BECKMAN COULTER Life Sciences

Biomek i-Series Automated SurePlex PCR and VeriSeq PGS Library Prep for Illumina®

Introduction

The SurePlex PCR and VeriSeq PGS (Preimplantation Genetic Screening) Library Prep for Illumina® is used to prepare up to 12 single-index OR up to 24 dual-index, single-read libraries from SurePlex amplification. The VeriSeq PGS solution begins with DNA amplification from a single embryonic cell or multiple embryonic cells using the SurePlex DNA Amplification Kit. Libraries are prepared from low DNA input (only 1 ng unpurified SurePlex double-stranded DNA. The VeriSeq PGS Library Prep uses an engineered transposome to simultaneously fragment and tag ("tagment") SurePlex input DNA, adding unique adapter sequences in the process. A limited-cycle PCR reaction uses the adapter sequences to amplify the SurePlex insert DNA. The PCR reaction also adds index sequences to both ends of the DNA, enabling single-indexed sequencing of up to 12 pooled libraries and dual-indexed sequencing of up to 24 pooled libraries on the MiSeq System.

In this flyer, we demonstrate the automated performance of Illumina® SurePlex PCR and VeriSeq Library Prep Kit on the Biomek i5 Span-8 Genomics Workstation. In comparison to the manual pipetting, the library prep Kit automated on Biomek platform provides:

- Reduced hands-on time and increased throughput
- Reduction in potential pipetting errors
- Standardized workflow for improved results
- Quick installation with ready-to-implement methods
- Knowledgeable support

Spotlight

Biomek i5 Span-8 Genomics Workstation

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

- Span-8 pod with 1-1000 uL pipetting capability
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with 25 positions
- Orbital Shakers, Peltiers, Span-8 tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)
- Optional enclosure

Figure 1. Biomek i5 Span-8 Genomics Workstation with optional enclosure on a Biomek Mobiile Workstation. Deck layout in the lower image.

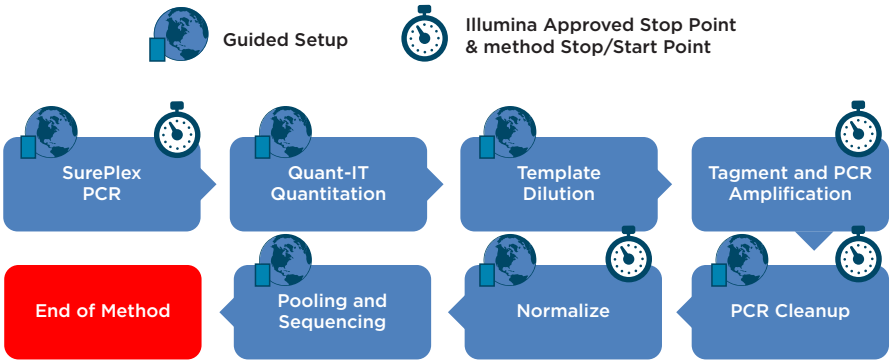

Figure 2. SurePlex PCR and VeriSeq Library Prep Kit workflow

Automated Method

Automation provides increased efficiency, reducing the hands-on time (Table 1). The method can be run using user interactive Guided Labware Setup with logical start and stop points assigned based on Illumina's recommendations, providing flexibility to the users in scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput.

Application is designed to run up to 96 total samples. The instrument has 2 Static Peltiers (one flat and one 96 PCR adaptor) and 1 Orbital Shaker.

Major Process Description

Sample Number	24	48	96
Instrument Setup Time (Total)	30 min	40 min	50 min
SurePlex PCR	20 min	39 min	1 hr 25 min
Template Quantitation	22 min	41 min	1 hr 20 min
Template Dilution, Tagment and PCR	36 min	1 hr 6 min	1 hr 51 min
Cleanup PCR	34 min	47 min	1 hr 13 min
Normalize	1 hr 9 min	1 hr 20 min	1 hr 44 min
Pooling	5 min	8 min	15 min
Method Run Time	**3 hr 36 min**	**5 hr 21 min**	**8 hr 38 min**
Total Time	**5 hr 51 min**	**7 hr 36 min**	**10 hr 53 min**

* Timing estimates include Thermocycling but do not include reagent thawing

Table 1. Estimated run times for VeriSeq for Illumina Library Prep Kit on the Biomek i5 Span-8 Genomics Workstation.

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.

