Biomek i-Series Automated Beckman Coulter Agencourt SPRIselect for DNA Size Selection

Introduction

The library preparation process for Next Generation Sequencing requires a uniform distribution of nucleic acid fragments within a specific size range. Using the Beckman Coulter Agencourt SPRIselect size selection method, the size distribution can be adjusted using left, right and double size selection to meet the specifications defined per application. Left size selection uses a high volume ratio of SPRIselect sample to remove smaller genomic material on the left side of the size distribution, keeping the larger fragments. The right size selection removes genomic material on the right side of the size distribution to keep smaller fragments. Double size selection on the other hand, removes genomic material on the left and on the right to keep "size selected" material in the interval yielding a tight fragment size distribution (Figure 1). First, the large fragments are removed and the supernatant is retained. Thereafter, a small volume of SPRIselect reagent is added and the supernatant is removed after magnetic separation. After an ethanol wash, elution buffer is added and the eluted material is transferred into a new plate. The SPRIselect method uses Agencourt's patented SPRI® paramagnetic bead-based technology to bind DNA to paramagnetic beads followed by washing away unwanted fragments (Figure 1). This magnetic separation makes the kit amenable to automation, as it eliminates the need for vacuum filtration or centrifugation. As a result, the process can be scaled for low to high throughput workflows. Here we demonstrate automated performance of Agencourt SPRIselect size selection method on the Biomek i5 Span-8 and Biomek i7 hybrid Genomics Workstations.

The Agencourt SPRIselect Kit automated on Biomek platforms provides:

- Reduced hands-on-time and increased throughput compared to the manual protocol
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Knowledgeable support for reagents, automation and methods all from single vendor
- File-driven, per well size selection

Spotlight

Biomek i5 Span-8 liquid handling platform

System features deliver trusted reliability and efficiency to increase walk-away time compared to the manual operation:

- Span-8 with 1-1000uL pipetting capability
- Independent 360˚ rotating gripper with offset fingers for efficient and reliable labware movement
- 25 positions for increased walk away time
- Active ALPs for controlling sample processing – Orbital shakers, peltiers and tip washing
- Optional items such as enclosure and on- deck integrations (e.g. ATC Thermo Fisher, Biometra T-Robot)

