Automated Illumina TruSeq Custom Amplicon Low Input Library Preparation Kit on the Beckman Coulter Biomek FX^P Automated Liquid Handler.

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Introduction and Method Description

The ability to rapidly sequence selected regions of a genome with high levels of coverage is essential for many applications and market segments ranging from the characterization of rare variants in FFPE cancer research to identification of genotype within large sample populations via Genotyping by Sequencing (GBS) in Agrigenomics. The Illumina TruSeq Custom Amplicon Low Input Library Preparation kit (FC-134-2001) allows for the characterization of up to 1536 amplicons spanning approximately 600kb in a single multiplex reaction for each sample. The kit supports input DNA amounts as low as 10 ng per sample and can be used with FFPE DNA samples depending on the quality of the sample. The kit is intended for use with customer designed panels developed using DesignStudio (**http://www.illumina.com/informatics/research/experimental-design/designstudio.html**).

In this technical note, we describe the automation of the Illumina TruSeq Custom Amplicon Low Input Library Preparation kit on the Beckman Coulter Biomek FX^P Dual Arm Multi-channel 96 and Span-8 automated liquid handler (Biomek FX^P). The automation method utilizes an intuitive HTML-driven user interface (UI) to provide a simplified and efficient user experience. The UI provides the user with a number of options to configure the automation workflow. The user may specify the use of internal controls provided in the Illumina TruSeq Custom Amplicon Low Input Library Preparation kit, the number of samples to process, and how much of the workflow to perform. The UI allows the user to configure a variety of options around index deployment, including deploying the i5/i7 primer tubes directly on deck or using a pre-prepped index plate and either using automatic index assignment to sample wells or utilizing a csv formatted file to specify the deployment of the indices. The UI also provides additional flexibility in allowing the choice between off-deck incubations using an external thermocycler or on-deck incubations with a Biometra TRobot thermocycler integrated to the Biomek FX^P liquid handler for maximized walk-away time. The method utilizes Guided Labware Setup to guide the user through setting up the instrument with clear instructions for the placement of labware and reagents on the deck. If desired, the method can be run through the Biomek Method Launcher run time interface for a simplified run time experience. The automation method can prepare up to 96 dual-indexed Illumina TruSeq Custom Amplicon Low Input libraries in approximately 6.5 hours including incubations and a 29 cycle PCR amplification.

Figure 1. Beckman Coulter Biomek FX^P

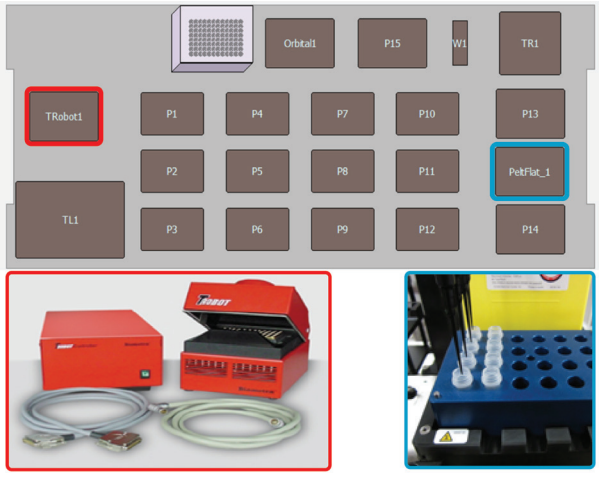

Figure 2. Biomek FX^P deck configuration (top). Biometra TRobot (bottom left) offers fully automated enzymatic incubations and thermocycling while the Static Peltier unit (bottom right) provides chilled enzyme master mix storage during the course of the run.

