LEXOGEN The RNA Experts

BECKMAN COULTER Life Sciences

Automation of Lexogen's QuantSeq 3' mRNA-Seq Library Prep Kits on Biomek FXp NGS Workstation

The Lexogen QuantSeq 3' mRNA-Seq Library Prep Kits for Illumina (FWD and REV) produce ready-to-sequence libraries from total RNA. Total RNA is first reverse transcribed with an oligo(dT) primer containing a 5' partial P7 sequencing adapter (Figure 1). RNA is then removed and second strand cDNA is synthesized using random primers that contain a 5' partial P5 sequencing adapter, and proceeds without strand displacement. This results in the generation of a single tag at the 3' end of each transcript that contains both partial sequencing adapters required for library amplification. After purification, the library is amplified by PCR to add unique indices for multiplexing and complete Illumina P5 and P7 sequencing adapters.

According to Lexogen's protocol, the QuantSeq 3' mRNA-Seq Library Prep kits provide the following features.

- Generate one 3' tag per transcript, for simplified data analysis removing the need for length normalization.
- QuantSeq libraries require about 10x less reads than whole transcriptome mRNA-Seq, enabling a higher level of sample multiplexing in one sequencing run.
- Free access to the QuantSeq Data Analysis Pipeline on the Lexogen platform is included with every kit.
- Low-cost alternative to microarrays for whole-transcriptome gene expression profiling.
- Applicable across wide variety of sample types, ranging from high quality to degraded / Formalin-Fixed Paraffin-Embedded (FFPE) RNA samples.
- For blood samples, the Globin Block add-on module enables globin depletion during library prep without any added protocol steps.

Figure 1. Lexogen QuantSeq 3' mRNA-Seq Library Prep Kit workflow.

Spotlight: Biomek FX^P

In this application overview, we describe the automation of Lexogen QuantSeq 3' mRNA-Seq Library Prep Kits on Biomek FX^P platform from Beckman Coulter Life Sciences.

Biomek FX^P automated Lexogen QuantSeq 3' mRNA-Seq Library Prep Kits provide:

- Reduced hands-on time and increased throughput.
- Reduction in pipetting errors.
- Standardized workflow for consistent results.
- Quick implementation with demonstrated method.

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

- Multichannel (96 or 384) and Span-8 pipetting provide flexible options for pipetting full plates, individual wells with variable volumes and custom arrays.
- Orbital Shakers, Peltiers, Span-8 and 96 channel tip wash stations for controlling sample processing.
- Optional on-deck thermal cycler integration.

Automated Method

The Biomek automated Lexogen Quantseq 3' mRNA-Seq Library Prep Kit protocol prepares 96 ready-to-sequence libraries in approximately 7 hours and 15 minutes. The user interface of the automated method includes Biomek Method Launcher, an optional Biomek SW package that simplifies the method implementation and reduces the introduction of errors during method setup.

Table 1. Estimated run times for automated Lexogen Quantseq 3' mRNA-Seq Library Prep Kit (FWD).

Process	Time**	
	16 reactions	96 reactions
Instrument setup*	30 mins	45 mins
Method Run	4 hrs 30 mins	6 hrs 30 mins
Total	**6 hr, 30 mins**	**7 hrs 15 mins**

* Hands-on time. Timing does not include reagent thawing and homogenization.

**All time data are rounded up to 15 minutes accuracy.

1. Biomek Method Launcher (BML)

BML is a secure interface for selecting methods without affecting method integrity (Figure 2). It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to carry out system maintenance.

