Automation of the Illumina Nextera® Rapid Capture Enrichment on the Biomek FX^P Dual Arm Multi-96 and Span-8 Workstation

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Abstract

As Next Generation Sequencing (NGS) costs decline, researchers and clinicians have embarked on experiments of increasing size and sophistication. The manual preparation of large numbers of sequencing libraries needed by these experiments represents a major bottleneck for many researchers. The Biomek FX^P Dual Arm Multi-96 and Span-8 Workstation (BiomekFX^P) puts every aspect of liquid handling required for automation of NGS sample preparation – including optimized pipetting for reagents and samples, cooling, shaking and thermo cycler integration to maintain protocol-defined environmental conditions – into a single, automated system capable of consistently providing high quality sequencing libraries for variety of NGS applications.

The Illumina Nextera Rapid Capture Enrichment Kits allows for the rapid sequencing over a reduced portion of the human genome including coding exons only (Exome), or all exons, untranslated regions and miRNAs (Expanded Exome). Briefly, Nextera Rapid Capture Enrichment Kits use an engineered transposon to fragment and tagment input DNA in a single reaction. After tagmentation, the PCR reaction amplifies the insert DNA while adding the i5/i7 indexes to enable dual-indexed sequencing and multiplexing of up to 96 samples. Following tagmentation, two rounds of hybridization with oligo baits corresponding to the targeted regions are used the remove non-target sequences from the library pool. A final round of amplification completes the enrichment process.

This application note describes the automation of the Nextera Rapid Capture Enrichment protocol on the Biomek FX^P automated liquid handler. The automation method can process up to 96 samples simultaneously into high quality libraries with consistent insert sizes in a single run lasting less than nine hours (Table 1) from as little as 50ng of input genomic DNA for each sample.

Automation Method

The Biomek FX^P Nextera Rapid Capture Enrichment automated method closely follows the Illumina manual protocol presented in Nextera® Rapid Capture Enrichment Guide (P/N FC-140-9001DOC). The automation method consists of five modules controlled by a single HTML-driven User Interface (UI). Module 1 performs the tagmentation, indexing PCR, and PCR cleanup to generate a Nextera library for each sample (up to 96). Module 2 allows the user to pool the libraries using a variety of strategies depending on the desired complexity of the pools and the number of libraries. Module 3 performs the setup for the first hybridization, which then runs for at least 90 minutes but can be allowed to run overnight. This process hybridizes the library fragments to the biotinylated oligo baits corresponding to the regions of interest. Module 4 then captures the targeted library fragments using streptavidin beads while the non-targeted regions are washed away. Following elution from the streptavidin beads, the first enrichment products are then subjected to a second round of hybridization setup in Module 4. Finally, Module 5 performs the second round of hybridization capture and the second PCR amplification of the enriched library pools. The automated method workflow is presented in Figure 1.

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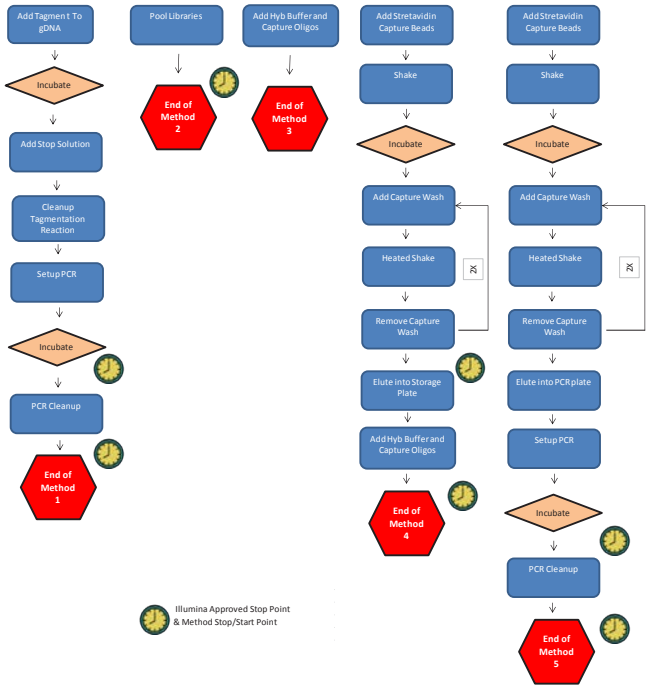

Figure 1: Biomek FX^P Nextera Rapid Capture Enrichment workflow.

