

Automated Directional RNASeq Library Preparation using the Biomek FX^P Liquid Handler.

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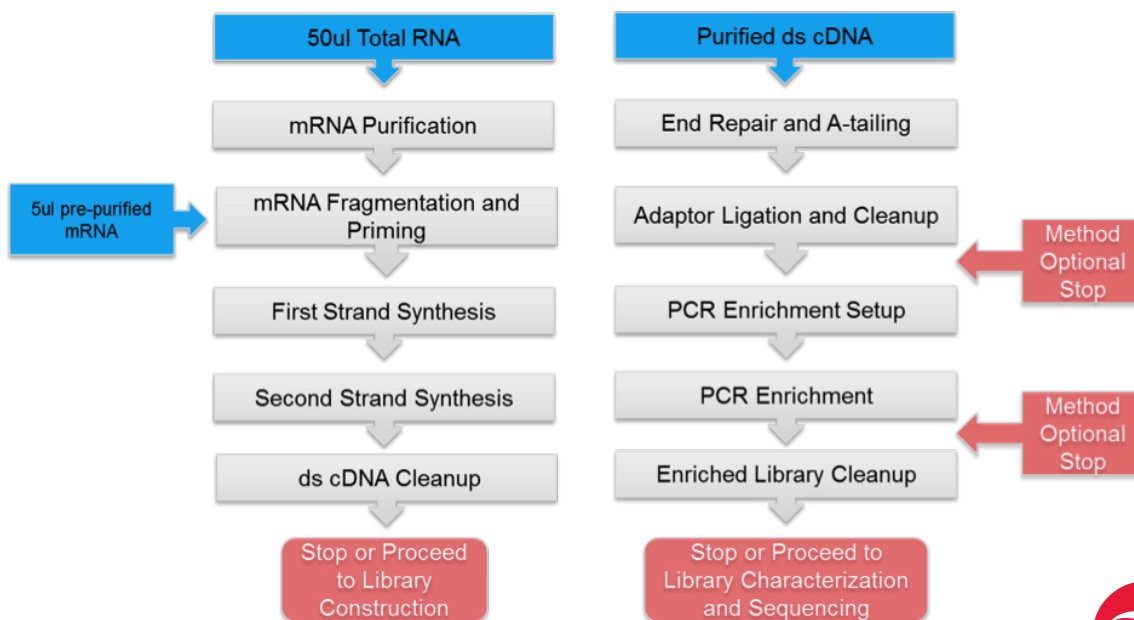
Introduction

The purpose of this tech-note is to describe the automation of the New England Biolabs NEBNext (Registered trademark) Ultra Directional RNA Library Prep Kit for Illumina on the Beckman Coulter Biomek FX^P automated liquid handler. The Biomek NEB Ultra Directional RNA Method was designed to work with NEBNext Ultra Directional RNA Library Prep Kit for Illumina protocol (Catalog Number E7420S/L), and provides a solution for simultaneously constructing up to 96 individually bar-coded directional RNASeq libraries per method run.

The method provides users with the option to utilize either off-deck incubations using an external thermocycler, or to perform incubations on-deck with a Biometra TRobot thermocycler integrated to the Biomek liquid handler. The method employs individual Span-8 probes to deliver enzyme and reagent transfers while DNA cleanup, wash, and elution transfers are performed using the multi-channel 96 pipetting head. A static Peltier unit ensures that enzyme master mixes are kept cool during the course of the method.

The data presented below includes a comparison of library construction using the Biomek FX^P method at 25ng and 100ng total RNA inputs. Automated sample preparation allows for the simultaneous construction of up to 96 directional RNASeq libraries in about 9.5 hours. For convenience, this method can be run in a single nine and one-half hour run, or as a two day run, with day 1 consisting of sample preparation through cDNA synthesis and day 2 consisting of the sample preparation through the end of library construction. Figure 1 shows the workflow of the NEBNext Ultra Directional RNA Library Prep Kit for Illumina protocol.

Figure 1. Automation Workflow. Blue indicates method inputs, red indicates potential stop points, and grey indicates method steps



Materials and Methods

The data was generated using the materials and methods described in the field installation guide.