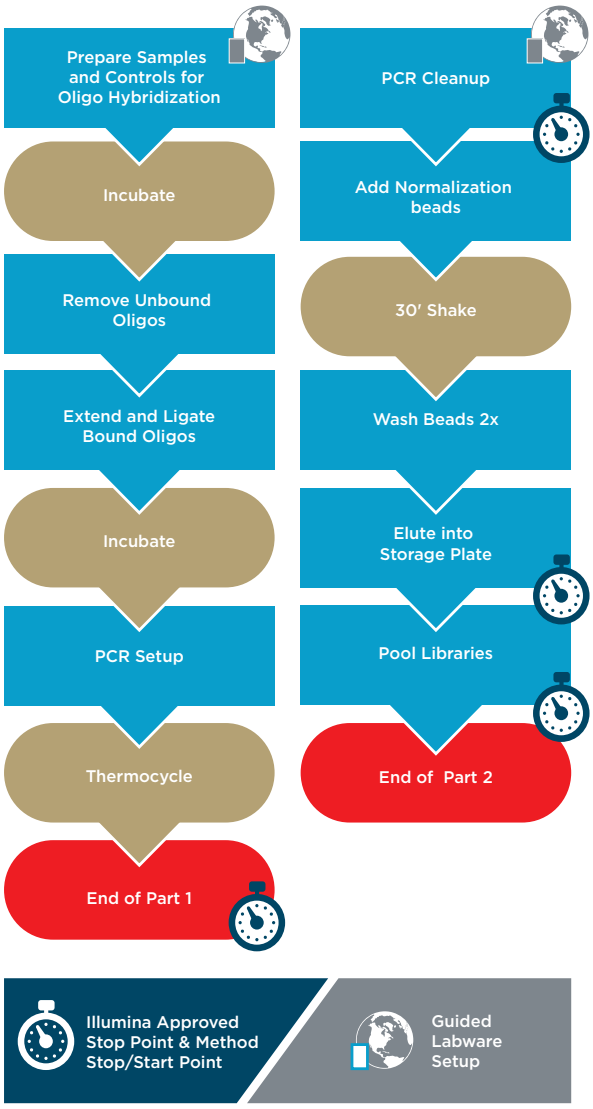

Figure 3. Biomek FX^P automation workflow for the Illumina TruSeq Custom Amplicon Low Input kit.

Major Process Description	Automated/Hands on Time		
	24 Samples	48 Samples	96 Samples
Library Construction: Part 1			
Method Run	3 hr, 16 min	3 hr, 41 min	4 hr, 20 min
Post Library Construction: Part 2			
Method Run	1 hr, 47 min	1 hr, 56 min	2 hr, 13 min
Total	**5 hr, 3 min**	**5 hr, 36 min**	**6 hr, 33 min**

**Timing includes 2 hr, 22 min of incubation and thermocycling. Timing does not include thawing of reagent

Figure 4. Biomek FX^P automation method time estimates for the Illumina TruSeq Custom Amplicon Low Input kit.

Experimental Design and Results

To test the automation method, two automated library construction runs were performed as follows. Universal Human Reference DNA (Promega) was quantified using PicoGreen (Life Technologies) in conjunction with the SpectraMax i3 (Molecular Devices) and a 2.5 ng/ul master stock was created. From this stock, 86 10 ng technical replicates were created in a PCR plate. Eight water only negative controls were included on the sample plate for a total of 94 sample wells. The inline controls provided in the Illumina TruSeq Custom Amplicon kit (2800M positive control and RS1 negative control) would also be set up during the course of the automation method. The Illumina TruSeq Cancer Amplicon Panel, a panel of 212 amplicons covering a total of 35kb of the human genome containing 48 oncogenes, was used as the amplicon panel for this experiment. Incubations and the 29 cycle PCR amplification were performed in an off-deck thermocycler. Following post-PCR purification, the pre-normalized libraries were assayed on the 2200 TapeStation (Agilent) using D1000 ScreenTape. A collection of electopherogram traces from the second automation run are shown in Figures 5, 6, and 7.

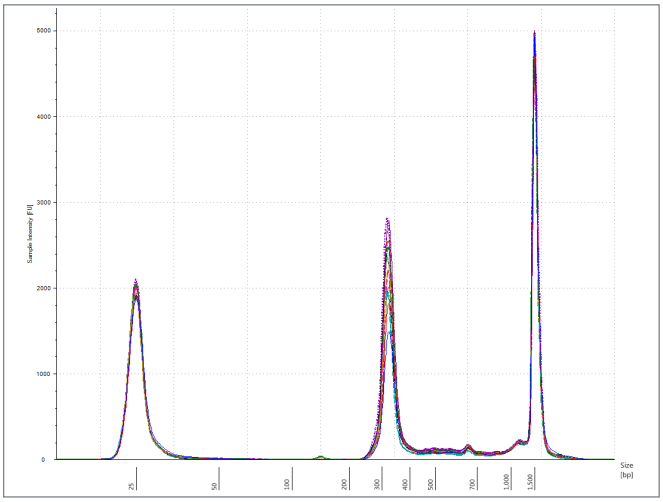

Figure 5. 14 TruSeq Custom Amplicon 10ng technical replicate TruSeq Custom Amplicon Low Input libraries

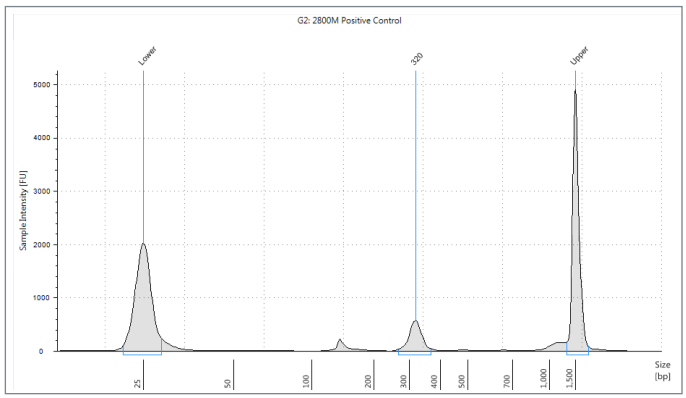

Figure 6. 2800M internal positive control TruSeq Custom Amplicon Low Input library.