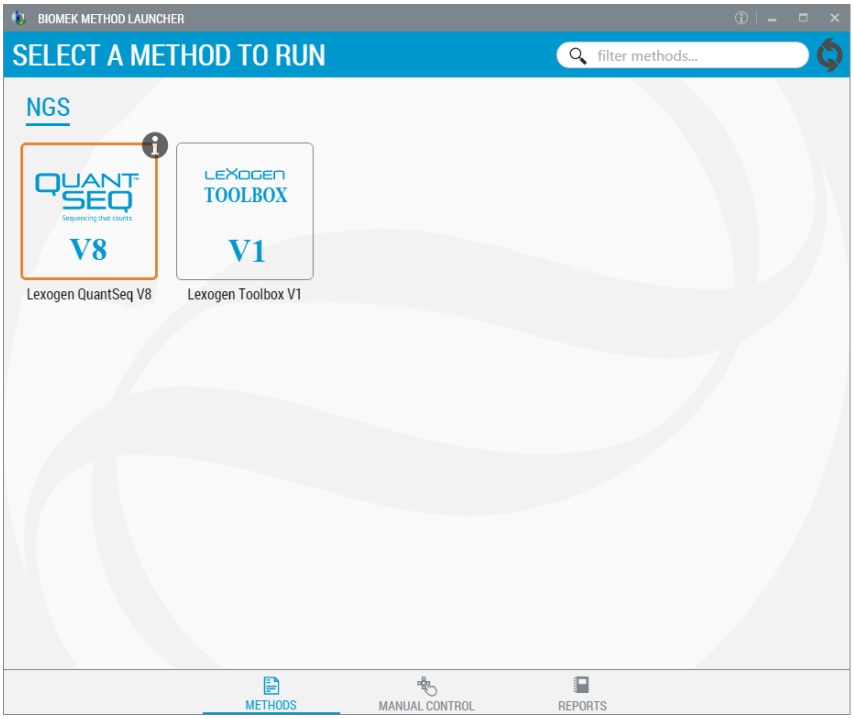

Figure 2. Biomek Method Launcher provides an easy interface to start the method.

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). The automated method has a modular design that provides users flexibility to schedule their day. The method allows processing of RNA samples of varying quality and quantity with either single or dual indexing. Spike-ins are also handled through automation, eliminating the need for manual dispensing. The automated method allows the use of on-deck or off-deck thermal cycling. With on-deck thermal cycling, the method provides the option to select the number of PCR cycles.

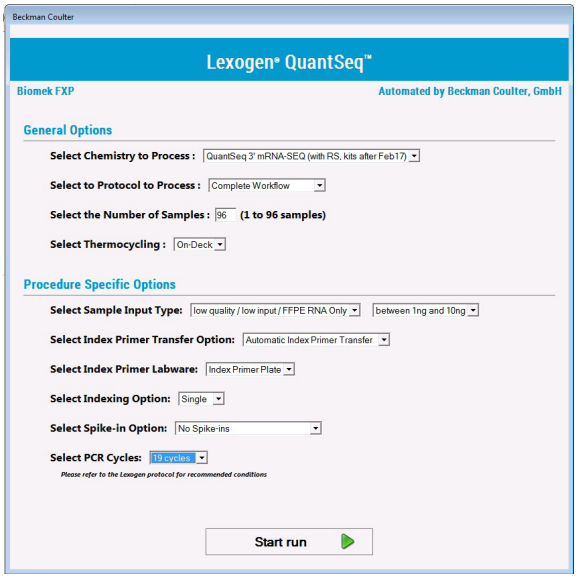

Figure 3. Biomek Method Options Selector indicates sample number and processing options.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS. It provides the user specific text and graphical setup instructions, with reagent volume calculation and step by step instructions to prepare reagents (Figure 4).

