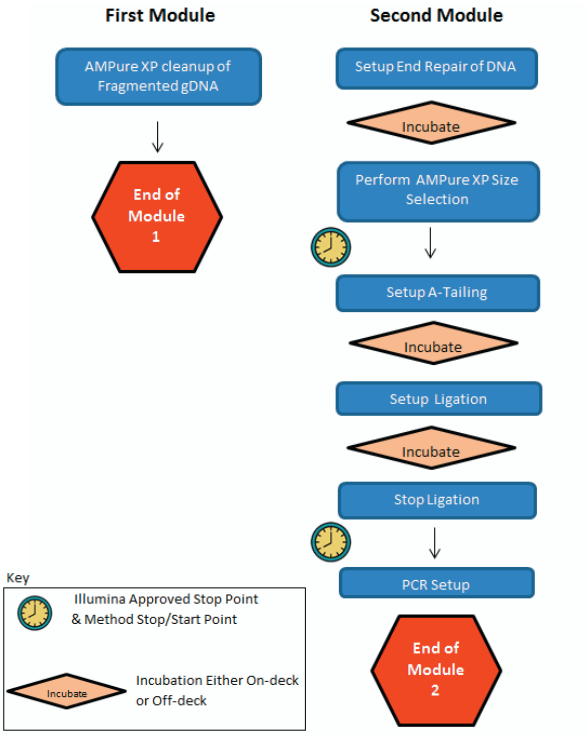
High-Throughput Illumina® TruSeq® Nano DNA Library Construction on the Biomek FX^P Dual-Arm Multi-96 and Span-8 Workstation

Abstract

The manual preparation of large numbers of sequencing libraries, which can be labor-intensive and time-consuming, is a bottleneck for many laboratories. Manual methods also carry a higher risk of human error and inconsistency. The Biomek FX^P Dual-Arm Multi-96 and Span-8 Workstation (BFX^P) puts every aspect of liquid handling required for automation of NGS sample preparation—including optimized pipetting for reagents and samples, cooling, shaking and thermocycler integration to maintain protocol-defined environmental conditions–into a single, automated system, while limiting manual handling of any potentially hazardous chemicals. It has the capability to consistently provide high-quality template libraries at a throughput needed to take advantage of the high capacity of NGS while providing a system flexible enough to meet a user's changing needs.

The Illumina TruSeq Nano DNA kit is a DNA library prep system for low sample inputs that provides a streamlined workflow that includes Solid Phase Reversible Immobilization (SPRI) bead-based size selection. This technical note describes the automation of the Illumina TruSeq Nano DNA kit on the Biomek FX^P for interrogation of low-input samples down to 100 ng. One to 96 samples can be run simultaneously, and setup requires only a few clicks of the mouse and 1-time pipetting of the required kit components into the reservoirs. The pipetting tools and software improve the ease and speed of reaction setup. The automated method consists of two modules: one for SPRI-based cleanup to purify sheared samples; and the other to prepare libraries all the way through PCR[1] enrichment. The end result is as many as 96 sequence-ready libraries from as little as 100 ng of DNA. The resulting high-quality libraries display consistent insert sizes, minimal adapter dimers, and low percent PCR duplication to provide efficient interrogation of samples with limited DNA. All of this was accomplished with minimal hands-on time (see Table 1).

Biomek FX^P T-Robot SPRIWorks



Workflow of the TruSeq Nano DNA method, highlighting the steps of the protocol and the Illumina-approved start and stop points built into the user interface and method.

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Table 1. Major Process Description.

Automated/Hands-On Time			
	24 Samples	48 Samples	96 Samples
Shear Cleanup			
Prepare Reagants/ Set Up Inst.	15 min	15 min	15 min
Method Run	40 min	43 min	49 min
Total	55 min	58 min	1 hrs 4 min
Library Construction			
Prepare Reagants/ Set Up Inst.	25 min	27 min	30 min
Method Run	5 hrs 43 min	6 hrs 4 min	6 hrs 36 min
Total	6 hrs 8 min	6 hrs 31 min	7 hrs 6 min

Timing does not include thawing of reagents and PCR thermocycling.

