APPLICATION NOTE

Automated Illumina® Stranded mRNA Prep, Ligation Kit on the Biomek i7 Dual Hybrid Workstation

Introduction

The Illumina Stranded mRNA Prep, Ligation kit is a simple RNA-seq solution for analysis of the coding transcriptome. It supports inputs as low as 25ng and up to 1000ng across multiple species. It enables users to precisely measure strand orientation, offers uniform coverage and high-confidence discovery of novel isoforms, allele-specific expression, and gene fusions.

It converts the messenger (mRNA) in total RNA into up to 384 dual-indexed libraries. Oligo(dT) magnetic beads purify and capture the mRNA molecules containing polyA tails. The purified mRNA is fragmented and copied into first strand complementary DNA (cDNA) using reverse transcriptase and random primers. In a second strand cDNA synthesis step, dUTP replaces dTTP to achieve strand specificity. The final steps add adenine (A) and thymine (T) bases to fragment ends and ligate adapters. The resulting products are purified and selectively amplified for sequencing on an Illumina system.

The automated method has a throughput of 96 samples on a Biomek i7 Dual Hybrid (DH) Automated Workstation.

The Illumina Stranded mRNA Prep, Ligation kit automated on the Biomek i7 DH platform provides:

- Reduced hands-on time in system setup and potential pipetting errors
- Quick installation with ready-to-implement methods
- Knowledgeable support from Illumina and Beckman Coulter Life Sciences

Spotlight

- Biomek i7 Hybrid (Multichannel 96, Span-8) Workstation features flexible configurations which increase user confidence and walk-away time.
- 1200 µL Multichannel head with 1-1000 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip for multichannel pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with up to 45 positions
- Shaking, heating/cooling, and tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermal cyclers)

Figure 1. Biomek i7 Dual Hybrid Workstation with optional Enclosure on a Biomek Mobile Workstation. Deck layout in the lower image.

* Can start here if using Pre-purified RNA

** Double Stranded

🛑 Illumina approved Stop point and method stop/start point

Figure 2. Illumina Stranded mRNA Prep, Ligation Kit automated workflow.

Automated method

Automation provides increased efficiency, reduction in human errors and hands-on time as compared to manual library preparation **(Table 1)**.

Kit Type	**Illumina Stranded mRNA Prep, Ligation kit**				
Method section	**Single strand and CDNA synthesis**	**A-tail + Anchor**	**Indexing +PCR**	**Cleanup**	**Total time**
Method Run ETC (Estimated time of Completion)	2 hr 18 min	1 hr 29 min	20 min	21 min	
Cycling times	2 hr 24 min	54 min	42 min	0	
Total Time (with on-deck ATC)	4 hr 42 min	2 hr 52 min	1 hr 2 min	21 min	8 hr 57 min

*Total timing estimates include thermocycling with on-deck ATC but do not include reagent thawing and deck set up.

Table 1. Estimated run times for automating the Illumina Stranded mRNA Prep, Ligation kit to make 96 libraries on the Biomek i7 DH Workstation.

The method can be run using Method Option Selector, which is an interactive user interface that supports modular design and logical start and stop points based on Illumina's recommendations. Guided Labware Setup helps with ease of deck setup and reagent information and DeckOptix Final Check software minimizes costly setup errors. Automated method provides flexibility to the users in scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput.

The method utilizes at least two cold positions, a shaker, and an optional on-deck thermocycler for automated amplificaiton and incubation steps.

The software provides several user-friendly features such as:

1. **Biomek Method Launcher (BML)**

   BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed.

