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Automation of library preparation for Chromatin immunoprecipitation (ChIP–Seq) on Biomek i7

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Identification of the importance of DNA–protein interactions, such as transcription factor binding and histone modifications, has expanded our understanding of gene regulation, genome integrity and chromatin organization. Chromatin Immunoprecipitation (ChIP) is a commonly used technique to study DNA–protein interactions and is based on the enrichment of DNA along with the associated proteins. In short, antibodies against the proteins of interest are used to isolate the proteins along with the bound DNA. Subsequently, this DNA is released and sequenced to gather information about where the protein of interest binds across the genome. The coupling of ChIP with high–throughput sequencing analysis (ChIP–seq) enables identification of DNA protein interactions in an unbiased manner. Compared to ChIP followed by microarray (ChIP–chip), ChIP–seq offers better data quality, higher resolution, less noise, higher genome coverage and broader dynamic range. As the cost of sequencing continues to decrease, ChIP–seq has become the method of choice to study DNA–binding proteins and histone modifications in a genome–wide manner with base pair resolution[1, 2].

Despite the advances in ChIP–seq technologies, there are limitations of using the technology in many applications. Primarily, the library preparation step for ChIP–seq is time consuming and cumbersome, especially when the required throughput is high[1,2]. Also, as the immunoprecipitated DNA samples are of very limited quantity, the library preparation method must be sensitive and reliable, in order to minimize sample loss. Due to these factors, the quality of the data obtained from ChIP–seq experiments varies according to the skill and experience of the user. To overcome this limitation, we automated the widely used Swift Accel–2S® DNA library kit for ChIP–seq on the Beckman Coulter Biomek i7 Hybrid Workstation. The Swift Biosciences Accel–2S® DNA library kit automated on the Biomek platform provides:

- Reduced hands–on–time for the user
- Increased throughput compared to manual operation
- Reduction in pipetting errors compared to manual operation
- Standardized workflow for improved consistency
- Quick start–up with ready–to–implement methods

Spotlight

Biomek i7 Hybrid (Multichannel 96, Span-8) Genomics Workstation System features delivers reliability and efficiency to increase user confidence and walk-away time, compared to manual operation (Figure 1):

- 300 µL or 1200 µL Multichannel head with 1-300 µL and 1-1200 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers optimize access to high-density decks
- 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. Biomek i7 Hybrid (Multichannel 96, Span-8) Workstation equipped with high capacity, 45 position deck

Automated Method

After initial deck setup, the automated Swift Biosciences Accel-2S® DNA library preparation method processes 96 samples in less than 7 hrs (Table 1). After the initial setup, the method requires no further user interaction. In order to simplify method editing, modifications, and set up the Demonstrated Method Interface consists of three components:

1. Biomek Method Launcher (BML)
2. Method Option Selector (MOS)
3. Guided Labware Setup (GLS)

Process	Time (96 samples; i7 Hybrid)
Instrument setup*	30 min
Method run	6 hr 40 mins
Total	7 hrs 10 mins

Table 1. Run times for Swift Biosciences Accel-2S® DNA library preparation method automated on Biomek i7 Hybrid workstation.

*Timing does not include kit reagent thawing and homogenization.