Method Details

Setting Up Library Construction

Setup for the automated library construction requires making a few selections in the Biomek FX^P user interface (Figures 1 and 2). The automated method is designed to closely follow the Illumina TruSeq Nano DNA protocol while maintaining the flexibility that Biomek software provides (see workflow on previous page). Built into the user interface are all the Illumina-approved start and stop points to allow the user: (1) to select which steps of the process to perform; (2) configure how many samples from 1 to 96 to prepare; and (3) how to handle incubations. Users are also given multiple options on how to configure adapters. The user interface enables users to select the adapter labware from a list that includes the Illumina DNA adapter plate (DAP), Illumina DNA adapter tubes, or a custom plate as defined by the user. Users may choose which adapters to use with which samples by selecting their own transfer files or by using the default setup. Setting up the PCR reaction to enrich for fragments with the proper adapters is also optional and allows for the volume of sample to change based on user needs (Figure 2).

Once all of the method parameters have been entered, the Biomek FX^P software provides a reagent calculator that tells the users which reagents are required, what volume of each is necessary, as well as how to prepare the reagents, when necessary (Figure 3). The volume required is updated based on the number of samples selected in order to minimize reagent loss.

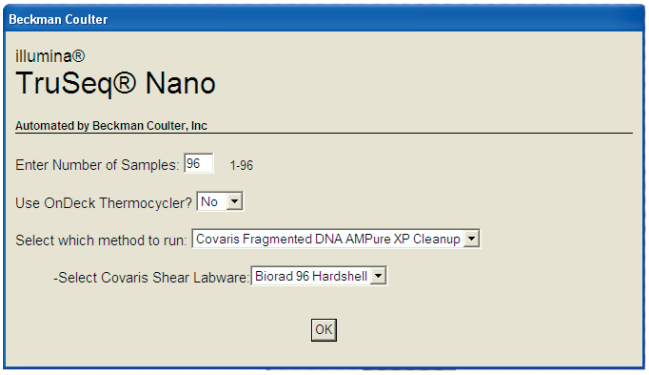

Fig. 1. User interface showing Shear cleanup option.

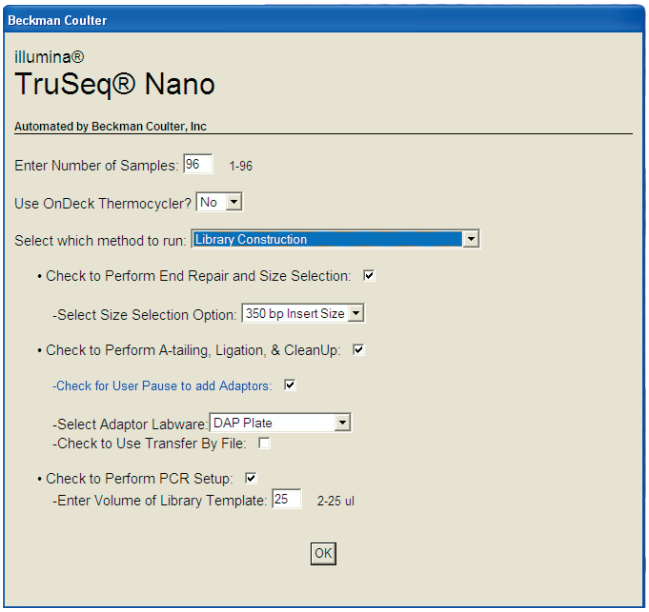

Fig. 2. User interface showing library construction options.

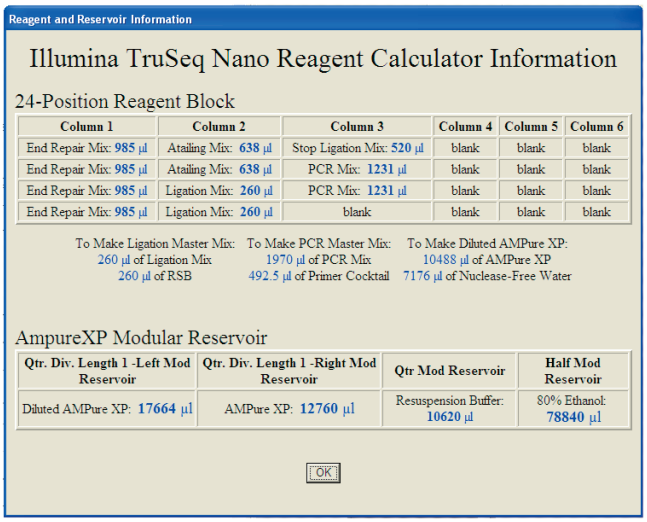

Fig. 3. Library construction reagent calculator.

Results

Sample Layout and Results

Genomic DNA from *E. coli* was sheared using a Covaris® protocol for 550 bp insert size. Twelve replicates of the sheared gDNA were distributed in a 96-well plate (as shown in Figure 4) and purified in a starting volume of 50 µl and an input concentration of 200 ng in a 96-well plate using AMPure XP.