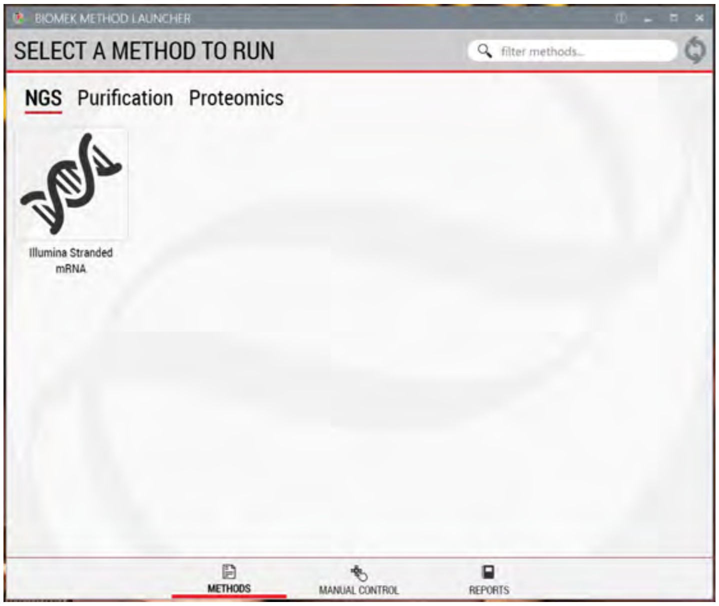
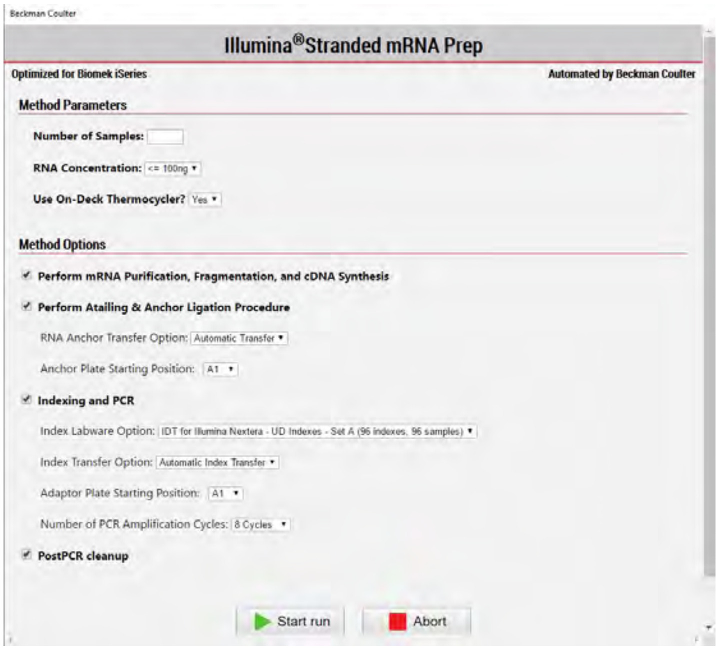


Figure 3. Biomek Method Launcher provides a straightforward interface to launch the method.

2. **Method Option Selector (MOS)**

   MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability and the ease of method execution.



Method Parameters

Number of Samples:

RNA Concentration: <= 100ng

Use On-Deck Thermocycler? Yes

Method Options

☑ Perform mRNA Purification, Fragmentation, and cDNA Synthesis

☑ Perform Atailing & Anchor Ligation Procedure

RNA Anchor Transfer Option: Automatic Transfer

Anchor Plate Starting Position: A1

☑ Indexing and PCR

Index Labware Option: IDT for Illumina Nextera - UD Indexes - Set A (96 indexes, 96 samples)