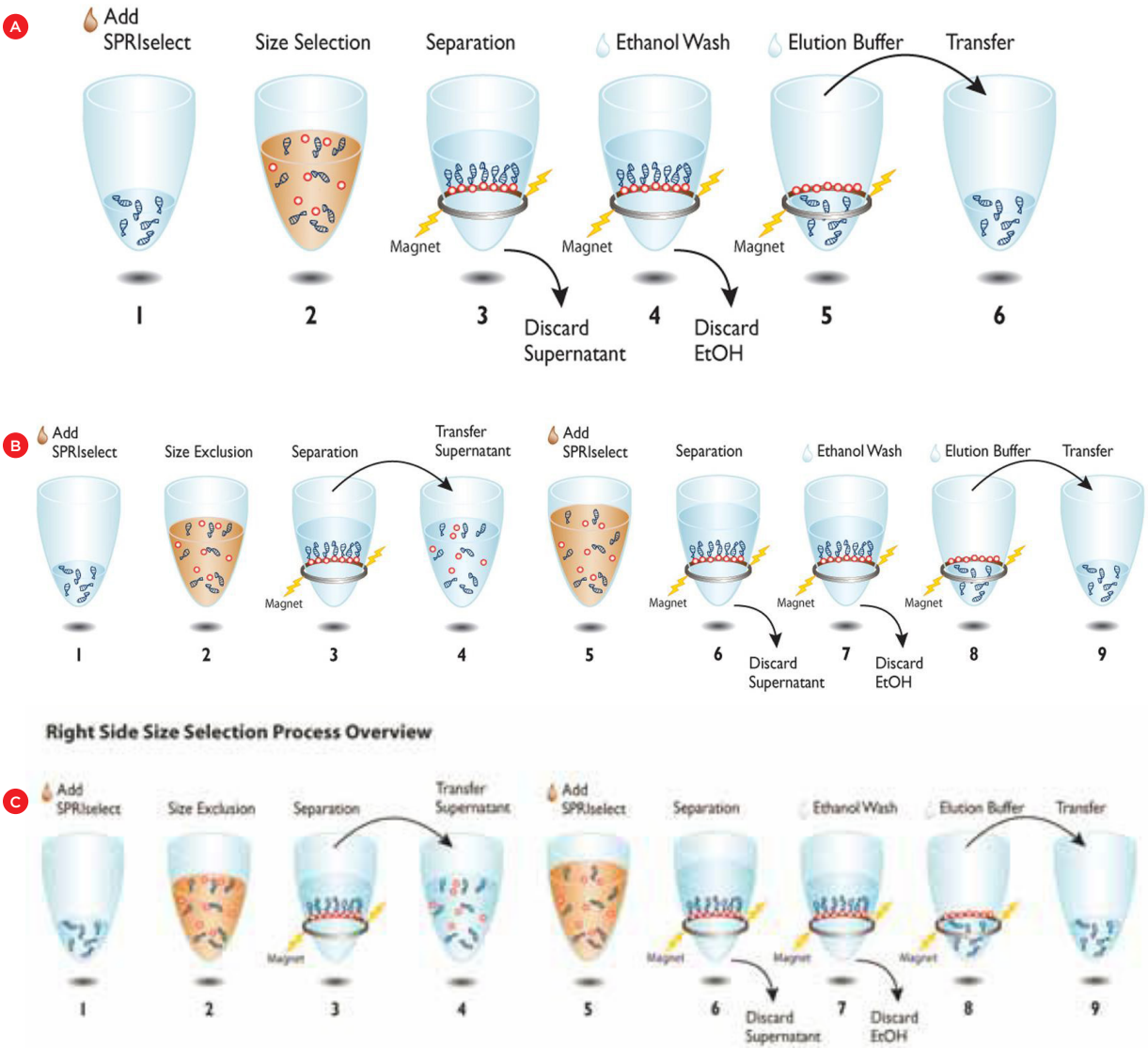

Figure 1. Beckman Coulter Agencourt SPRIselect Size Selection Method Workflows.

Left (a) right (b) and double (c) size selection.

Automated method

Automation provides increased efficiency, reducing the hands on time (Table 1). The use of Biomek Method Launcher simplifies the method implementation and reduces the introduction of errors during method setup.

Process	Time: i7 Hybrid method (96 samples)			Time: i5 Span-8 method (96 samples)		
	Left size selection	Right size selection	Double size selection	Left size selection	Right size selection	Double size selection
Instrument setup*	15 mins	15 mins	15 mins	15 mins	15 mins	15 mins
Method run	34 mins	44 mins	45	52 mins	1 hr 10 mins	1 hr 13 mins
Total	49 mins	59 mins	1 hr	1 hr 7 mins	1 hr 25 mins	1 hr 28 mins

Table 1. Estimated run times for Agencourt SPRIselect Size Selection Method.

*Timing does not include reagent thawing and homogenization. Note: i7 Hybrid method uses both Span-8 and Multichannel pods.

1. Biomek Method Launcher (BML)

BML is a secure interface for selecting 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 interference, if needed.

Figure 2. Biomek Method Launcher provided 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. To reduce the time of manual setup, the method provides options to aliquot reagents into processing plates, from the reservoirs. For each workflow, there are two options to input the size ratios. The size ratios can be uploaded in a custom csv file, providing the flexibility to use different ratios for different samples (Figure 4). Alternatively, if the "one size" option is selected, the ratios can be specified in the MOS. Additionally, the timings of size selection procedure and the volumes can be customized through the MOS, to suit the user needs.

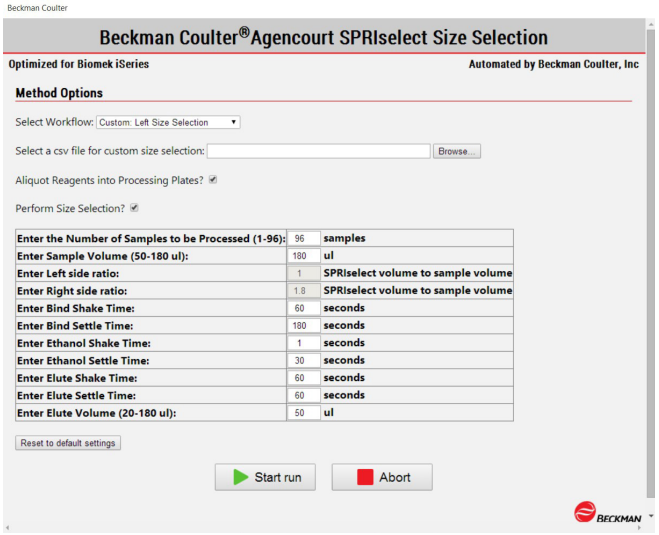

Figure 3. Biomek Method Options Selector indicate sample number and processing options

Sample	SampleWell	SizeOptionLeft	SizeOptionRight	LeftRight	SelectPlate1ID
Sample1	A1	0.2	0.1	0.1	Cleanup_plate1
Sample2	B1	0.2	0.1	0.1	Cleanup_plate1
Sample3	C1	0.2	0.1	0.1	Cleanup_plate1
Sample4	D1	0.2	0.1	0.1	Cleanup_plate1
Sample5	E1	0.2	0.1	0.1	Cleanup_plate1
Sample6	F1	0.2	0.1	0.1	Cleanup_plate1
Sample7	G1	0.2	0.1	0.1	Cleanup_plate1
Sample8	H1	0.2	0.1	0.1	Cleanup_plate1
Sample9	A2	0.2	0.1	0.1	Cleanup_plate1
Sample10	B2	0.2	0.1	0.1	Cleanup_plate1
Sample11	C2	0.2	0.1	0.1	Cleanup_plate1
Sample12	D2	0.3	0.1	0.2	Cleanup_plate1
Sample13	E2	0.3	0.1	0.2	Cleanup_plate1
Sample14	F2	0.3	0.1	0.2	Cleanup_plate1
Sample15	G2	0.3	0.1	0.2	Cleanup_plate1
Sample16	H2	0.3	0.1	0.2	Cleanup_plate1
Sample17	A3	0.3	0.1	0.2	Cleanup_plate1
Sample18	B3	0.3	0.1	0.2	Cleanup_plate1
Sample19	C3	0.3	0.1	0.2	Cleanup_plate1