	1	2	3	4
A	Rep 1	Rep 9		
B	Rep 2	Rep 10		
C	Rep 3	Rep 11		
D	Rep 4	Rep 12		
E	Rep 5			
F	Rep 6			
G	Rep 7			
H	Rep 8			

Fig. 4. Sample plate map for 12 samples run.

The cleaned gDNA were processed by selecting the options in the user interface for the Illumina adapter tubes and the 550 bp selection, along with off-deck incubation. For PCR enrichment, 20 µl of sample was used. The samples were purified and quantified using the AMPure XP and Kapa Biosystems Library Quant qPCR Setup method. To check the quality of the libraries, a 1:100 dilution of the first 11 libraries was checked using Agilent Bioanalyzer 2100 for size distribution. Figure 5 displays consistent library size and distribution of the 11 libraries and no detectable adapter dimer peak at ~120 bp. Amplification of the libraries resulted in an average yield of 269 nM resulting in more than enough library to process for sequencing (Figure 6).

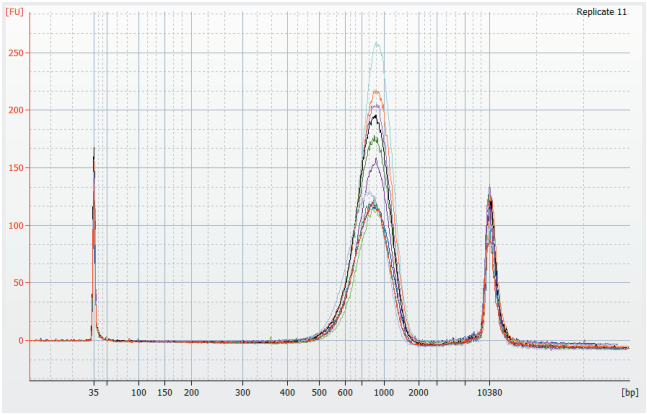

Fig. 5. Agilent Bioanalyzer 2100 trace of 1:100 dilutions of the prepped libraries.

	Source (Library) Plate Concentration				nM
	1	2	3	4	5
A	255.8	203.5	NA	NA	NA
B	284.7	298.3	NA	NA	NA
C	299.7	339	NA	NA	NA
D	289.9	325.5	NA	NA	NA
E	225.7		NA	NA	NA
F	278.9		NA	NA	NA
G	216.4		NA	NA	NA
H	216.1		NA	NA	NA

Fig. 6. Kapa qPCR quantitation results shown in nM concentration.

Table 2. Library-Quality Statistics Generated by the BaseSpace® Resequencing Analysis Pipeline (basespace.illumina.com) Set to Default Parameters.

Library	% Aligned Read 1	Read 1 Q30	% Aligned Read 2	Read 2 Q30	Frag Length Median	St.Dev Frag length	% Duplicate
Rep 1	88.43%	98.06%	87.86%	94.37%	567	144	0.2%
Rep 2	88.66%	98.04%	88.29%	94.82%	583	148	0.2%
Rep 3	88.61%	97.95%	88.17%	93.96%	616	154	0.2%
Rep 4	88.29%	98.02%	88%	94.84%	598	147	0.2%
Rep 5	89.04%	98.02%	88.75%	94.87%	594	148	0.2%
Rep 6	89.25%	98.02%	88.94%	94.97%	594	146	0.2%
Rep 7	89.08%	98%	88.79%	94.79%	597	149	0.2%
Rep 8	89.04%	97.99%	88.50%	94.18%	603	150	0.2%
Rep 9	89.14%	98.10%	88.60%	94.14%	554	142	0.2%
Rep 10	89.03%	98%	88.72%	94.90%	590	144	0.2%
Rep 11	89.20%	97.97%	88.77%	94.57%	593	148	0.2%
Rep 12	89.20%	98.02%	88.85%	94.81%	579	144	0.2%

As shown in Table 2, the libraries produced with the TruSeq Nano method are highly consistent with one another in terms of percent alignments to the reference genome and quality scores. Median fragment lengths (593 bp) are also highly reproducible between replicates. Percent PCR Duplicates—a key quality metric that measures the amount of artifacts produced via PCR—was quite low for the libraries built using the BFX.[P] On balance, these metrics show that the libraries produced are high-quality and sequenceable.