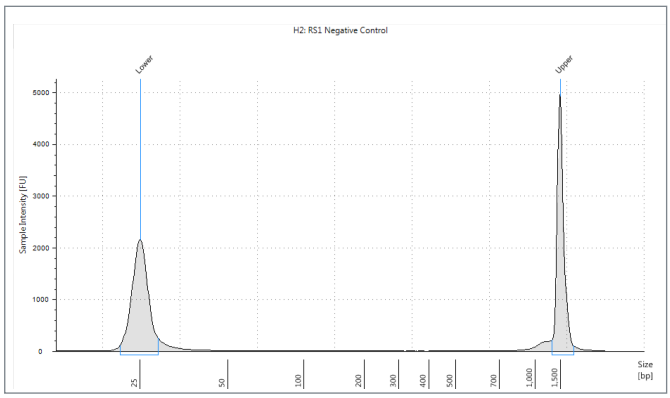

Figure 7. RS1 internal negative control TruSeq Custom Amplicon Low Input library

Following post-PCR cleanup, the libraries created in each automation run were subjected to bead-based normalization and sequenced on an Illumina MiSeq system using a 600 cycle v3 MiSeq sequencing kit. The first run yielded a total of 26.2 million pass filter reads with 94.1% of reads Q30 or higher. Run 2 yielded 25.1 million pass filter reads with 94.3% of reads Q30 or higher. Libraries were analyzed on BaseSpace (basespace.illumina.com) using the TruSeq Amplicon App with the TruSeq Cancer Amplicon manifest with default parameters. Average read mapping was 94% for Run 1 and 95% for Run 2 respectively. Percent mapped reads are shown in Figure 8 for Run 1 and Figure 9 for Run 2.

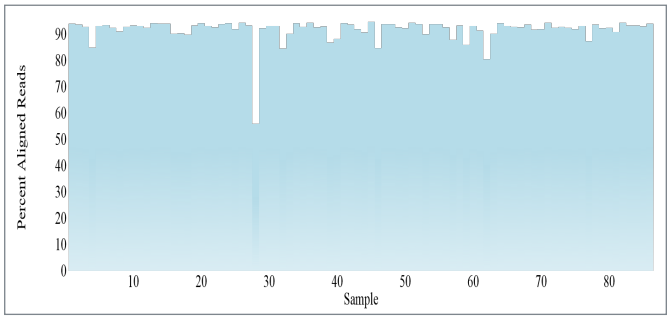

Figure 8. TruSeq Custom Amplicon Low Input Run 1 Percent Alignment

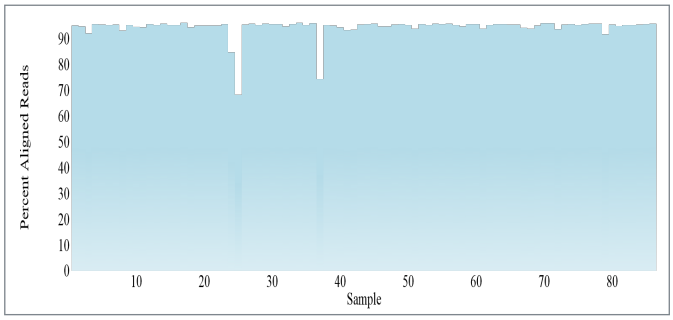

Figure 9. TruSeq Custom Amplicon Low Input Run 2 Percent Alignment

In Run 1, average amplicon coverage was 2401X ± 386.7X, while average amplicon coverage in Run 2 was 2368X ± 512X. Average uniformity of coverage was greater than 96% for both runs. Amplicon panel coverage is shown for each library for Run 1 (Figure 10) and Run 2 (Figure 11).