Index Transfer Option: Automatic Index Transfer

Adaptor Plate Starting Position: A1

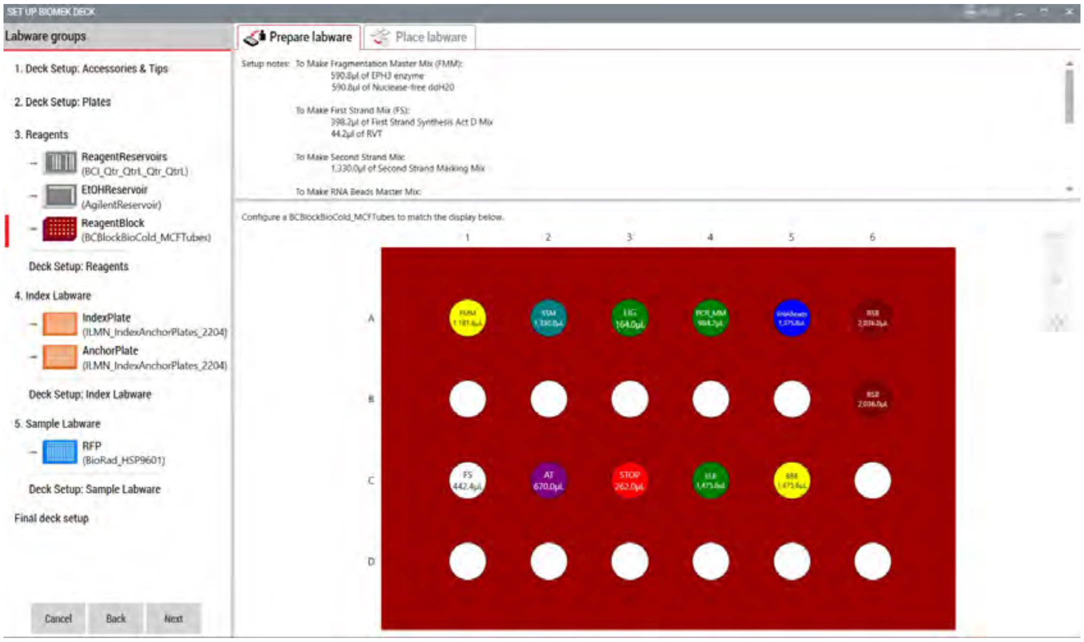
Number of PCR Amplification Cycles: 8 Cycles

☑ PostPCR cleanup

Figure 4. Illumina Stranded mRNA Prep, Ligation Method Option Selector.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS and provides the user specific graphical setup instructions for labware placement with reagent volume calculation and step by step instructions to prepare reagents.



Setup notes: To Make Fragmentation Master Mix (FMM):
590.8µl of EPH3 enzyme
590.8µl of Nuclease-free ddH20

To Make First Strand Mix (FS):
398.2µl of First Strand Synthesis Act D Mix
44.2µl of RVT

To Make Second Strand Mix:
1,330.0µl of Second Strand Marking Mix

To Make RNA Beads Master Mix:

Configure a BCBlockBioCold_MCFTubes to match the display below.

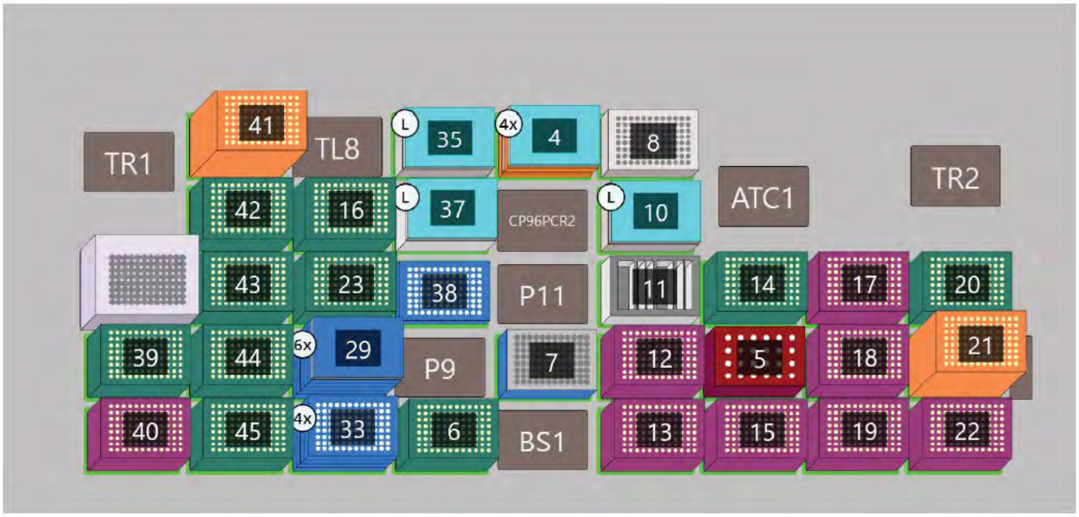


Figure 5. Guided Labware Setup provides recipe notes, indicates reagent volumes (upper image), and guides the user for correct deck setup (lower image).

