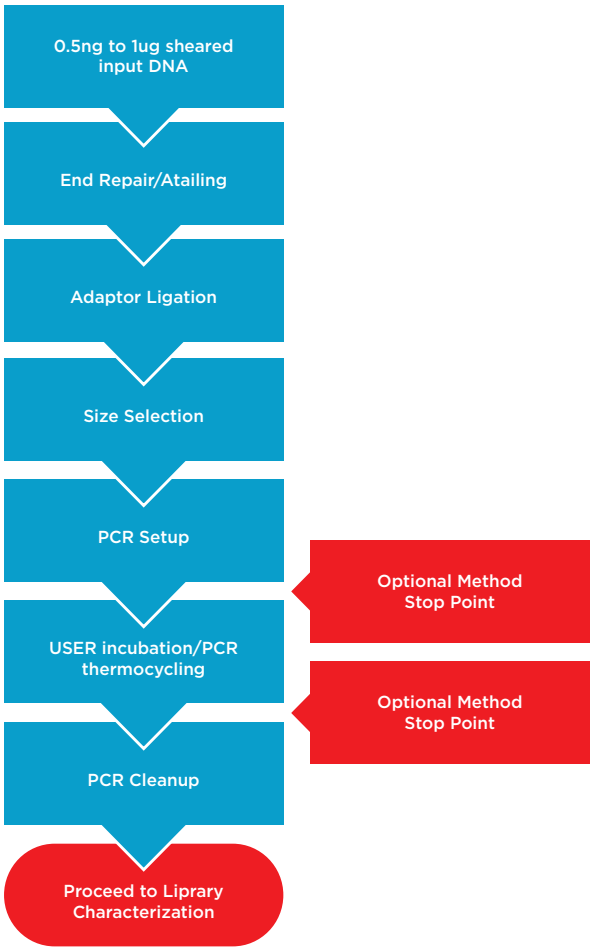

Figure 3. NEBNext Ultra II DNA workflow automated on the Biomek FX^P.

Major Process Description	Automated/Hands on Time		
	24 Samples	48 Samples	96 Samples
Library Construction: Part 1			
Method Run	2 hr, 27 min	2 hr, 36 min	3 hr, 3 min
PCR Setup: Part 2			
Method Run	0 hr, 8 min	0 hr, 12 min	0 hr, 16 min
Post PCR Cleanup: Part 3			
Method Run	0 hr, 33 min	0 hr, 36 min	0 hr, 43 min
Total Run Time	**3 hr, 7 min**	**3 hr, 23 min**	**4 hr, 1 min**

**Timing does not include thawing of reagents or thermocycling

Figure 4. Biomek FX^P NEBNext Ultra II DNA method time estimates.

Experimental Design

Genomic DNA samples from three species of chili peppers (*Capsicum annuum, Capsicum baccatum,* and *Capsicum chinense*) were obtained from Dr. Padma Nimmakayala at West Virginia State University. These samples, in addition to Universal Human Reference DNA (Promega), were quantified using PicoGreen (Life Technologies) in conjunction with the SpectraMax i3 (Molecular Devices). 2 ug of pepper gDNA and 4 ug of human gDNA were resuspended in 10 mM Tris buffer and sheared to 400bp using a Covaris S220 instrument with the manufacturer's recommended settings. For the first automated method run, three 500 ng technical replicates of each of the pepper species and seven 500 ng human technical replicates were used as templates for library construction. This automation run utilized a 1:2 dilution of the NEBNext Adaptor, size selection for 400-500bp inserts and five cycles of PCR amplification. In the second automation run, three 20 ng technical replicates and three 0.5 ng technical replicates of each of the pepper species, seven 20 ng human technical replicates and seven 0.5 ng human technical replicates were used as templates for library construction. The second automation run utilized a 1:25 dilution of the NEBNext adaptor, no size selection, and 15 cycles of PCR amplification. Libraries were multiplexed during PCR using the NEBNext Multiplex Oligos for Illumina (96 Index Primers, catalog number #E6609). The resulting libraries from both automation runs were grouped onto a single plate for Post-PCR cleanup. The clean libraries were then assayed on the 2200 TapeStation (Agilent) using DNA High Sensitivity ScreenTape. Automation libraries were delivered to New England Biolabs for sequencing. Following quantification on the 2100 Bioanalyzer (Agilent), a library pool of all 48 libraries was created and sequenced on the Illumina NextSeq 500 using a 2x150bp high output run. A cluster density of 248K/mm^2 was achieved, 87.7% of which passed filter. Of the estimated 174.2 Gb of sequence produced, 143.2 Gb (82%) were high quality (Q30 or higher).

Results

Library yield and size distribution

Library size distribution was consistent between replicates. Low input libraries (20 ng and 0.5 ng) have a broader size distribution compared to 500 ng inputs, as the lower input libraries were not size selected. Library yield for 500 ng libraries was lower than the yield obtained manually, possibly due to greater sample loss during size selection. The 20 ng library yields were very high, most likely due to using higher than recommended number of PCR cycles to be able to process 20 ng and 0.5 ng inputs in one 96-well plate (Figure 5).