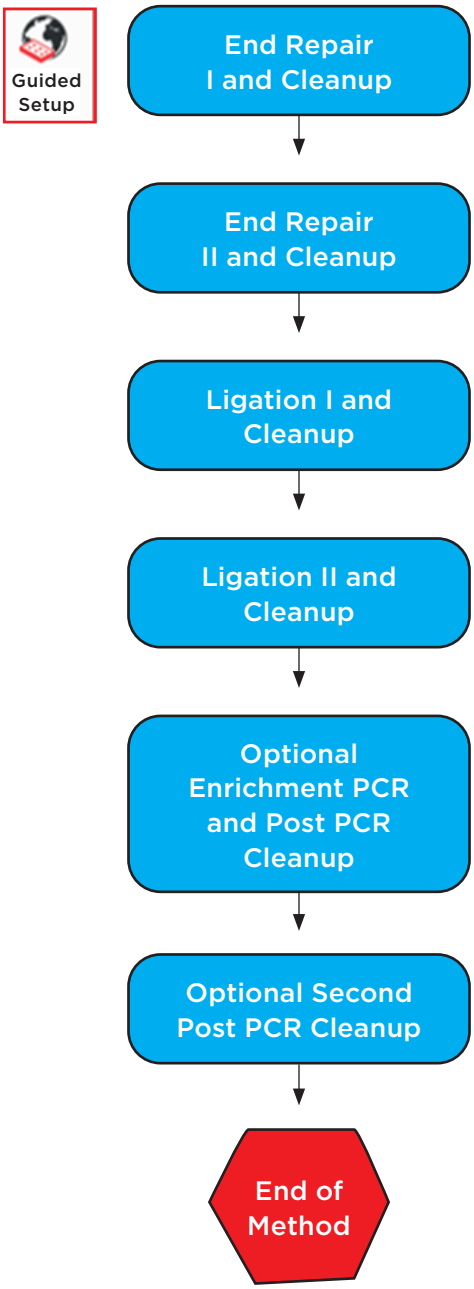

Figure 2. Automated Swift Biosciences Accel-2S® DNA library preparation workflow using Biomek i7 Hybrid workstation.

1. Biomek Method Launcher (BML)

BML is a secure interface for executing methods without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interfere with the method if needed.

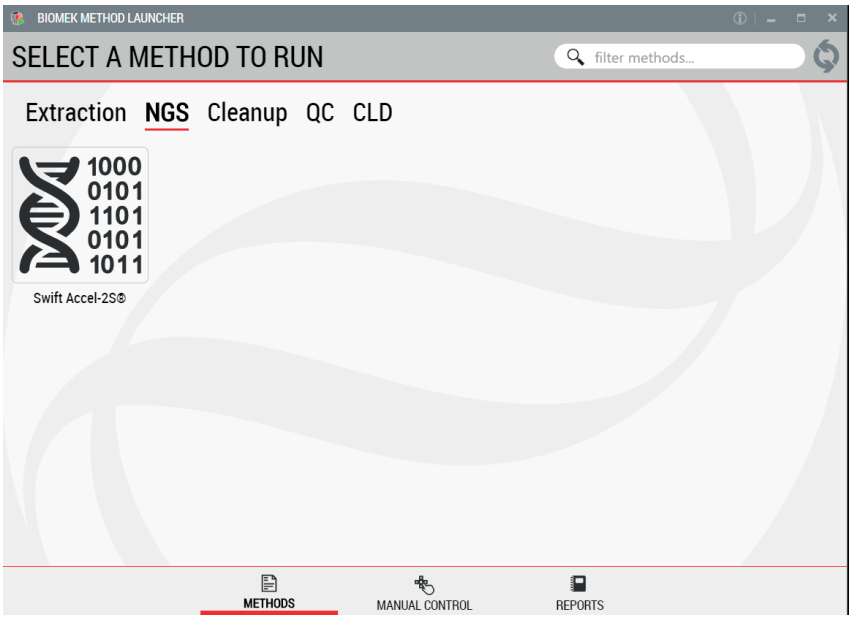

Figure 3. BML provides an easy interface to start the method.

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. The automated method is designed in a modular manner, to allow for ease of deployment in the laboratory based on the user's needs both before and after PCR amplification. Apart from the ChIP-seq workflow, the Swift Biosciences Accel-2S® DNA library preparation kit can be used for PCR-free whole genome sequencing and targeted sequencing by hybridization capture. This can be accomplished with a wide range of input quantities and a variety of sample types, including microbial samples, FFPE, and cell-free DNA (cfDNA). The Swift Accel-2S for Biomek i7 automated method represents a complete implementation of all the sample flexibility that is available in the manual protocol, including the following options:

- Ability to process any number of samples from 1 to 96
- Ability to start the method with either low input gDNA (< 10 ng), high input gDNA (10–250 ng), FFPE DNA, ChIP DNA, or cfDNA
- Variable size selection options for 200bp, 350bp, 450bp, or library preparation for hybridization capture
- Support for a variety of indexing strategies, including Single Indexed Adapter Kits, MID-Indexed Adapter Kits, Combinatorial Dual Indexed Adapter Kits, 2S Dual Indexing Kit, and the 2S Unique Dual Indexing Kit
- Automatic index transfer or file-driven custom index transfers
- Variable Enrichment PCR cycles