Major Process Description	Automated/ Hands on Time		
	24 Samples	48 Samples	96 Samples
Tagmentation Through 1st PCR Setup: Method 1			
Prepare Reagents/Set up Inst	15 min	15 min	15 min
Method Run	1 hr, 32 min	1 hr, 44 min	2 hr, 8 min
Total	**1 hr, 47 min**	**1 hr, 59 min**	**2 hr, 23 min**
Pooling: Method 2	**24 Samples**	**48 Samples**	**96 Samples**
Prepare Reagents/Set up Inst	5 min	5 min	5 min
Method Run	0 hr, 2 min	0 hr, 3 min	0 hr, 5 min
Total	**0 hr, 7 min**	**0 hr, 8 min**	**0 hr, 10 min**
1st Hyb Setup: Method 3	**6 samples**	**12 samples**	**24 samples**
Prepare Reagents/Set up Inst	15 min	15 min	15 min
Method Run	0 hr, 5 min	0 hr, 8 min	0 hr, 13 min
Total	**0 hr, 20 min**	**0 hr, 23 min**	**0 hr, 28 min**
1st Capture Through 2nd Hyb Setup: Method 4	**6 samples**	**12 samples**	**24 samples**
Prepare Reagents/Set up Inst	15 min	15 min	15 min
Method Run	1 hr, 52 min	1 hr, 58 min	2 hr, 7 min
Total	**2 hr, 7 min**	**2 hr, 13 min**	**2 hr, 22 min**
2nd Capture Through 2nd PCR Cleanup: Method 5	**6 samples**	**12 samples**	**24 samples**
Prepare Reagents/Set up Inst	15 min	15 min	15 min
Method Run	2 hr, 52 min	2 hr, 59 min	3 hr, 8 min
Total	**3 hr, 7 min**	**3 hr, 14 min**	**3 hr, 23 min**
Complete Method	**7 hr, 25 min**	**7 hr, 54 min**	**8 hr, 43 min**

**Timing does not include thawing of reagents or thermocycling

Table 1: Biomek FX^P Nextera Rapid Capture Enrichment time estimates based on number of samples processed.

The Biomek FX^P Nextera Rapid Capture Enrichment automated method utilizes an HTML-driven User Interface (UI) with a wide array of options, some of which are module specific and others that are more general. Some general options include the selection of which module to perform, which types of devices being used for incubation steps (on-deck peltiers for incubations combined with off-deck thermocycling for PCR or off-deck thermocycling for both incubations and PCR), selection of labware to be used for PCR and master mix deployment, and the number of samples/pools to be processed. Module 1 (Tagmentation through 1st PCR Cleanup), allows the user to select how much of the tagmentation process to perform, as well as specifying how the i5/i7 index primers are to be deployed (either on deck in tube format or pre-aliquoted by the user in a 96-well plate). Module 2 (Pooling) allows the user to specify how the libraries are to be pooled (by column, by row, or with a custom pooling strategy specified by a .csv formatted text file), the pool complexity, and the volume of each library to add to the pool(s). Module 3 (1st Hybridization Setup) allows the user to specify the labware to be used for hybridization (96 well plate or tube rack). An image of the UI is shown in Figure 2.

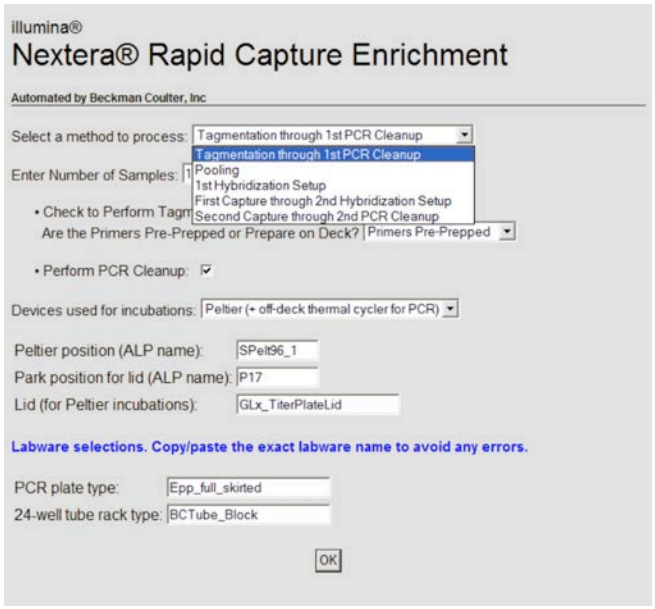

Figure 2: Biomek FX^P Nextera Rapid Capture Enrichment UI.