4. DeckOptixFinal Check (DFC)

Prior to starting a run, DFC (included with BML) analyzes the deck to reduce setup errors and prevent a failed experiment caused by the user, such as missing or misplaced labware or using the wrong tip or type of plate.

Experimental design

To demonstrate capabilities to perform a 48-sample library preparation on a Biomek, we prepared libraries from universal human RNA (UHR). 16 replicates each of 75ng, 50ng, and 25ng input were processed on a single plate and subjected to 14 cycles of PCR amplification.

Results

Final libraries were analyzed post-PCR cleanup on a D1000 screen tape with an Agilent TapeStation 4200 instrument. The libraries were pooled and then sequenced on an Illumina NextSeq550Dx item #20005715 using NextSeq 500/550 High Output Kit v2.5 (300 Cycles), item #20024908. Libraries were subsampled to 30M reads and analyzed using the BaseSpace RNA-Seq Alignment App v2.0.1. Duplicates are reported at 4M subsampled pair-end reads passing filter (PF). The average size for libraries prepared with all 3 inputs was 320bps **(Figure 6a, 6b, 6c)** which is as expected. The average yields for the 75ng, 50ng and 25ng inputs were 16.5ng/µL, 8.9ng/µL and 4.7ng/µL respectively. The performance metrics for the automated Biomek library were compared to previously generated data by Illumina **(Table 2)** and are comparable. The mRNA analyses demonstrates that the distribution feature for RNA extracted corresponds to either mRNA or other RNAs that are not rRNAs **(Figure 7)**.