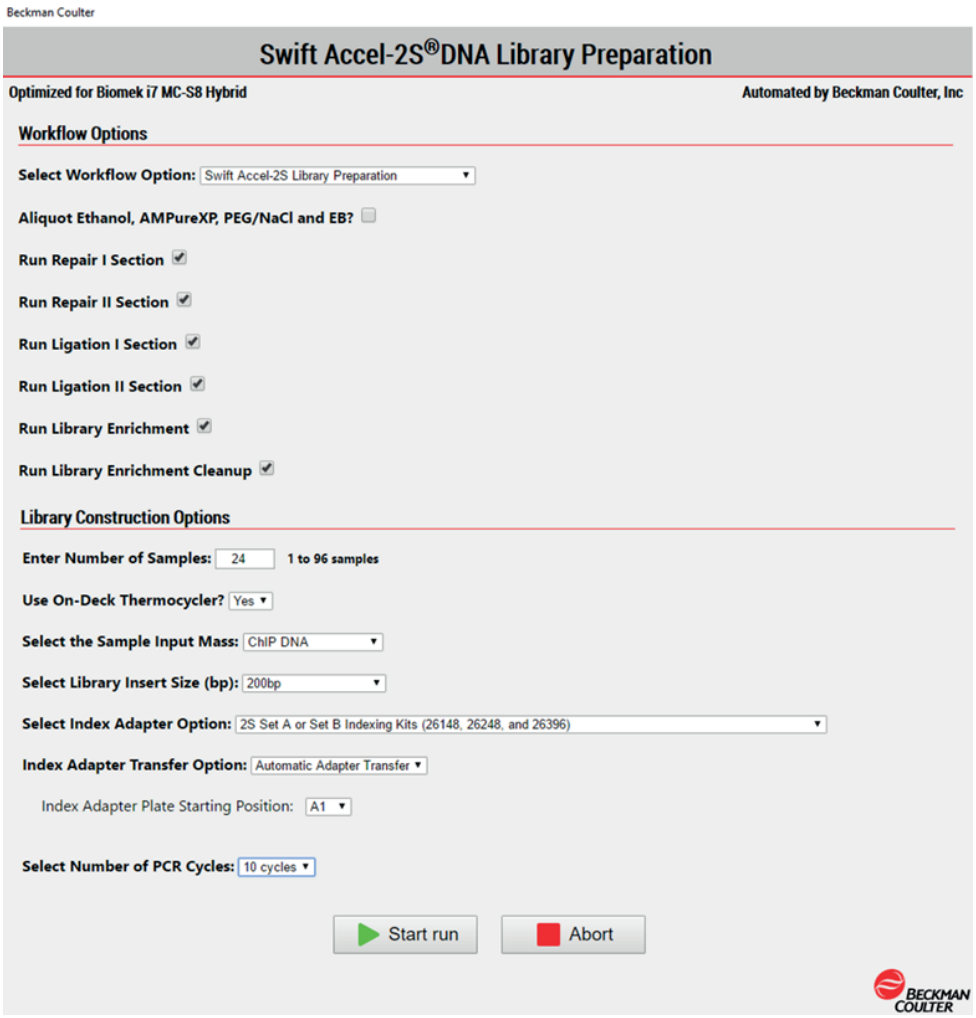

Figure 4. Biomek MOS is used to define sample number and processing options for the Swift Biosciences Accel-2S® DNA library kit workflow.

3. Guided Labware Setup (GLS)

GLS is used to inform the user how to load the deck prior to running the experiment. GLS is generated based on the options that are selected by the user in the MOS. It provides user-specific text and graphical setup instructions, along with reagent volume calculations and step-by-step instructions to prepare reagents for the library preparation workflow to be performed.

