



# Biomek Automated Genomic Sample Prep Accelerates Research

## Biomek i-Series Automated Illumina TruSeq® Stranded Total RNA Sample Preparation Kit Protocol

### Introduction

Illumina TruSeq® Stranded Total RNA Sample Preparation Kit protocol converts total RNA into a template library of known strand orientation (Illumina TruSeq Stranded Total RNA Library Prep Guide). First, rRNA and other high abundance transcripts are targeted for removal using a combination of biotinylated Ribo-Zero® probes and streptavidin removal beads (Illumina TruSeq Stranded Total RNA Library Prep Guide). Then the RNA is fragmented and copied into cDNA using a dUTP replacement strategy that maintains strand information in the library, followed by the addition of a single 'A' base and ligation of the adapter. The final PCR step enriches the products and creates the cDNA library for sequencing. The Illumina TruSeq Stranded total sample preparation Kit protocol enables analysis of both coding and noncoding RNA of known origin. Knowing the strand origin improves transcript annotation accuracy and alignment efficiency. Ribo-Zero probes have been developed targeting a variety of high-abundance transcripts for various species and tissues, including human, mouse, and rat rRNA, globins from blood-derived RNA, and plant rRNAs from plant RNA. In this technical note, we automate the Illumina TruSeq Stranded Total RNA Sample Preparation Kit protocol on Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation.

When compared to manual operations, The Illumina TruSeq Stranded Total RNA Library Prep Kit automated on Biomek platform provides:

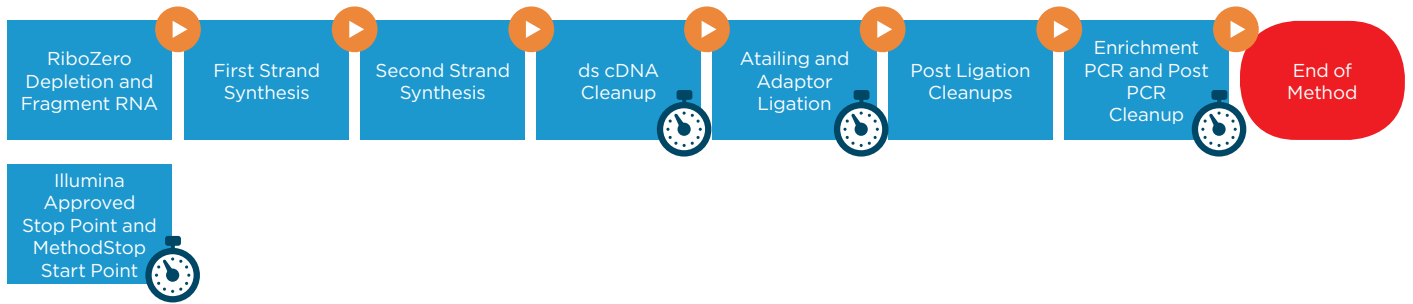
- Reduced hands-on-time and increased throughput- Option to run the method end-to-end with only setup and tear-down touch points
- Reduction in pipetting errors
- Reduction of cost by using low reagent volumes
- Quick implementation with demonstrated methods

### Spotlight

Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation System features deliver reliability and efficiency to increase user confidence and walk-away time

- 300uL or 1200uL Multichannel head with 1-300uL or 1-1200uL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers optimizes access to high density decks
- High deck capacity with 45 positions
- Orbital Shakers, peltiers, span-8 and 96 channel tip washing for controlling sample processing
- Spacious open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)





**Figure 1.** Illumina TruSeq Stranded Total RNA Sample Preparation Kit protocol

## Automated method

Automation of Illumina TruSeq® Stranded Total RNA Sample Preparation Kit protocol provides efficient sample preparation and increased throughput with minimal hands-on time (Table 1). The automated method is designed to minimize exposure to hazardous reagents (e.g. Actinomycin D) by separately collecting the reagents for convenient disposal as biohazardous waste. Through the use of Biomek method launcher the users can easily implement and adapt the method to suit their needs.