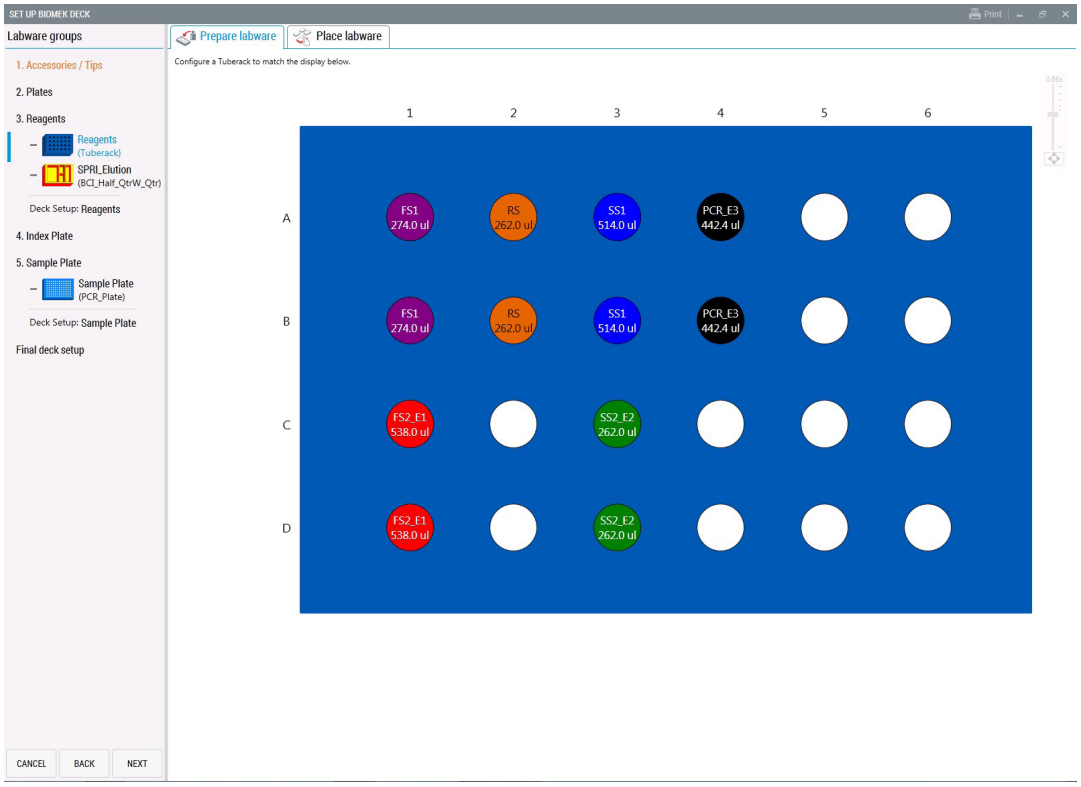

Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup options.

Experimental Design

The automated method was carried out using the Lexogen QuantSeq FWD Kit for Illumina (Cat. No. 015), with 500 ng of Universal Human Reference RNA (UHRR, Agilent Technologies) as input. Altogether 16 libraries were prepared, including 14 UHRR replicates and 2 no-input controls (NIC). All libraries were amplified with 13 PCR cycles. Samples were indexed using Lexogen's i7 indices 7081 to 7096. Library quality and yields were assessed by High Sensitivity DNA Assay (Bioanalyzer, Agilent Technologies).

Results

Bioanalyzer results indicate that all input samples generated libraries of consistent size distribution profiles. The two NIC wells show no library traces as expected (Figure 5).

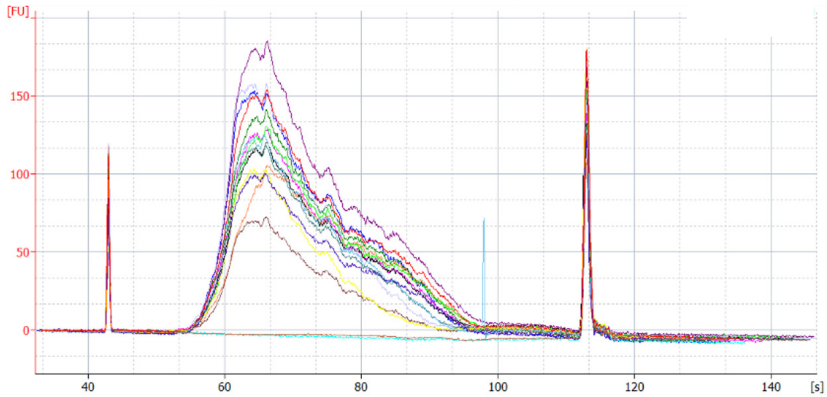

Figure 5. Electropherogram (Sample intensity in fluorescence units vs. time in seconds (s)) of Agilent Bioanalyzer. Different colors correspond to different inputs. Library size (in base pairs) increases with time.