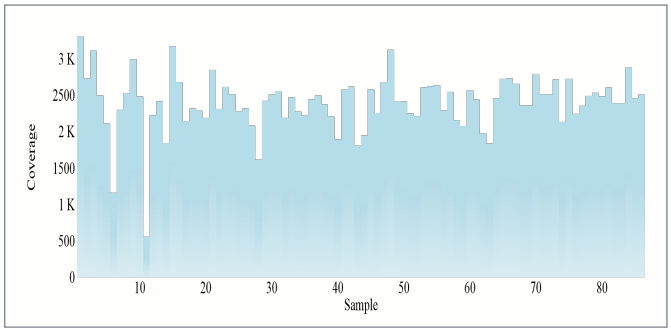

Figure 10. Amplicon panel coverage by library from TruSeq Custom Amplicon Low Input Run 1.

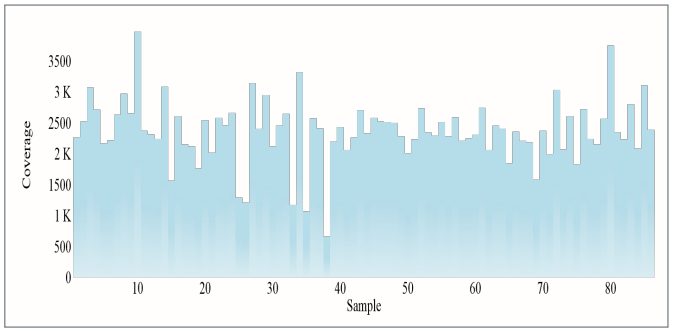

Figure 11. Amplicon panel coverage by library from TruSeq Custom Amplicon Low Input Run 2.

When looking at amplicon coverage for individual regions of interest, the results from Run 1 and Run 2 show no significant difference between Run 1 and Run 2 (F = 1.008, p=0.47), as shown in Figure 12.

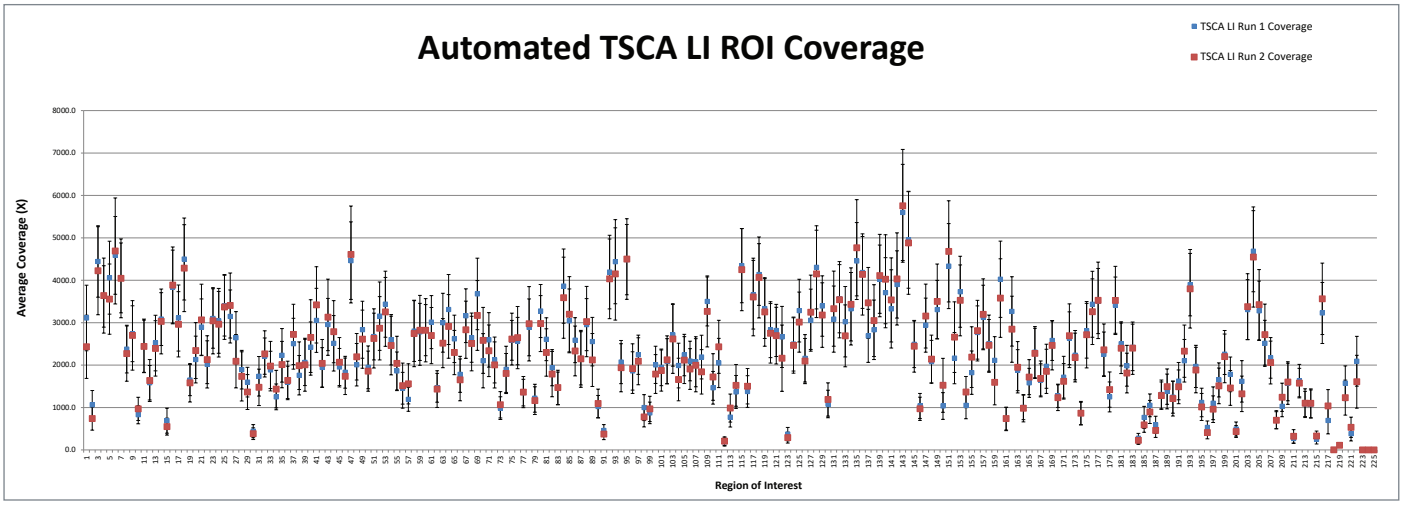

Figure 12. Average coverage of each TruSeq Amplicon Cancer Panel Region of Interest (n=86 for each run). Error bars are calculated standard deviation.

Conclusion

We have demonstrated that the Biomek FX^P automation method for Illumina TruSeq Custom Amplicon Low Input Library Preparation generates high quality, sequence ready libraries that provide deep, uniform coverage of the TruSeq Custom Amplicon Cancer Panel.

Research use only. Contact Beckman Coulter sales consultant for a system quotation at **www.beckman.com**.
The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at **beckman.com**

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