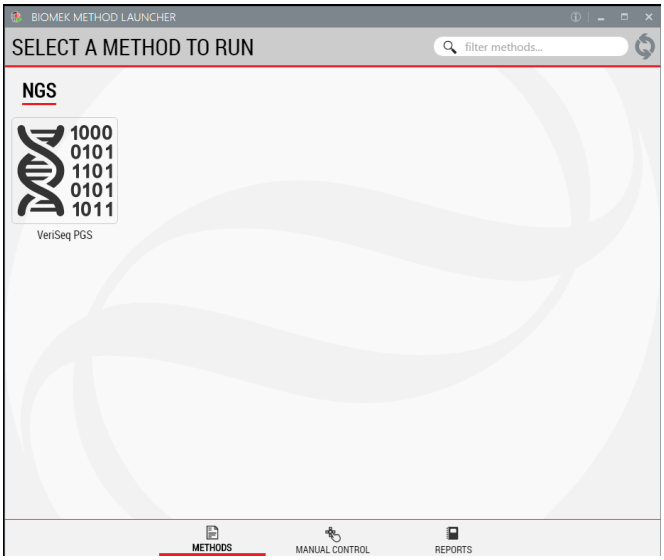

Figure 3. Biomek Method Launcher provides an easy interface to launch the method

2. Method Options Selector (MOS)

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

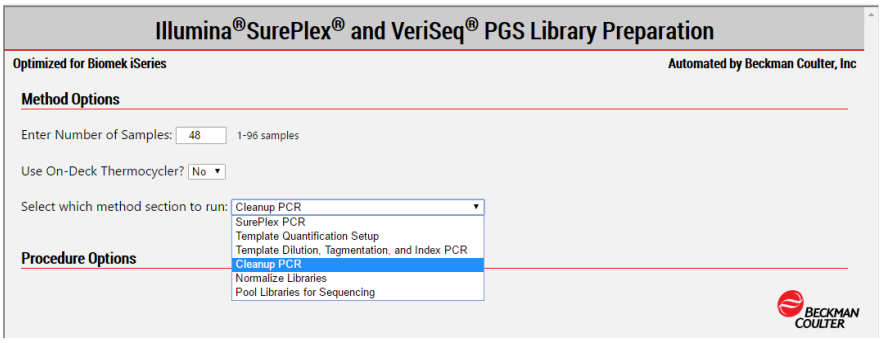

Figure 4. VeriSeq for Illumina Method Options Selector enables us to process from PCR to final library pooling with logical start and stop points