Yields for UHRR and NIC libraries were calculated for the regions 100 - 150 bp (linker-linker, LL), and 150 – 1000 bp (Library, time equivalent 55.78 – 95.51 seconds). Individual library values as well as mean and standard deviation for UHRR and NIC samples are presented in Table 2. Average UHRR library prep yields were high (3.26±0.67 ng/µl). No visible LL peaks were present in UHRR libraries, and quantification of the LL range accounted for ≤1 % of total yield. This demonstrates efficient library generation and purification were achieved by the automated method.

Table 2. Summary of library yields. Yields for the libraries were calculated for the regions 100 - 150 bp (linker-linker, LL), and 150 – 1000 bp (electropherogram time equivalent 55.78 – 95.51 seconds).

Plate Ref.	Sample Type	Library				LL			
		% of Total	Average Size [bp]	Conc. [ng/µl]	Molarity [nM]	% of Total	Average Size [bp]	Conc. [ng/µl]	Molarity [nM]
A1	UHRR	98	336	3.88	20.78	0	143	0.00831	0.0878
B1	UHRR	97	344	3.74	19.71	0	136	0.01908	0.2123
C1	NIC	3	163	0.00083	0.0079	6	125	0.00196	0.0237
D1	UHRR	96	340	3.60	19.12	1	133	0.0297	0.3371
E1	UHRR	97	365	3.94	19.60	0	138	0.00996	0.1093
F1	UHRR	96	341	2.98	15.72	0	138	0.0133	0.1464
G1	UHRR	97	348	3.19	16.58	0	139	0.01057	0.1150
H1	UHRR	97	347	4.08	21.39	0	141	0.01065	0.1141
A2	UHRR	96	308	3.33	18.67	1	136	0.02293	0.2553
B2	UHRR	96	328	3.20	17.29	0	136	0.01944	0.2172
C2	UHRR	97	318	2.10	11.46	0	142	0.00803	0.0855
D2	UHRR	96	307	1.94	10.84	1	135	0.01291	0.1445
E2	UHRR	97	326	2.85	15.37	0	145	0.00712	0.0742
F2	UHRR	99	345	3.62	19.08	0	146	0.00586	0.0609
G2	NIC	0	344	0.00005	0.0002	0	0	0	0
H2	UHRR	97	349	3.33	17.36	0	138	0.01	0.1205
Average UHRR		**96.85**	**334.85**	**3.27**	**17.35**	**0.23**	**139.08**	**0.01**	**0.15**
Average NIC		**1.50**	**253.50**	**0.00**	**0.00**	**3.00**	**62.50**	**0.00**	**0.01**
SD UHRR		**0.90**	**16.80**	**0.64**	**3.17**	**0.44**	**4.03**	**0.01**	**0.08**
SD NIC		**2.12**	**127.99**	**0.00**	**0.01**	**4.24**	**88.39**	**0.00**	**0.02**

Summary

We have demonstrated the successful automation of Lexogen's Quantseq 3' mRNA-Seq Library Prep protocol on the Biomek FX^P Workstation. Our assessments of size and yield indicate that the prepared libraries are in a good condition for sequencing. Our automated protocol streamlines and simplifies library preparation by reducing hands-on time. In addition, Biomek Method Launcher provides a user friendly interface where the user can easily customize and run the method, without introducing errors during the initial method setup. This data-demonstrated method is available for distribution. Please contact Beckman Coulter Life Sciences to learn more.

2023-GBL-EN-100730-V1