



Biomek Automated Genomic Sample Prep Accelerates Research

Biomek i-Series Automated Illumina TruSeq DNA PCR-Free

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. The process can be laborious and error-prone and therefore is ideal for Biomek automation. Choose from low to high throughput Biomek liquid handling workstations to automate all steps of the workflow providing the option for a complete walk-away solution. The methods are proven to deliver sequence-ready libraries through demonstrated performance using scientifically relevant samples. These ready-to-implement methods are compatible with either the LT or HT versions of the Illumina TruSeq DNA PCR-Free Sample Preparation kits (Illumina P/N FC-121-3001, FC-121-3002 or FC-121-3003).

This automated application is available on all Biomek platforms and provides:

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

Spotlight: Biomek i5 Span-8 Genomics Workstation

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

- 1-1000uL pipetting capability
- Independent 360° rotating gripper with offset fingers
- 25 positions
- Orbital Shakers, Peltiers and Tip washing for controlling sample processing
- Optional Items
 - Enclosure
 - On-Deck Thermocycler Integration (Biometra T-Robot, Thermo ATC)

T-Robot



Figure 1. Biomek i5 Span-8 Genomics Workstation with optional enclosure on a Biomek Cart. Deck layout with optional on-deck thermocycler for increased walk away time.

Demonstrated Method Interface (DMI):

Three simple modules that provide the user full instructions to better ensure error-free method setup and provides users maximum flexibility for scheduling their day

1. **Biomek Method Launcher (BML)** — secure interface for selecting methods without affecting method integrity and manual control



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



Figure 3. Manual Control can be run through the launcher interface

2. **Method Options Selector (MOS)** — Select run-time options and maximize flexibility in daily scheduling and method execution



Figure 4. Illumina TruSeq DNA PCR-Free Method Options Selector showing the different features and run options. Users can choose to run any number of samples between 1 and 96. The method is broken into modules based on Illumina's 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.

3. **Guided Labware Setup (GLS)** — Generated based on options selected in the MOS, and provides the user specific text and graphical setup instructions with reagent calculation

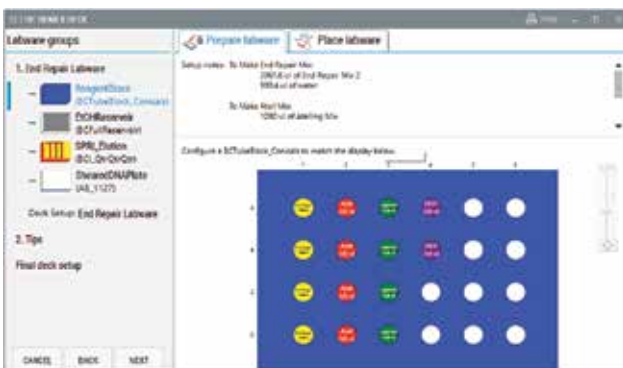


Figure 5. Guided Labware Setup showing reagent volumes and setup notes and guides the user for correct deck setup

Modular Design:

Built-in flexibility for scalable throughput and scheduling.

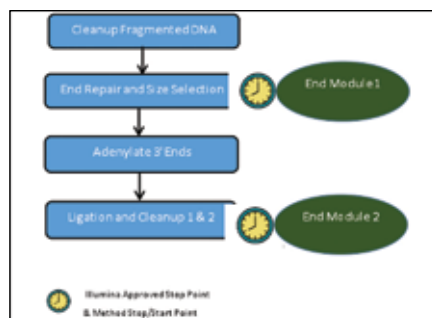


Figure 6. Illumina TruSeq DNA PCR-Free workflow.

| Major Process Description | 24 Samples | 48 Samples | 96 Samples |
|---|---------------------|----------------------|----------------------|
| End Repair | 1 hr 46 mins | 2 hrs 8 mins | 2 hr 50 min |
| A tailing, Ligation | 1 hr 26 mins | 1 hr 47 mins | 2 hr 28 mins |
| Hands on Time | 15 mins | 15 mins | 15 mins |
| Total Method Run Time | 3 hr 26 mins | 4 hrs 10 mins | 5 hrs 32 mins |
| **Timing does not include thawing of reagents | | | |

Table 1. Illumina TruSeq DNA PCR-Free estimated run times on i5 Span-8 Genomics Workstation. The method is broken into modules based on Illumina's recommended stop points. Users can choose to run between 1-96 samples and have the flexibility to run specific modules or full method.

Experimental Design and Results

Genomic DNA (Promega 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. 2µg of the sheared DNA was loaded in a volume of 50µl. Library quality was assessed using the Agilent TapeStation High Sensitivity D5000 kit.

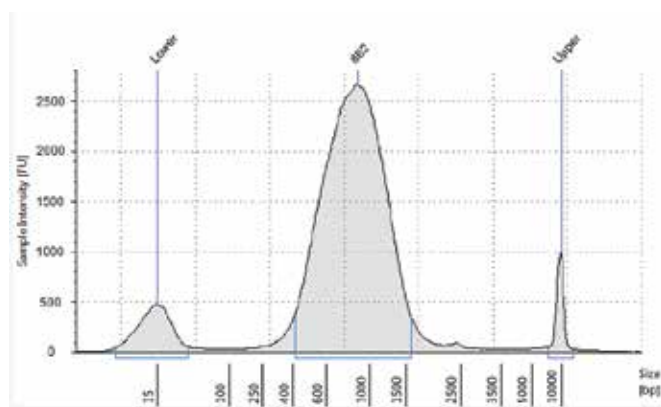


Figure 7. Final prepared library tracing on Agilent TapeStation using HS D5000 kit. The library size is comparable to Illumina's protocol and no adapter dimers at 120bp. qPCR yield was ~9nM.

Summary

The Illumina TruSeq DNA PCR-Free Sample Preparation kit automated on the new i-Series Workstation was demonstrated to provide a robust, flexible and efficient walk-away sample prep resulting in sequence-ready libraries.

Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions. Data shared in this document was obtained during development.

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