3. Guided Labware Setup (GLS)

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

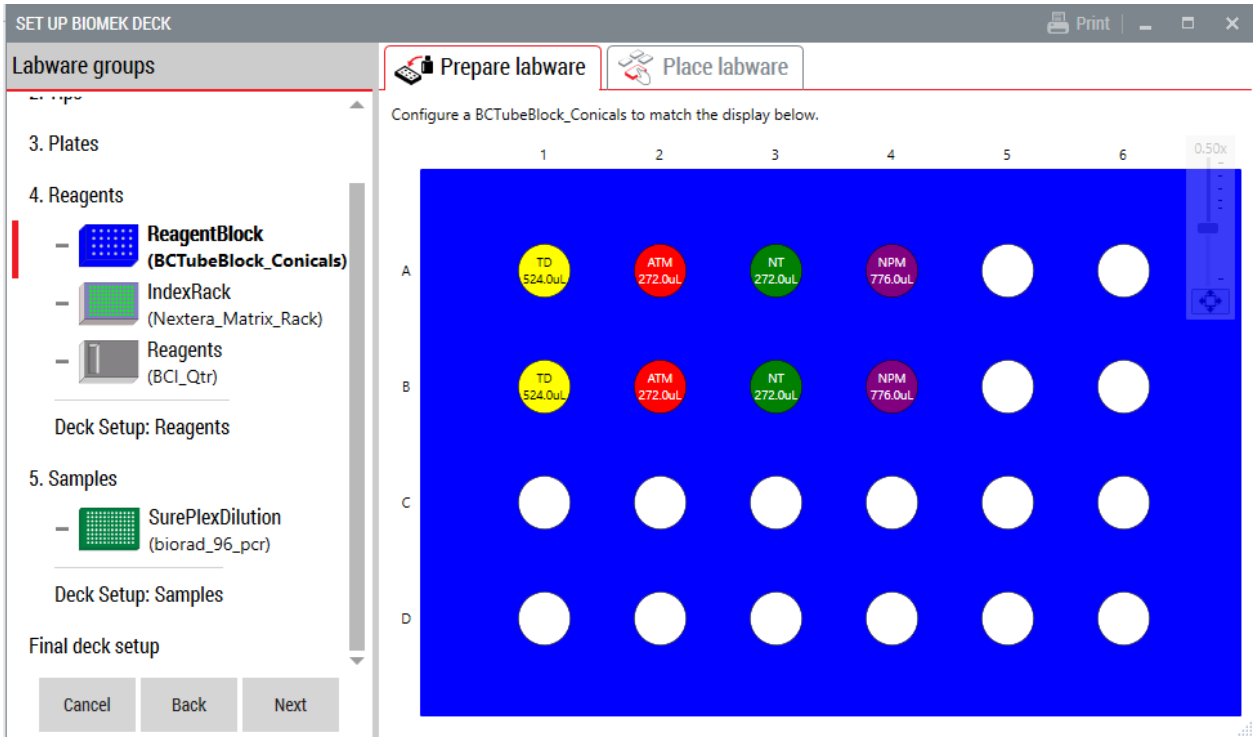

Figure 5A. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

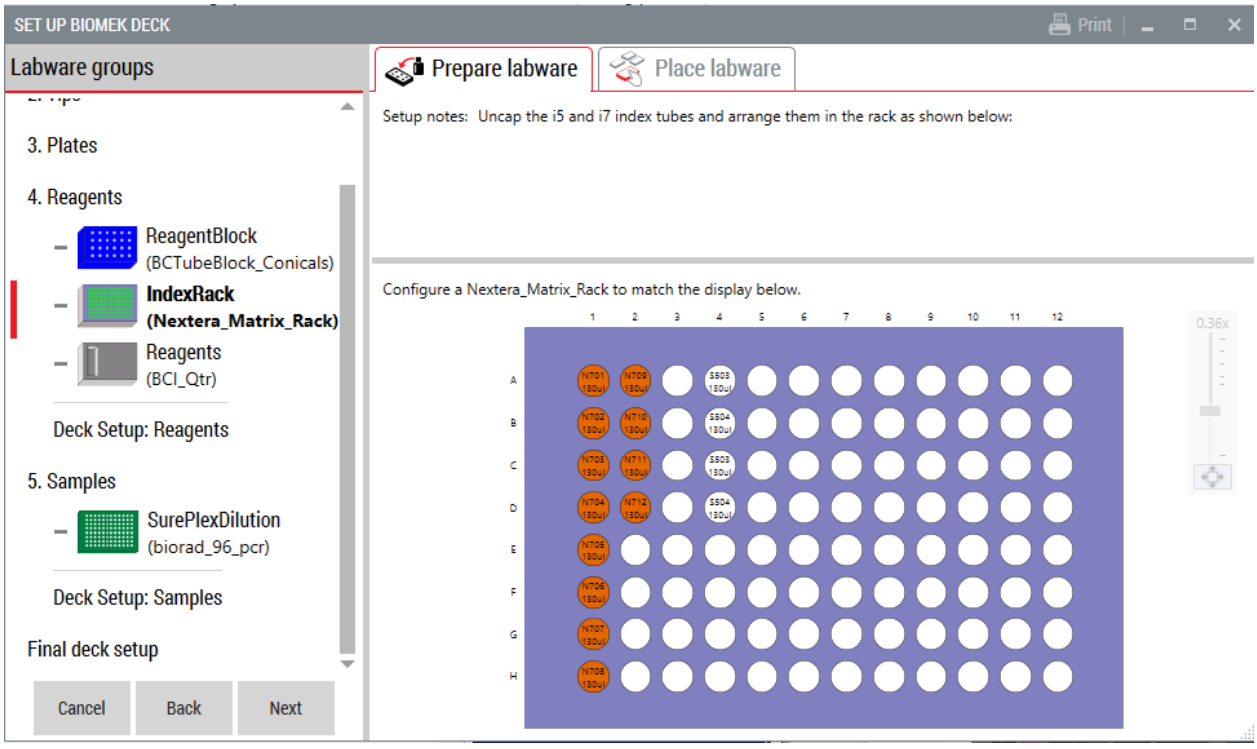

Figure 5B. Guided Labware Setup showing Indexes layout

Experimental Design

The Illumina VeriSeq PGS Kit was run with a batch of 24 samples with replicates of Male 1-Cell, Female 1-Cell and water. Qubit quantification results after cleanup showed at least sufficient yield and even high yield (>1.5ng/uL: high yield, 1-1.5ng/uL:sufficient yield).