In addition to the user interface, the automated Illumina PCR Free 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 3.

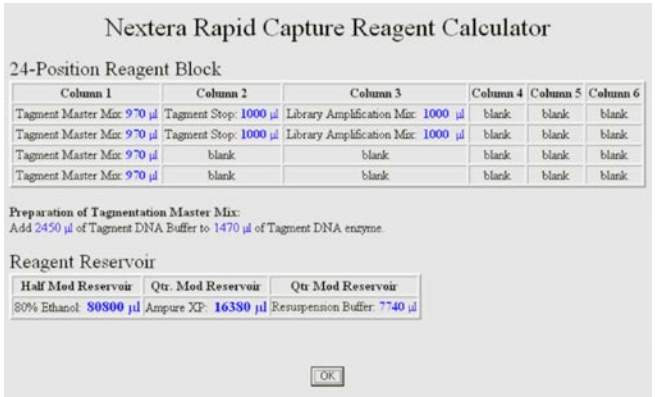

Figure 3: Biomek FX^P Nextera Rapid Capture Enrichment Reagent Calculator

Experiment Design and Results

Human Genomic DNA (Promega) was quantified using Quant-iT PicoGreen (Life Technologies). Eighty-four 50ng technical replicates and four 70ng technical replicates were arrayed in a 96 well plate for library construction using the automated method. The plate layout is shown in Figure 4. Following library construction but prior to pooling and enrichment 15 of the technical replicate libraries were assayed on the Agilent 2200 TapeStation using a D1000 High Sensitivity ScreenTape (Figure 5).

Column/Row	1	2	3	4	5	6	7	8	9	10	11
A	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng
B	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	70ng	70ng
C	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng
D	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng
E	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng
F	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng
G	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	70ng	70ng
H	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng	50ng

Figure 4: Nextera Rapid Capture plate layout.

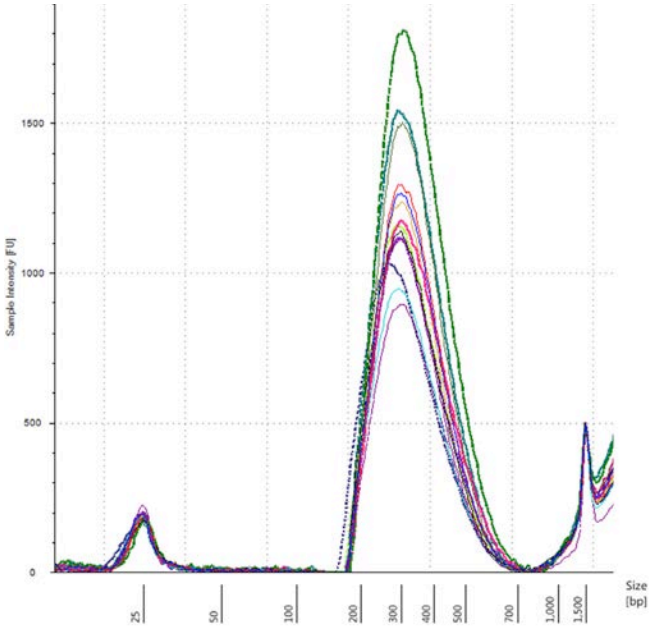

Figure 5: Nextera Rapid Capture Automated Libraries.

Libraries were quantified using Quant-iT PicoGreen (Life Technologies) and 500ng from each library in a row was added to a pool for a total of eight pools. Library pools were concentrated using Amicon Ultra-0.5 centrifugal filter units per the Illumina protocol and then placed back on the deck for two cycles of hybridization and capture using the automated method. Following the second amplification cleanup, the library pools were quantified using Quant-iT PicoGreen (Life Technologies), the results of which are shown below in Table 2.

Pool	Average Concentration (ng/ul)	Concentration (nM)
1	52.23	197.84
2	44.99	170.41
3	38.90	147.36
4	37.15	140.73
5	29.42	111.44
6	31.67	119.97
7	36.58	138.57
8	38.97	147.62

Table 2: Library pool yields from the Nextera Rapid Capture automated method.