Results and Discussion

25ng and 100ng Universal Human Reference (UHR) RNA (Agilent Technologies) was used as input for the New England Biolabs NEBNext Ultra Directional RNA Library Prep Kit for Illumina on the Beckman Coulter Biomek FX^P automated liquid handler. UHR RNA was assayed on the Agilent Bioanalyzer 2100 using an RNA6000 Pico kit (PN# 5067-1513) prior to the run to ensure high sample quality (data not shown). The purified adapter ligated library was amplified to 15 cycles per instruction of the New England Biolabs NEBNext Ultra Directional RNA Library Prep Kit for Illumina protocol using the Bio-Rad S1000 Thermal Cycler (Bio-Rad., Inc).

The quality and size distribution of the library was analyzed using the Agilent DNA High Sensitivity kit (PN# 5067-4626) and Agilent Bioanalyzer 2100. Representative Bioanalyzer 2100 results are shown in Figure 2. The library concentrations were measured using KAPA SYBR Fast Universal 2X qPCR Master Mix library quantification kits (PN# KK4824, KAPA Biosystems), and are summarized in Figure 3.

Figure 2. 25ng UHR RNA (left) and 100ng UHR RNA (right) NEBNext Ultra Directional RNA libraries for Illumina sequencing.

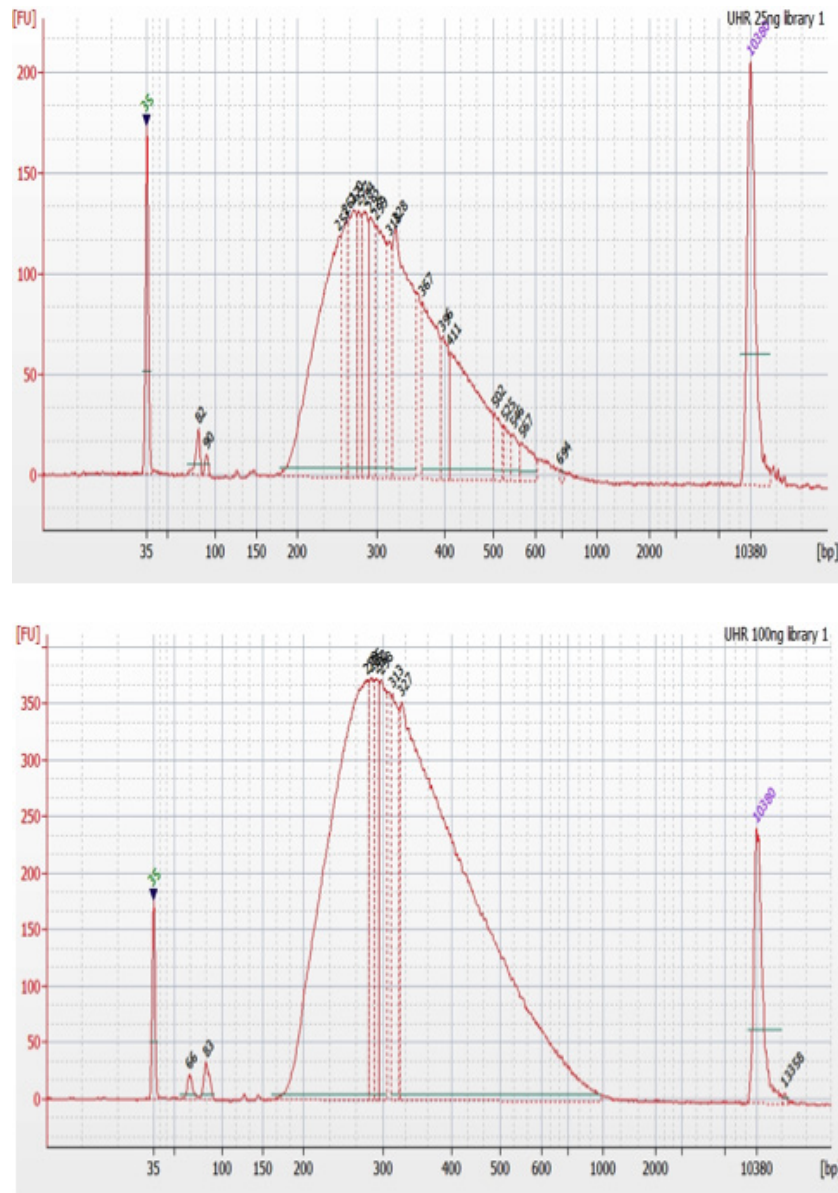
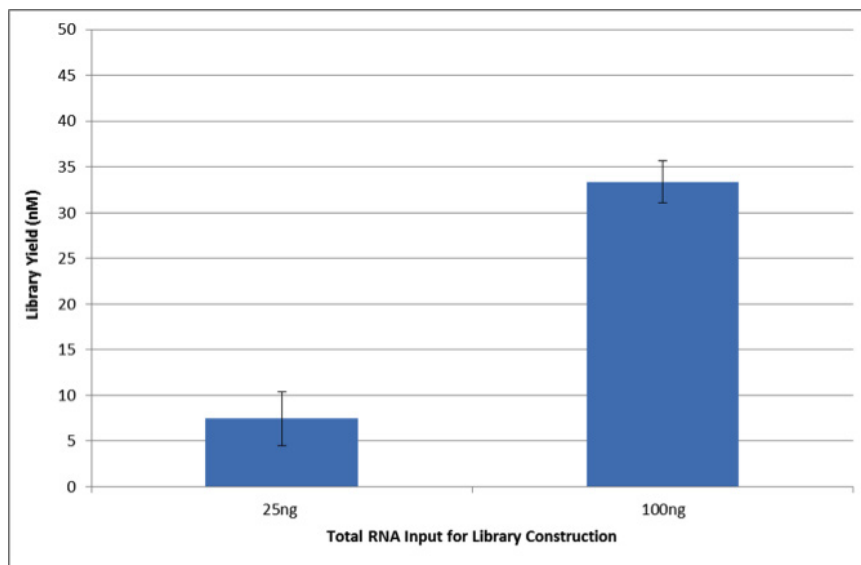
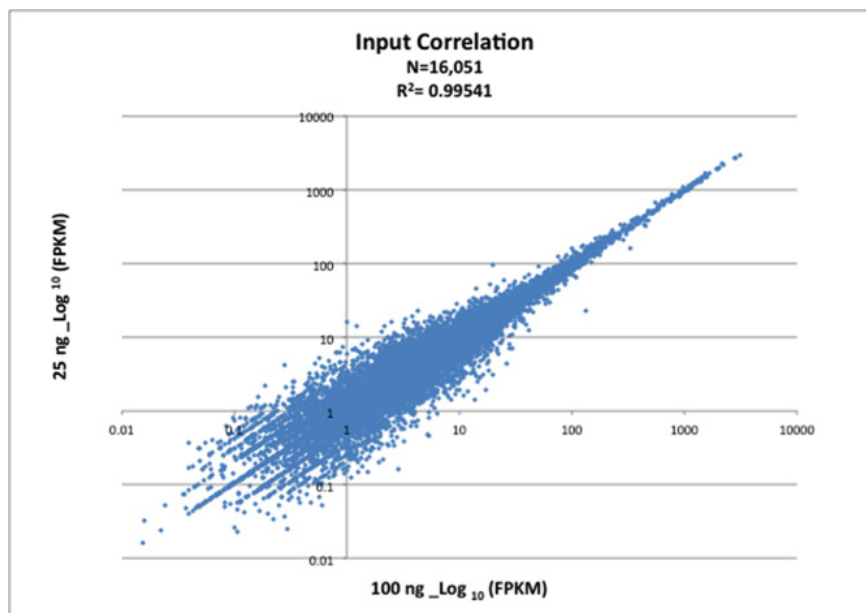


Figure 3. qPCR quantification of 25ng UHR RNA (left) and 100ng UHR RNA (right) NEBNext Ultra Directional RNA libraries for Illumina sequencing. Error bars indicate standard deviation.



Libraries were sequenced at New England Biolabs using a MiSeq 2x100 cycle paired end sequencing run. Reads were assayed using FastQC and aligned to the reference genome (HG19 build) using Tophat2. Following mapping, Cufflinks was used to measure transcript abundances in terms of fragments per kilobase of exon per million fragments mapped (FPKM). Bioinformatics analysis was performed at New England Biolabs using a local instance of Galaxy (<https://usegalaxy.org>). As shown in Figure 4, there is a high degree of correlation between 25ng and 100ng total RNA input libraries.