Results

A robust and reliable automation solution for NGS library prep is essential to take full advantage of Illumina's sequencing technology. Automation saves up valuable time and money and helps the researchers to obtain an efficient process with more walk-away time.

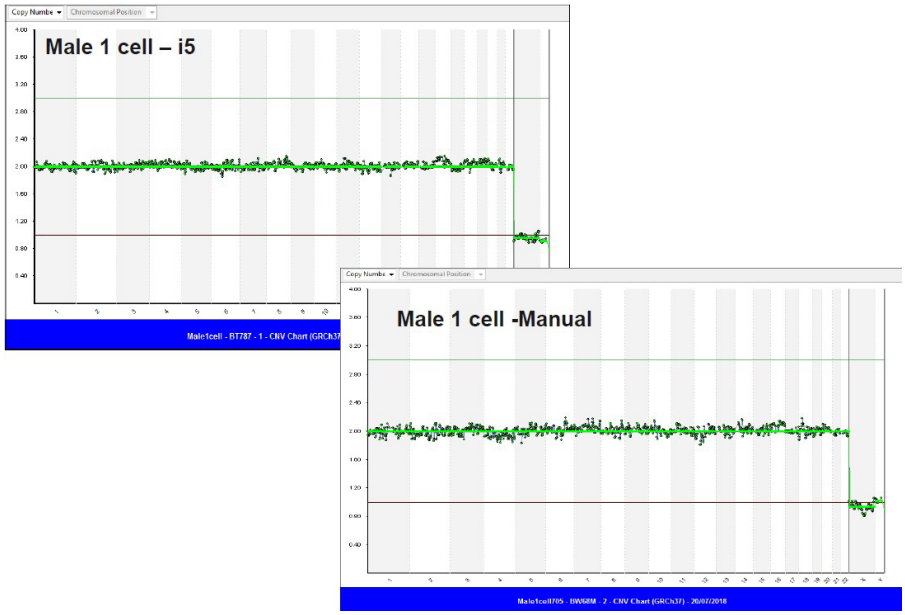

Figure 6. Copy Number Variant (CNV) profile of the male sample compared with Biomek i5 and manual run

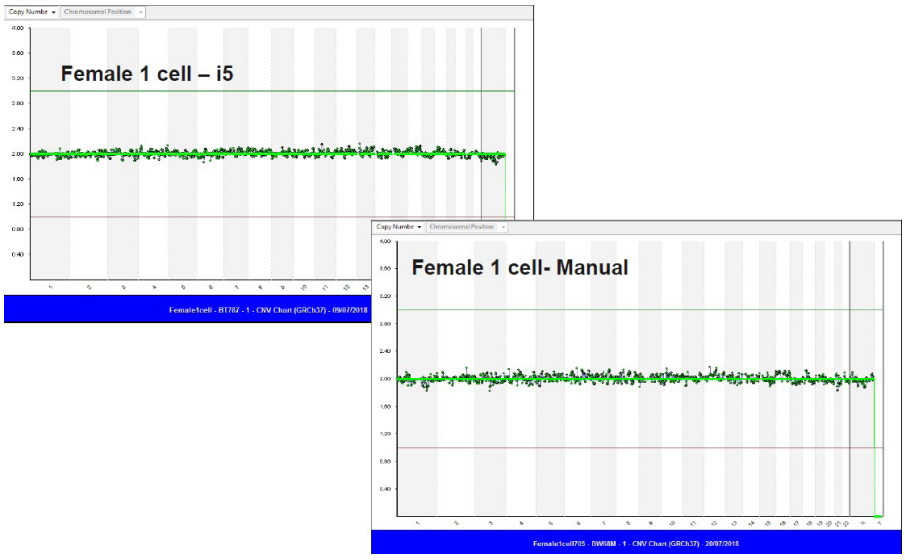

Figure 7. Copy Number Variant (CNV) profile of the female sample compared with Biomek i5 and manual run

Summary

We've demonstrated automation of VeriSeq for Illumina Library preparation kit on the Biomek i5 Span-8 Genomics Workstation. The solution delivers libraries that yield comparable results. It is flexible and scalable thus providing fast and efficient library construction.

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