



Biomek i-Series Automated Illumina TruSeq® DNA PCR-Free Library Prep Kit

Introduction

The Illumina TruSeq® DNA PCR-Free Sample Preparation workflow is designed to generate paired-end libraries without the need for PCR amplification. Solid Phase Reversible Immobilization (SPRI) bead chemistry is utilized throughout the protocol for size selection and sample cleanup steps (Figure 1, TruSeq® DNA PCR-Free Library Prep Reference Guide). The laborious and error-prone nature of this process makes it ideal for automation. In this application overview, we demonstrate the automated performance of Illumina TruSeq® DNA PCR-Free sample preparation kits protocol on Beckman Coulter Biomek i5 Span-8 and i7 Hybrid workstations.

Biomek i-Series automated Illumina TruSeq® DNA PCR-Free protocol provides:

- Standardized workflow for improved results
- Reduction in costly errors
- Reduced hands-on-time and increased throughput and
- Quick implementation with ready-to-implement methods delivered by knowledgeable support teams

Spotlight: The Biomek i7 Hybrid (Multichannel, Span-8) Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time compared to manual operation

- 300 uL or 1200 uL Multichannel head with
 1-300 uL and 1-1200 uL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom arrays of samples
- Independent 360° rotating gripper with offset fingers
- 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. thermocyclers)







Figure 1. Ilumina TruSeq® DNA PCR-Free workflow

Automated method

The chart below provides throughput and turn-around time for the Biomek NGS platforms used to automate Ilumina TruSeq® DNA PCR-Free.The data for the application overview was generated on i7 Hybrid.

| PROCESS | TIME | | |
|-------------------|---------------------------|---------------------------|-----------------------------|
| | i7 Hybrid (96 samples) | i5 Span 8 (96 samples) | Biomek 4000 (24 samples) |
| Instrument setup* | 30 mins | 30 mins | 15 mins |
| Method run | 5 hrs 15 mins | 7 hrs 6 mins | 5 hrs and 16 mins |
| TOTAL | 5 hrs 45 mins | 7 hrs 36 mins | 5 hrs 31 mins |

^{*}Timing does not include reagent thawing and homogenization

 Table 1. Estimated run times for the protocol on the Biomek Workstations.

Demonstrated Method Interface (DMI)

DMI composed of three simple modules that offer the user full instructions to reduce the occurrence of errors during method setup and maximize flexibility for scheduling their day.

1. Biomek Method launcher (BML)

A secure interface for selecting methods without affecting method integrity (Figure 2).

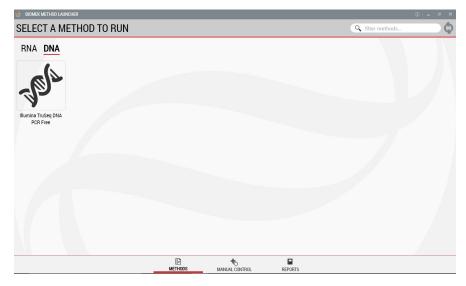


Figure 2. Biomek Method Launcher provides an easy interface to start the method

2. Method Options Selector (MOS)

Allows the users to select run-time options and maximize flexibility in daily scheduling and method execution. Users can choose to run any number of samples between 1 and 96. The method is broken into modules based on Illumina recommended stop points, so the user has the flexibility to run specific modules or full method. The MOS also provides the option for processing either 350bp or 550bp library insert size. Adaptors can be transferred by using Illumina 96-well DNA adapter plate or custom adapter labware. Adapter transfers can be automatic or by user-defined transfer file (Figure 3).

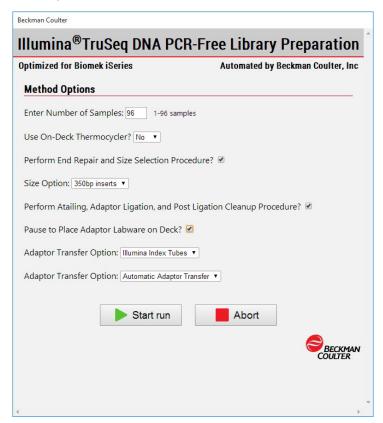


Figure 3. Biomek Method Options Selector indicates sample number and processing options

3. Guided Labware Setup (GLS)

Generated based on options selected in the MOS, and provides the user specific text and graphical setup instructions including reagent calculation (Figure 4).

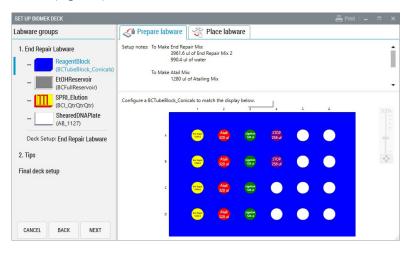


Figure 4. Guided Labware Setup displays required reagent volumes and setup notes to guide the user on correct deck setup

Experimental Design and Results

Genomic DNA (PromegaTM P/N: G3041) was sheared using a Covaris S220 focused ultrasonicator utilizing the settings defined in the Illumina TruSeq DNA PCR-Free Sample Preparation Kit protocol for a 550bp insert size for 12 replicates. 2 μ g of the sheared DNA was loaded in a volume of 50ul. Library quality was assessed using the Agilent TapeStation 2200 with High Sensitivity D5000 kit (Figure 5).

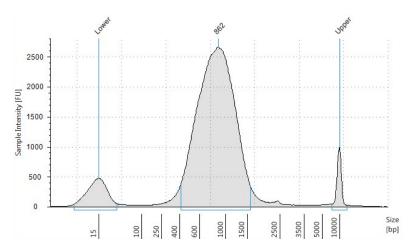


Figure 5. Final prepared library size on Agilent TapeStation using HS D5000 kit

Summary

The Illumina TruSeq® DNA PCR-Free sample preparation kit has been automated on multiple Biomek platforms to provide options for throughput and walk-away time. The automated method enables preparation of up to 96 sequence-ready libraries simultaneously. The Method Options Selector (MOS) and Guided Labware Setup (GLS) guide the user step by step through the method.



Data obtained during development

Beckman Coulter Life Sciences Biomek Automated Workstations and genomic reagent kits are not intended or validated for use in the diagnosis of disease or other conditions.

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