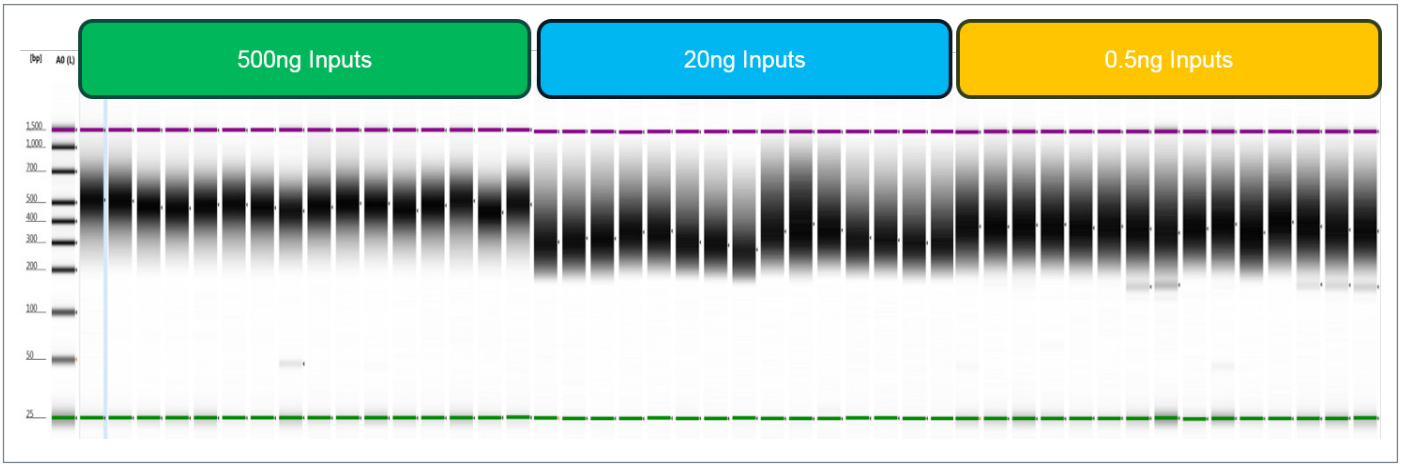

Figure 5. Library yield and size distribution. NEBNext Ultra II DNA libraries produced using the Biomek FX^P automated method.

Sequencing Metrics

Automated NEBNext Ultra II DNA libraries generated high quality sequencing data (high percentage of aligned reads, low percentage of adaptor-dimer), comparable to manual libraries.

Human gDNA Input (ng)	Total Reads (PE 2X150bp)	% Reads Aligned	% Reads Aligned in Pairs	Mismatch Rate	Error Rate	Indel Rate	% Chimeras	% Adapter
500	462,742,899	89.80	97.61	0.0141	0.0077	0.0008	2.08	2.14
20	595,106,746	74.82	96.44	0.0247	0.0134	0.0020	1.48	0.03
0.5	509,436,880	77.63	97.03	0.019834	0.0103	0.00177	1.42	1.57

Figure 6. Sequencing metrics. Human libraries were sequenced to 10X coverage on the Illumina NextSeq®500 instrument. Reads were mapped to the GRCh37 reference using Bowtie 2.1.0.

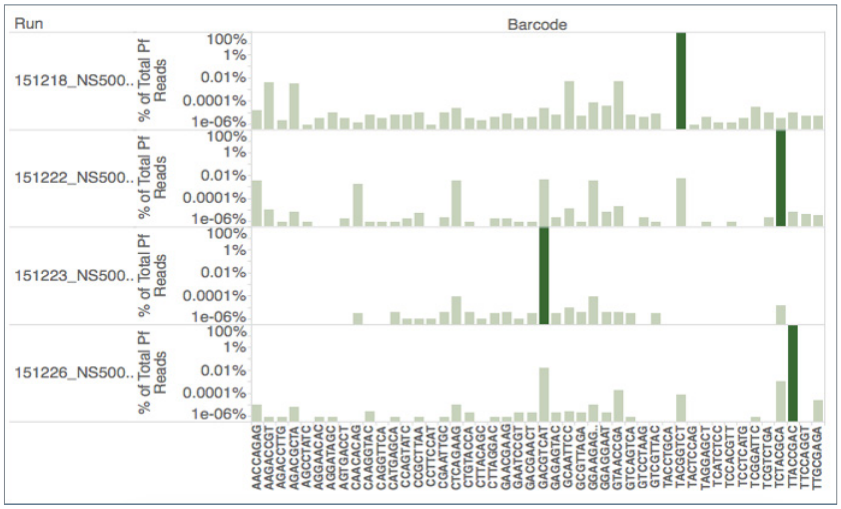

Figure7. Cross Contamination analysis.

Cross Contamination

Four libraries were selected from a set of 48 simultaneously prepared libraries. Each was individually sequenced on a NextSeq 500 (~ 100M reads each). Each library was assessed using Picard's Extract Illumina Barcodes with strict settings (0 mismatches allowed) and input files containing all 48 barcodes used during the automated library prep run. No significant contamination was observed (<0.02%) (Figure 7).

Conclusion

We have demonstrated that high quality, sequence-ready, DNASeq libraries are generated from the using the Biomek FX^{P} automated method in conjunction with the NEBNext Ultra II DNA Library Kit for Illumina, even with very low input amounts.

References

1. The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.
2. Contact Beckman Coulter sales consultant for a system quotation at **www.beckman.com**

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at **beckman.com**

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