Library Pools 2 and 8 were each sequenced on the Illumina MiSeq using MiSeq v2 300 cycle kits, which generated 11.7 million pass filter reads and 11.6 pass filter reads respectively. For both runs, over 93% of pass filter reads were successfully identified. Sequencing data was analyzed using the Illumina BaseSpace BWA Enrichment v1.0 application[1] with the hg19 human reference genome. An average of 97.8% of the reads from each of the 22 individual libraries from both pools aligned with the reference genome, for an average of 214,064,683 for each library. When using the analysis default of 150bp padding for each target region, an average padded base enrichment of 75.6% is achieved. Figure 6 displays total aligned bases, padded target aligned bases, and padded base enrichment for each of the 22 individual libraries.

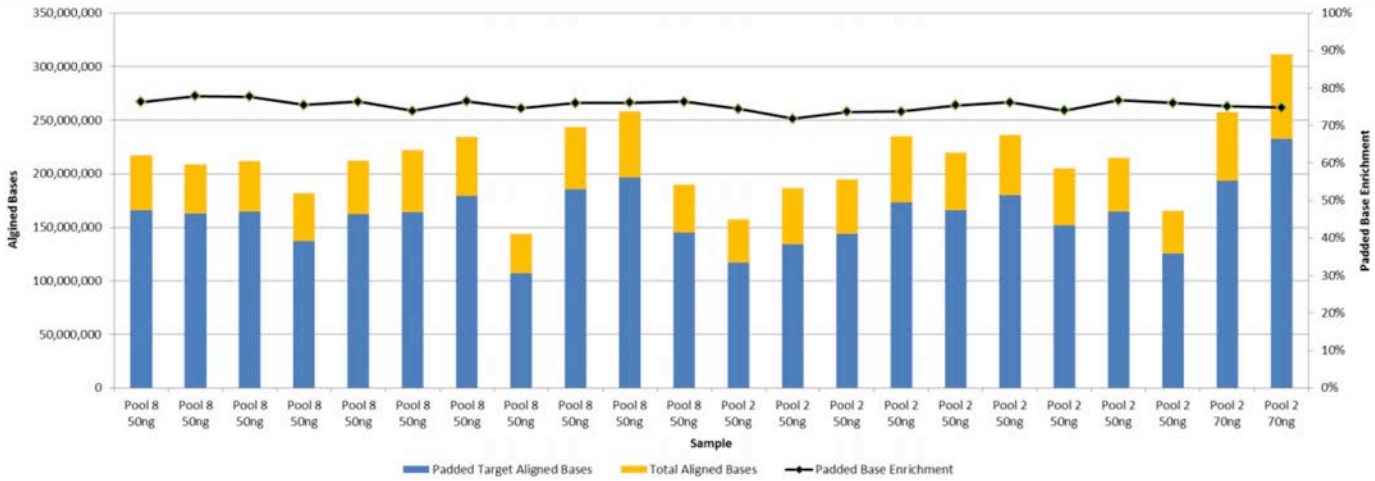

Figure 6: Base alignment and enrichment for 22 sequenced sample libraries.

Coverage across the targeted regions in each of the 22 sequenced sample libraries was very similar, with an average of 2.9X coverage achieved from the two MiSeq runs. Figure 7 shows the mean region coverage depth and uniformity of coverage across all targeted regions for each of the 22 libraries sequenced.

Conclusion

With the continued adoption of exome sequencing for a variety of applications in research and in the clinic, the demand for consistent, time efficient exome library preparation will increase in demand. The Biomek FX^P Nextera Rapid Capture Enrichment automated method provides a robust solution for the creation of Nextera Rapid Capture Enrichment libraries that display at high degree of coverage uniformity and base enrichment for the genomic regions targeted.