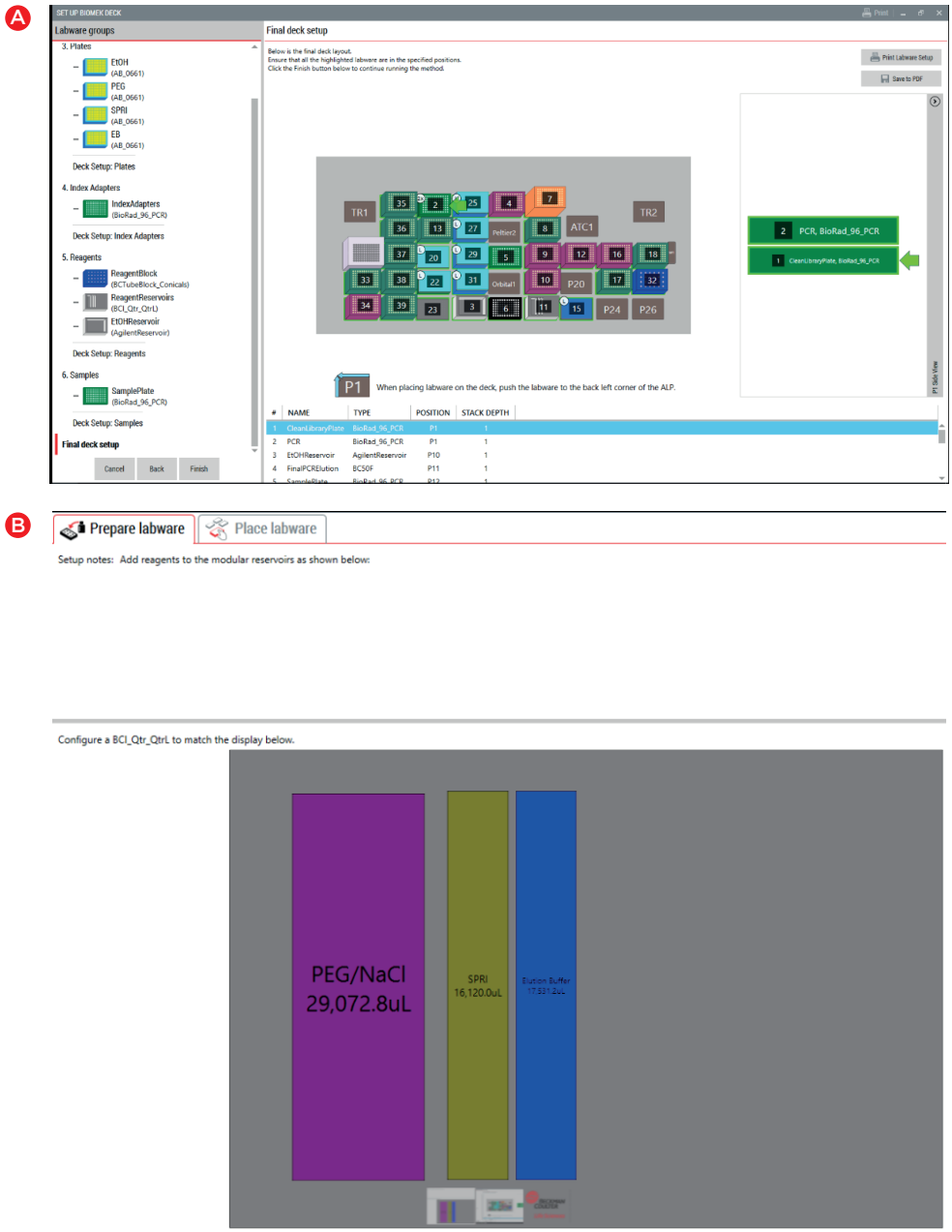

Figure 5. (a–b) GLS indicates correct deck setup and reagent volumes for user-defined experiment.

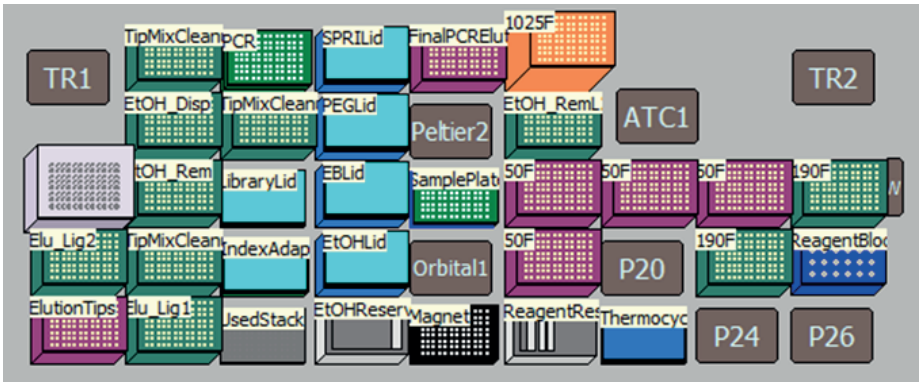

Figure 6. The Biomek i7 deck layout for automated Swift Biosciences Accel-2S® DNA library preparation workflow (96 samples).