Figure 4. An example .csv file used for the custom size selection

3. Guided Labware Setup (GLS)

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

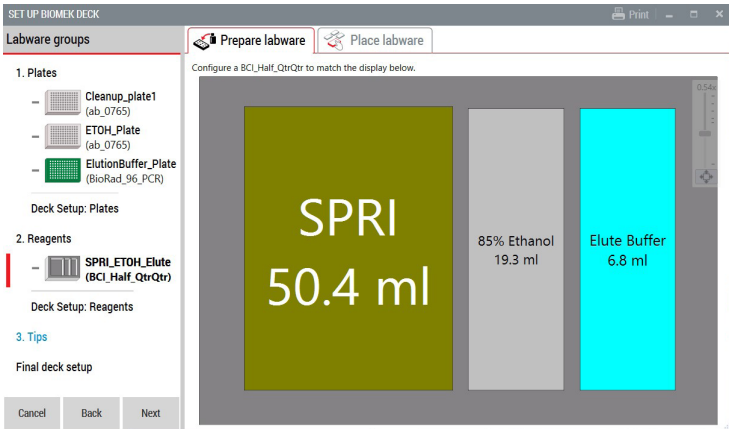

Figure 5. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

Experimental design

Promega human genomic DNA (100 µg) was sheared to fragments of 600 bp average size using a Covaris E210 instrument. The selected SPRIselect volume to sample volume ratios are indicated in the Table 2. Each size selection was done in three replicates. The data was collected using automated Agencourt SPRIselect size selection method on i7 hybrid workstation. The size distribution of shears and size selected fragments were evaluated using Agilent TapeStation D5000 screen Tape.

Selection type	SPRIselect volume to sample volume ratio	Expected Fragments
Left	0.65	Larger fragments ~700-900 bp average
Right	0.95	Smaller fragments ~150-100-200 bp average
Both	Left:0.9; Right:0.5	A tight distribution of fragments ~300-350 bp

Table 2: The size ratios used in the automated Agencourt SPRIselect size selection protocol

Results

All three size selections yielded the fragments of the required size (Figure 6).

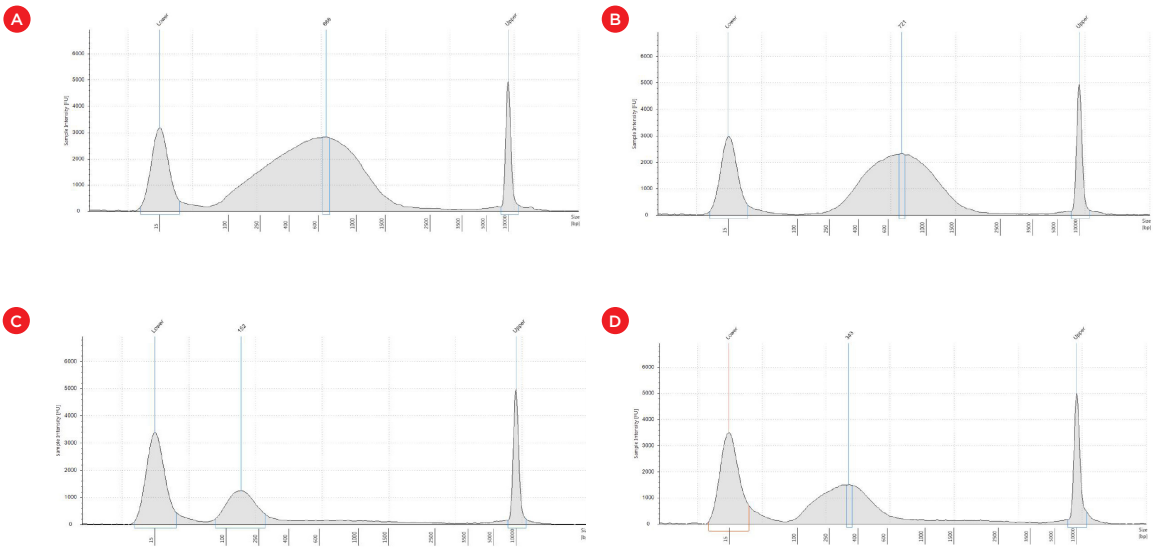

Figure 6. Fragment distribution in Agilent TapeStation corresponding to (a) Covaris shears: average size 668 bp, (b) left size selection: average size 721 bp, (c) right size selection: average size 152 bp and (d) double size selection: average size 343 bp. X axis: size (bp); Y axis: Sample intensity.

Summary

We demonstrated the successful automation of Agencourt SPRIselect size selection method on the Biomek i5 Span-8 and Biomek i7 Hybrid Workstations. Automation enabled quick and efficient size selection with the per well size- selection option. The Biomek Method Launcher provides a user friendly interface to run the method, without introducing errors.

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