Introduction

The Illumina TruSeq Exome Library Prep kit is a workflow to generate indexed paired-end libraries and is specially designed to work with Formalin Fixed Paraffin Embedded (FFPE) samples. 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 is therefore 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 Illumina TruSeq Exome Library Preparation kits (Illumina P/N FC-150-001, FC-150-002 or FC-150-003).

This application is automated on multiple Biomek workstations 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 i7 Hybrid Genomics Workstation

System features provide highest throughput and flexibility of all Biomek Genomics workstations for maximized efficiency and reliability to increase user confidence and walk-away time

- 300 uL or 1200 uL Multichannel head with 1-250 uL and 1-1000 uL pipetting range
- Span-8 with 0.5-5000uL pipetting capability
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Two Independent 360° rotating gripper with offset fingers
- 45 positions
- Orbital Shakers, Peltiers and Tip washing for controlling sample processing
- Optional Enclosure

Figure 1. Biomek i7 Hybrid Genomics Workstation with optional enclosure on a Biomek Cart. 45 position deck capacity maximizes efficiency and increases 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

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

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
Modular Design:
Built-in flexibility for scalable throughput and scheduling.

**Figure 6.** Illumina TruSeq Exome workflow.

<table>
<thead>
<tr>
<th>Major Process Description for 96 samples/8 pools*</th>
<th>Section 1</th>
<th>Section 2</th>
<th>Section 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruSeq Exome</td>
<td>6 hrs 23 mins</td>
<td>2 hrs 16 min</td>
<td>3 hrs 28 mins</td>
</tr>
<tr>
<td>Hands On Time</td>
<td>30 mins</td>
<td>15 mins</td>
<td>15 mins</td>
</tr>
</tbody>
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**Table 1.** Illumina TruSeq Exome turnaround time on i7 Hybrid Genomics Workstation. The method is modular design based on Illumina’s recommended stop points. Users can choose to run between 1-96 samples and 1 to 8 pools.

**Experimental Design and Results**

DNA was extracted from FFPE samples using FormaPure DNA Extraction Kit from Beckman Coulter. Horizon Quantitative Multiplex Reference Standard for FFPE (HD200), breast (FFPE 76 and FFPE 96), lung (FFPE 110 and FFPE 121) and liver (FFPE 107) blocks were used to generate libraries using Illumina’s TruSeq Exome Kit and sequenced with NextSeq. Library quality was assessed using the Agilent TapeStation High Sensitivity D5000 kit.

**Figure 7.** Library Yield in ng as measured by Quant-IT Picogreen assay.
Summary

The sequencing run generated 112.9M passed filter reads with 96% of those reads identified. 80% of targeted regions were covered to the depth of at least 20X for the FFPE block samples, while over 95% of targeted regions for the Horizon sample were covered at a depth of 20X. In conclusion, Biomek i-Series liquid handlers offer a flexible solution for complex NGS workflows with challenging sample types.