Experimental design

ChIP was performed using a standard ChIP-seq protocol[3] from 1X106 MV411 cells. Chromatin was incubated with antibodies targeting transcription factors MED1, MYC, MYB, SPI1, as well as POLII. ChIP DNA samples were quantified by Qubit Fluorometer prior to library preparation on the Biomek i7 using Swift Biosciences Accel-2S® DNA library preparation reagents at the Molecular Biology Core Facilities at Dana-Farber Cancer Institute. Finished libraries were quantified by Qubit Fluorometer, Agilent TapeStation 4200 and RT-qPCR using the Kapa Biosystems library quantification kit. Uniquely dual indexed libraries were pooled and sequenced on an Illumina NovaSeq 6000 with paired-end 50 bp reads. Reads were aligned to the UCSC hg19 reference genome assembly using bwa (v0.7.17) and peak calling was performed using MACS2 (v2.1.2)[4-5].

Results

For 14 of 16 samples, ChIP DNA samples were below the detection limit of Qubit fluorometer (ThermoFisher,). Following library preparation using Swift Biosciences Accel-2S® DNA reagents on the Biomek i7, sequencing library yields observed were greater than 7 ng/ul for each sample tested (Table 2). These libraries were then characterized by Agilent TapeStation (Agilent, Figure 8) in order to analyze the quality of the libraries that were prepared on the i7. As shown in Figure 7, the libraries exhibited the expected size distributions. Notably, there were no adapter dimers detected in any of the libraries. All libraries produced >20M read pairs with high mapping rates (>86%) against the hg19 human reference genome (Figure 8) with low duplication rates; PCR bottleneck coefficients > 97 (Figure 9)[6]. Taken together, these results indicate that automation using the Beckman i7 Hybrid workstation maintains the advantages of the Accel-2S® kit, specifically minimal duplication and adapter dimerization while maintaining high DNA yields.

Sample	Concentration (ng/µL)	Input Amount	Library Yield (ng/µL)
MBCF1	Too Low	undetectable	19
MBCF2	Too Low	undetectable	13.2
MBCF3	0.734	7.34	46.4
MBCF4	Too Low	undetectable	10.9
MBCF5	Too Low	undetectable	27.6
MBCF6	Too Low	undetectable	30.2
MBCF7	Too Low	undetectable	24.4
MBCF8	Too Low	undetectable	13
MBCF9	Too Low	undetectable	29.2
MBCF10	Too Low	undetectable	19.5
MBCF11	Too Low	undetectable	15.1
MBCF12	Too Low	undetectable	7.42
MBCF13	Too Low	undetectable	10.6
MBCF14	Too Low	undetectable	7.7
MBCF15	0.116	1.16	22.2
MBCF16	Too Low	undetectable	21.6

Table 2. DNA quantification following ChIP and subsequent Swift Biosciences Accel-2S® DNA library preparation. DNA concentrations were measured using Qubit fluorometer (ThermoFisher).