Process	Time	
	24 Samples	96 Samples
Prepare Reagents, Set up Instrument*	15 mins	30 mins
cDNA Synthesis	3 hrs, 51 mins	4 hrs, 32 mins
Library Construction	2 hrs, 60 mins	3 hrs, 46 mins
<b>Total*</b>	<b>7 hrs, 6 mins</b>	<b>8 hrs, 48 mins</b>

\*Timing estimate includes incubations and thermocycling. Timing estimate does not include reagent thawing

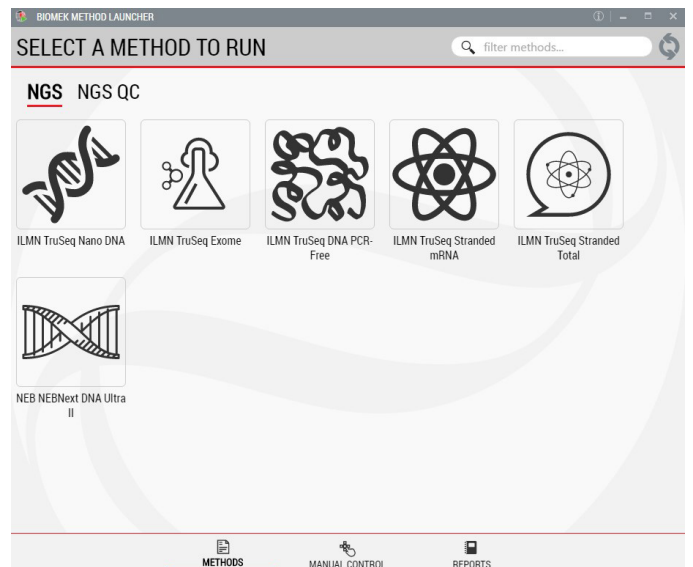
**Table 1.** Estimated run times for Illumina TruSeq® Stranded Total RNA sample preparation Kit protocol on the Biomek i7 Dual Hybrid Genomics Workstation.

### 1. Biomek Method Launcher (BML): User friendly interface

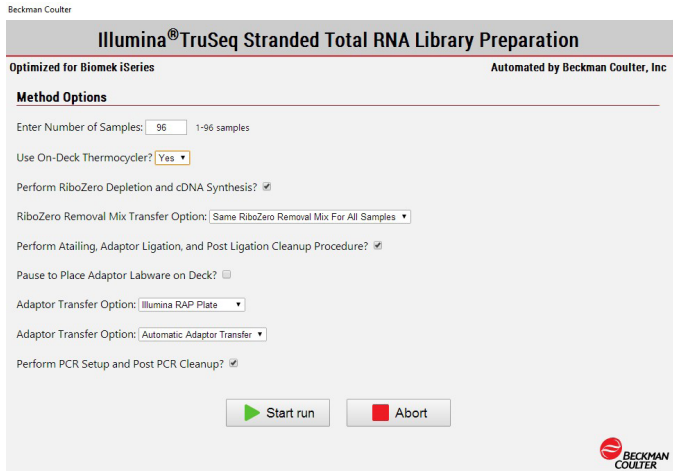
BML is a user friendly interface for securely launching the method without introducing errors during method setup (Figure 2). Through BML, users can monitor the progress of the run, off site.

### 2. Method Options Selector (MOS): superior flexibility

MOS provides several sample number and sample processing options (e.g. Ribo-Zero removal mix transfer options, adaptor transfer options, thermocycling options) to maximize flexibility of the method. MOS is arranged in a modular manner, providing workflow optimization (Figures 1, 3). Ability to start parts of the method based on the user constraints and logical start and stop points assigned based on Illumina’s recommendations, allows users to recover from errors without having to start from the beginning of the method (Figures 1, 3). Users have the option to perform per-well Ribo-Zero depletion with different Ribo-Zero removal mixes or treat all wells with same Ribo-Zero Removal Mix. The thermocycling steps can be done either off-deck or on-deck using an automated thermocycler (ATC Thermo Fisher; Figure 4).



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



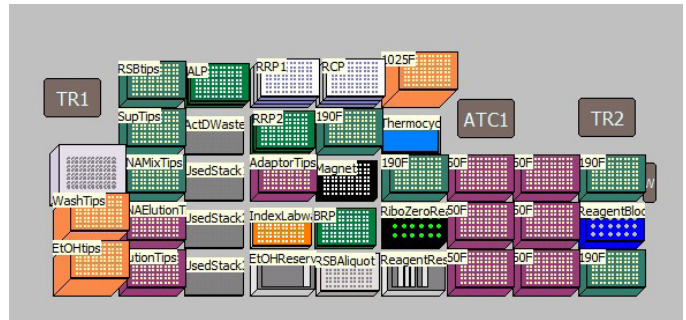
**Figure 3.** Biomek Method Options Selector indicates sample number and processing options