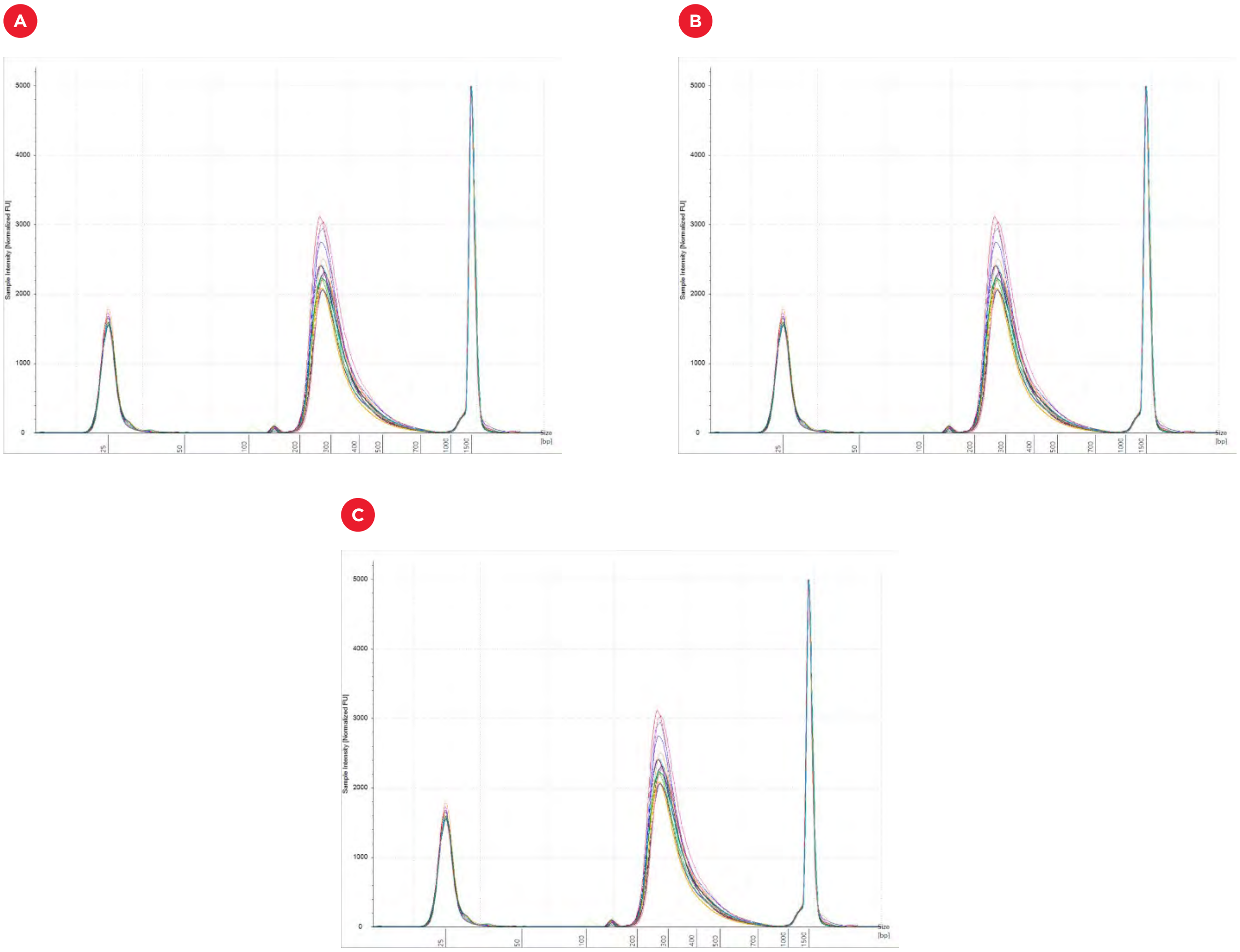


Figure 6. Traces for libraries on Agilent TapeStation High Sensitivity D1000 tape **(A)** 12, 75ng libraries. **(B)** 15, 50ng libraries. **(C)** 15, 25ng libraries.

	ILMN	BCLS	BCLS	BCLS	ILMN
Starting input	100	75	50	25	25
% rRNA	1.8%	0.60%	0.73%	0.53%	1.5%
% Strandedness	99.4%	98.6%	98.6%	98.5%	99.4%
Median CV of coverage	0.46	0.53	0.52	0.52	0.47
% Duplicates	3.7%	2.5%	2.8%	3.4%	3.3%
% Aligned	97.8%	92.8%	94.0%	95.5%	97.8%

Table 2. Performance metrics for Illumina Stranded mRNA Prep, Ligation kit compared between automated prep on Biomek i7 workstation versus existing data from basespace. Libraries were subsampled to 30M reads and analyzed using the BaseSpace RNA-Seq Alignment App v2.0.1. Duplicates are reported at 4M subsampled pair-end reads passing filter (PF).

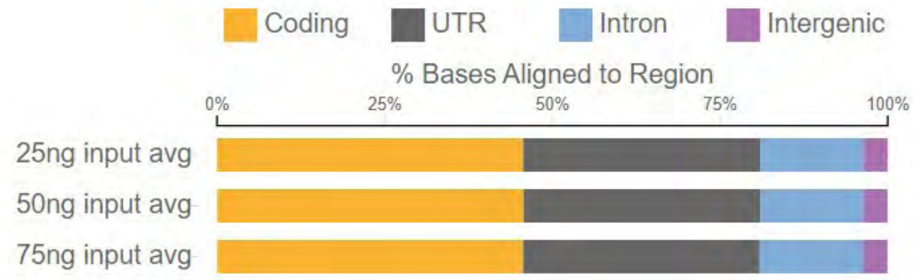


Figure 7. Distribution of mapped reads from sequenced samples.

Summary

We've demonstrated that automation of Illumina Stranded mRNA Prep, Ligation kit on the Biomek i7 Dual Hybrid Workstation is a fully walk-away solution that provides an efficient, flexible and scalable solution for any size lab. The automation solution delivers libraries that yield quality results in downstream workflows and saves valuable time and money.

The Illumina Stranded mRNA Prep, Ligation kit is for Research Use Only. The Illumina Stranded mRNA Prep, Ligation kit is not for use in diagnostic procedures. Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this protocol, including but not limited to warranties of fitness for a particular purpose or merchantability or that the protocol is non-infringing. All warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter. This protocol is for demonstration only and is not validated by Beckman Coulter.

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