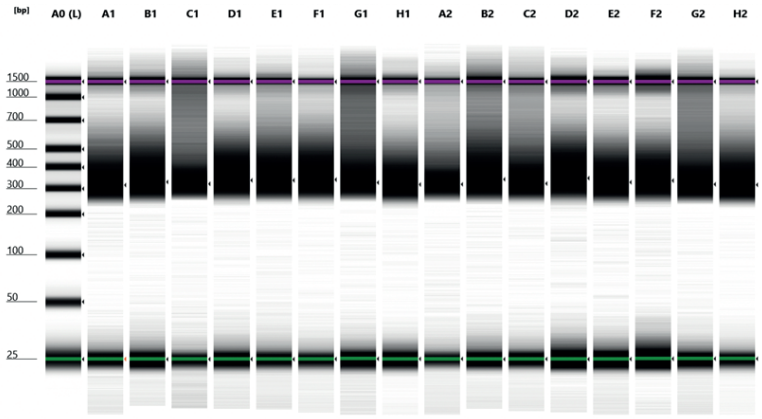

Figure 7. TapeStation (Agilent) Gel Library Fragment Size Distribution Profiles. A0: ladder, A1–H2: DNA libraries prepared using Accel-2S® DNA kit on Biomek i7 Hybrid workstation.

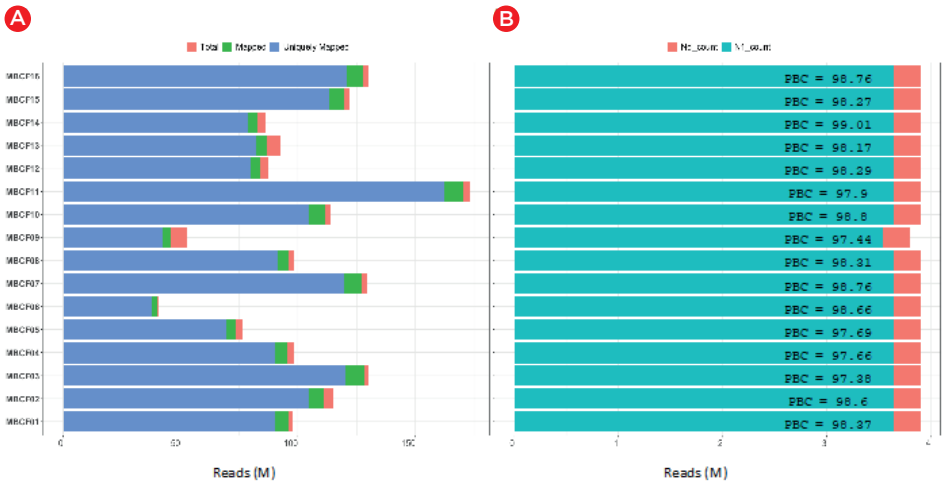

Figure 8. Read Alignment Summary: (a) Total reads, mapped reads, and uniquely mapped reads for each sample. (b) The number of genomic locations with at least one unique mapping read (Nd), the number of genomic locations with exactly one uniquely mapping read (N1) from a down-sampled number of total reads and the PCR bottleneck coefficients (PBC).

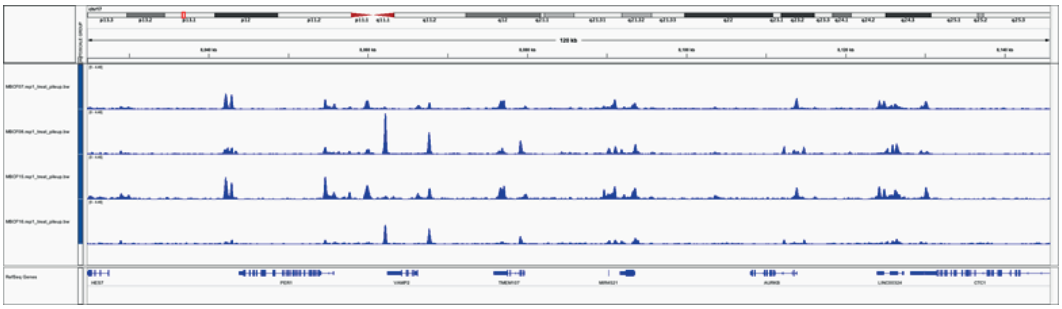

Figure 9. ChIP Peak Coverage Plots. Read coverage plots are shown for samples MBCF07, MBCF08, MBCF15, and MBCF16.

Summary

ChIP–seq is a widely used method for studying DNA–protein interactions and gene expression regulation. Following the immunoprecipitation step, DNA yields are often very low, and the samples are often quite precious. Here we show how automating the Swift Accel–2S® DNA kit using a Biomek i7 Hybrid workstation can robustly produce high quality ChIP libraries from low or undetectable amounts of ChIP DNA at scale.

References

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