### 3. Guided Labware Setup (GLS): step by step instructions

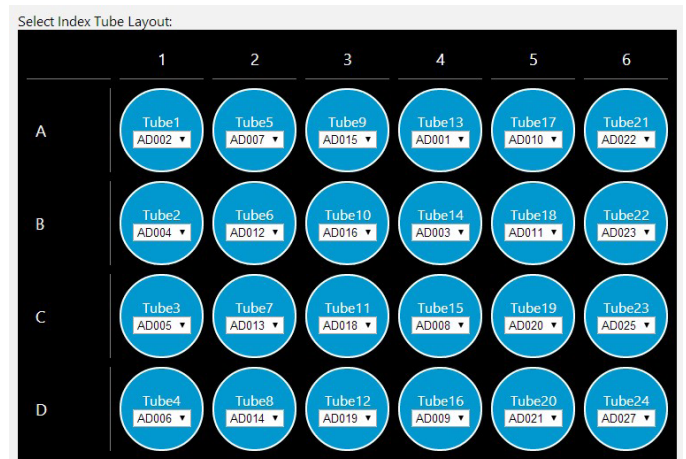
GLS provides the user specific step-by-step graphical setup instructions for reagent volume calculations, preparation of reagents and placement of labware on the deck (Figure 5). The GLS guides the users to place Illumina low throughput and high throughput adaptor labware on deck along with custom adaptor plates (Figure 6). The steps are generated based on the options selected in MOS. For instance, selecting automatic adaptor transfer creates dataset driven adaptor ID logs, indicating which adaptor has been assigned to which sample through Biomek software Data Acquisition and Reporting Tool (DART). DART gathers data and synthesizes runtime information from Biomek log files to capture all sample manipulations during the course of the method. Alternatively, users also have the option to customize adaptor assignments by uploading a .csv file. The method also supports placing Illumina LT and HT adaptor labware on deck along with custom adaptor selection. An optional pause step is included for placing adaptors on deck.



**Figure 5.** Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup



**Figure 4.** Deck Layout for TruSeq Stranded Total RNA sample preparation Kit protocol on Biomek i7 Dual Hybrid for 96 samples with on-deck thermocycling option



**Figure 6.** Guided Labware Setup enables selecting index tube layout

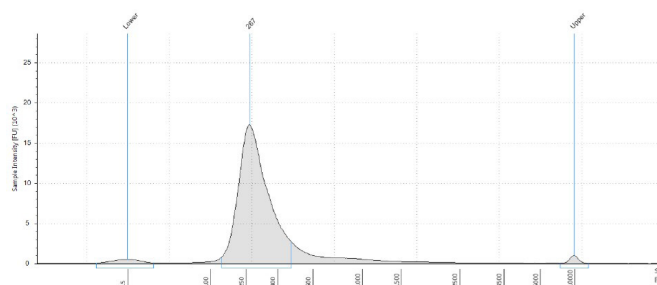
### Experimental design

Universal human reference total RNA (UHR 2 µg/µl, 1 µg/µl, 500 ng/µl, 100 ng/µl; 2 replicates from each) and RNA samples extracted using Beckman Coulter Agencourt RNAdvance Tissue Kit (Breast 58 ng/ul, Lung 156 ng/ul and Liver 29 ng/ul with 2 technical replicates each) was used for the automation of Illumina TruSeq® Stranded total RNA sample preparation Kit protocol on the Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation using 10 PCR cycles. After the preparation, the libraries were analyzed on Agilent TapeStation 2200 with Agilent High Sensitivity D5000 ScreenTape system.

## Results

Agilent TapeStation results indicated that the majority of the prepared libraries are of expected size (Approximately 260 bp, TruSeq Stranded total RNA sample preparation Guide; Table 2; Figure 7). As indicated by Illumina, the cDNA yield increased with the amount of input RNA (Table 2).

Sample ID	TapeStation size (bp)	Yield (pg/μl)
UHR 2 μg Rep1	267	11500
UHR 2 μg Rep2	277	18200
UHR 1 μg Rep1	271	4910
UHR 1 μg Rep2	269	7170
UHR 500 ng Rep1	266	3390
UHR 500 ng Rep2	257	2880
UHR 100 ng Rep1	279	988
UHR 100 ng Rep2	271	742
Breast Rep1	250	2360
Breast Rep2	238	4050
Lung Rep1	253	366
Lung Rep2	257	451
Liver Rep1	226	4010
Liver Rep2	222	784



**Figure 7.** Electropherogram (Sample intensity vs. size in base pairs) of Agilent TapeStation corresponding to 2μg replicate 1 showing the libraries around expected size of the marker

**Table 2.** Library quantification of automated Illumina TruSeq® Stranded Total RNA Sample Preparation kit protocol using Agilent TapeStation 2200

## Summary

We demonstrated the automation of the Illumina TruSeq® Stranded Total RNA Sample Preparation kit on Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Genomics Workstation. Our assessments (Size and yield) indicate that the prepared libraries are in a good condition for sequencing. Our Automated protocol increases the library preparation efficiency by reducing the 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.

Data obtained during development

Biomek i-Series Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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AAG-276806.17