Conclusion

For optimal sequencing results, high quality RNA should be used for any RNASeq experiment.

The use of Beckman Coulter automation solutions in conjunction with the New England Biolabs NEBNext Ultra Directional RNA Library Prep Kit for Illumina allows for the construction of high quality, reproducible directional RNASeq libraries from limiting sample inputs.

Automation Labware

PART NUMBER	MANUFACTURER	DESCRIPTION	QUANTITY REQUIRED FOR 96 SAMPLES
B01124	Beckman Coulter, Inc.	Biomek Span-8 P1000 Tips, Pre-sterile with Barrier	2
379503	Beckman Coulter, Inc.	Biomek Span-8 P250 Tips, Pre-sterile with Barrier	3
A21586	Beckman Coulter, Inc.	Biomek P50 Tips, Pre-sterile with Barrier	5
717256	Beckman Coulter, Inc.	Biomek AP96 P20 Tips, Pre-sterile with Barrier	2
717253	Beckman Coulter, Inc.	Biomek AP96 P250 Tips, Pre-sterile with Barrier	4
372790	Beckman Coulter, Inc.	Quarter Reservoir	6
534681	Beckman Coulter, Inc.	Reservoir, Half	3
372795	Beckman Coulter, Inc.	Frame for Reservoirs [†]	2
A32782	Beckman Coulter, Inc.	Agencourt® SPRIPlate® 96R - Ring Super Magnet Plate [‡]	1
A83054	Beckman Coulter, Inc.	BCI Tube Block [‡]	1
C5064	Acme Automation	ReactorAdaptor96Flat ^{†**}	1
AB-1127	Fisher Scientific	Abgene 96-Well Storage Plate, Square Well, 1.2 mL	5
16466-042	VWR	2mL SuperClear™ Screw Cap Microcentrifuge Tubes- Conical Bottom	16 tubes
HSP-9641	Bio-Rad	Hard-Shell® Thin-Wall 96-Well Skirted PCR Plates	8
MSL-2022	Bio-Rad	Arched Auto-Sealing Lids*	1

†One Time purchase

* For on-deck thermocycling only

** For on-deck Adaptor_Plate only

Biomek ALPs

PART NUMBER	MANUFACTURER	DESCRIPTION
719654	Beckman Coulter, Inc.	Span-8 Wash ALP
719363	Beckman Coulter, Inc.	Multichannel Wash Station including pump and tubing
379448	Beckman Coulter, Inc.	Orbital Shaker ALP, Single Position
719590	Beckman Coulter, Inc.	Span-8 Disposal ALP
719357	Beckman Coulter, Inc.	Static 1x1 ALP Platform
719361	Beckman Coulter, Inc.	Static Peltier ALP
719948	Beckman Coulter, Inc.	4x3 ALP kit
719366	Beckman Coulter, Inc.	Biomek FX Device Controller

Auxiliary Equipment

PART NUMBER	MANUFACTURER	DESCRIPTION
G2940CA	Agilent Technology	Agilent 2100 Bioanalyzer
5067-4626	Agilent Technology	High Sensitivity DNA kit

User-Supplied Reagents

PART NUMBER	MANUFACTURER	DESCRIPTION
Not available	User Preferred	Elution Buffer - Nuclease free water, TE, 10mM Tris, pH 8.5
A63881	Beckman Coulter, Inc.	AmpureXP
AB00138-01000	American Bioanalytical	Ethanol
E7420S (24 rxns) or E7420L (96 rxns)	New England Biolabs	NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®
E7335L (Set 1, 96 rxns) or E7500L (Set2, 96 rxns)	New England Biolabs	NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1 or 2)
E7490L (96 rxns)	New England Biolabs	NEBNext Poly(A) mRNA Magnetic Isolation Module
A1410	Sigma	Actinomycin D (dissolved in dimethylsulfoxide [DMSO] to 5 µg/µl)

*Note: unless otherwise indicated, the recommended manufacturer is Beckman Coulter.



NEBNext is a registered trademark of New England Biolabs.

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