Conclusion

Reliable and efficient automation solutions for NGS library construction are essential in order to take full advantage of Illumina's powerful Next Generation Sequencing technology. Biomek Automation standardizes the process, reducing the potential for human error, which saves time and reagent cost and gives researchers valuable, worry-free, walk-away time. The Biomek FX[P] automated method is capable of creating up to 96 TruSeq Nano DNA libraries ready for PCR thermocycling in just over 8 hours from low-input samples.

Author

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Reagents Used		
Part Number	**Manufacturer**	**Description**
N/A	User-Preferred	Elution Buffer Nuclease-Free Water, TE Buffer
A63882	Beckman Coulter, Inc.	AMPure XP
A66514	Beckman Coulter, Inc.	RNAClean XP
AB00128-01000	American Bioanalytical	Ethanol
FC-121-4003	Illumina	TruSeq Nano DNA HT kit
FC-121-4002	Illumina	TruSeq Nano DNA LT kit
AM6050	Life Technologies	Human Reference Brain RNA
740000	Agilent Technology	Universal Human Reference RNA
KK4835	Kapa Biosystems	Library Quantification Kit—Illumina/ABI
14380	USB® Corporation	Stock *E. coli* gDNA

Consumables Used			
Part Number	**Manufacturer**	**Description**	**Qty for 96-Sample Run**
717253	Beckman Coulter	Biomek AP96 P250 Tips, Pre-Sterile with Barrier (case of 10 racks)	2 Tip Boxes
379503	Beckman Coulter	Biomek Span-8 P250 Tips, Pre-Sterile with Barrier (case of 10 racks)	2 Tip Boxes
A21586	Beckman Coulter	Biomek P50 Tips, Pre-Sterile with Barrier (case of 10 racks)	8 Tip Boxes
717256	Beckman Coulter	Biomek AP96 P20 Tips, Pre-Sterile with Barrier (case of 10 racks)	2 Tip Boxes
B01124	Beckman Coulter	Biomek Span-8 P1000 Tips, Pre-Sterile with Barrier, 1025 µl (case of 5 racks)	1 Tip Box
372790	Beckman Coulter	Quarter Reservoir (case of 48)	3 Reservoirs
372788	Beckman Coulter	Quarter Reservoir, Divided by Length (case of 48)	2 Reservoirs
372786	Beckman Coulter	Half Modular Reservoir	1 Reservoir
372795	Beckman Coulter	Reservoir Frame* (1 each)	1 Frame
A32782	Beckman Coulter	Agencourt SPRIPlate 96R—Ring Super Magnet Plate*	1 Magnet
A83054	Beckman Coulter	Blue Heater/Chiller 24-Well Block*	1 Tube Block
373661	Beckman Coulter	24-Position Black Microfuge Tube Rack**	1 Tube Rack
373696	Beckman Coulter	Insert, Tube, 11 mm, White, for 1.5 mL Microfuge Tubes** (case of 25)	24 Inserts
16466-042	VWR	2 mL SuperClear® Screw Cap Microcentrifuge Tubes	19 Tubes
AB-1127	Thermo Scientific	ABgene 96-Well Plate, Square Well, 1.2 mL	4 Plates
HSP-9641	Bio-Rad	Hard-Shell® Thin-Wall 96-Well Skirted PCR Plate	11 Plates
MSL-2022	Bio-Rad	Arched Auto-Sealing Lids***	1 Lid
MSP-1003	Bio-Rad	Microseal 'P' Replacement Pads***	2 Pads
4312063	Applied Biosystems®	MicroAmp® Splash-Free 96-Well Base**	1 Base

* One-time purchase.
** Dependent on adapter labware options.
*** Required for on-deck thermocycling.

Equipment Used		
Part Number	**Manufacturer**	**Description**
G2940CA	Agilent Technology	Agilent 2100 Bioanalyzer
5067-4626	Agilent Technology	High-Sensitivity DNA Kit

Biomek FX^{P} Dual Arm Multi-96 and Span-8 Workstation Configuration Used[2]		
Part Number	**Manufacturer**	**Description**
719654	Beckman Coulter	ALP, Tip Wash, 8-Channel
719363	Beckman Coulter	96-Well Wash Station
379448	Beckman Coulter	ALP, Shaking, Orbital, Single-Position, Biomek
719590	Beckman Coulter	Waste, Span-8, ALP
719357	Beckman Coulter	ALP, Standard Single-Position
719361	Beckman Coulter	ALP, Cooling/Heating, Single-Position, Biomek
719948	Beckman Coulter	ALP, High-Density, 12-Position, 4x3
719366	Beckman Coulter	Biomek Device Controller
Request a Quote	Beckman Coulter	Biometra T-Robot Integration

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.

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