Software Used

1. BaseSpace: basespace.illumina.com

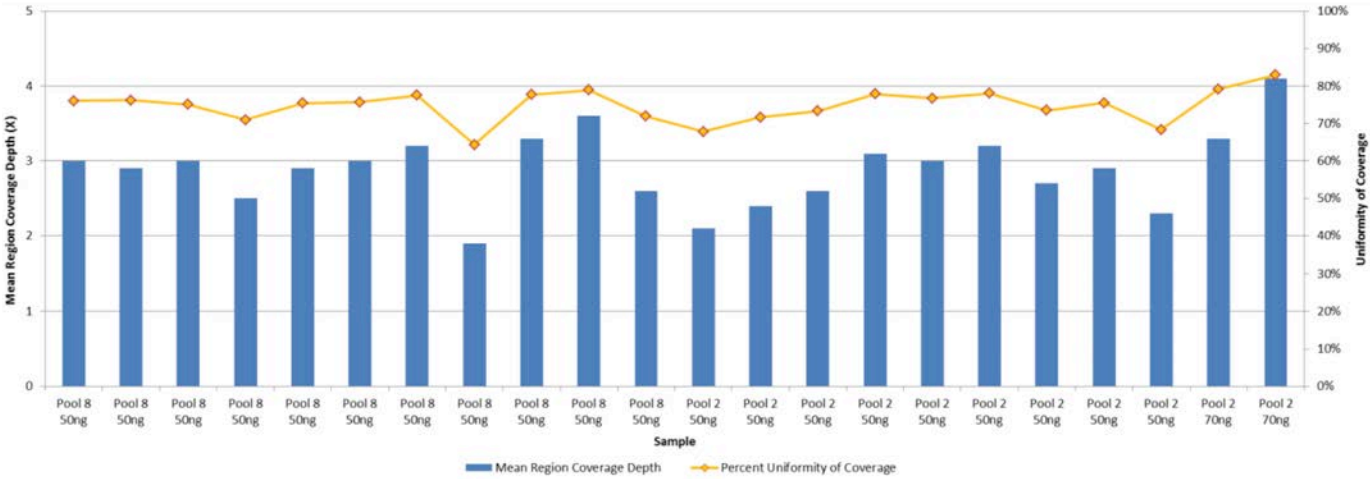

Figure 7: Region coverage depth and uniformity for 22 sequenced sample libraries.

Reagents Used

MANUFACTURER	DESCRIPTION	PART NUMBER
User Preferred	Elution Buffer- Nuclease Free water, TE Buffer	N/A
Beckman Coulter	AMPure XP	A63881
American Bioanalytical	Ethanol	AB00515-00500
Illumina	Nextera Rapid Capture Exome	FC-140-1003

Equipment Used

PART NUMBER	MANUFACTURER	DESCRIPTION
G2964AA	Agilent Technology	Agilent 2200 TapeStation
5067-5584	Agilent Technology	High Sensitivity D1000 ScreenTape Kit

Consumables Used

MANUFACTURER	DESCRIPTION	MANUFACTURER PART #	TOTAL FOR 96 SAMPLE RUN
Beckman	Biomek Span-8 P1000 Tips, Pre-sterile with Barrier	B01124	3
Beckman	Biomek Span-8 P250 Tips, Pre-sterile with Barrier	379503	5
Beckman	Biomek P50 Tips, Pre-sterile arrier	A21586	12
Beckman	Biomek AP96 P250 Tips, Pre-sterile with Barrier	717253	8
Beckman	Quarter Reservoir	372790	4
Beckman	Reservoir, Half	534681	2
Beckman	Quarter Reservoir, Divided by Length	372788	2
Fisher Scientific	Abgene 96-Well Storage Plate, Square Well, 1.2 mL	AB-1127	9
VWR	2mL SuperClear Screw Cap Microcentrifuge Tubes-Conical Bottom	16466-042	27
Eppendorf	Twin.Tec PCR Plate 96, skirted, clear	951020401	9
Beckman*	Frame for Reservoirs	372795	2
Beckman*	Agencourt SPRIPlate 96R - Ring Super Magnet Plate	A32782	1
Beckman*	BCI Tube Block	A83054	1
Beckman*/ Millipore	24-Position Tube Rack (Beckman) with Amicon Ultra-0.5 filter unit (Millipore)	373661/UFC503008	1
Beckman*	BCI Tube Block	A83054	1
Beckman*	BCI Round Bottom Adaptor-Peltier	A49568	1

* denotes one time purchase

Biomek Configuration Used

PART NUMBER	QTY	MANUFACTURER	DESCRIPTION
719948	1	Beckman Coulter, Inc.	ALP, High-Density, 12-Position, 4x3
719357	5	Beckman Coulter, Inc.	ALP, Standard Single-Position
379448	1	Beckman Coulter, Inc.	ALP, Shaking, Orbital, Single-Position
A93938	1	Beckman Coulter, Inc.	Static Peltier ALP
A93942	1	Beckman Coulter, Inc.	Shaking Peltier ALP
719654	1	Beckman Coulter, Inc.	Span-8 Wash ALP
719590	1	Beckman Coulter, Inc.	Waste, Span-8, ALP
719363	1	Beckman Coulter, Inc.	Biomek Disposable Tip Wash ALP, 96-Channel
719366	1	Beckman Coulter, Inc.	Biomek FX Device Controller

Figure 8:
Biomek FX[P] deck configuration.

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