Automation of NGS Sample Size Selection using SPRIselect

Technical Information Bulletin

Automation of NGS Sample Size Selection using SPRIselect on the Biomek 4000 Laboratory Automation Workstation

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Abstract

To streamline the NGS sample preparation process, Beckman Coulter has recently launched the SPRIselect Reagent Kit to allow NGS sample size selection to be automated on Biomek liquid handling platforms for high throughput and high efficiency sample preparations. The data presented here demonstrates the automation of the SPRIselect Kit on the Biomek 4000 Workstation. The demonstrated method will prepare 96 samples in 96-well plate format for small to medium throughput laboratories.

SPRIselect is a SPRI-based (Solid Phase Reversible Immobilization) paramagnetic bead technology. To demonstrate the capabilities of SPRIselect, three different shears of E. Coli gDNA ranging between 100 – 2000bp were pooled into a "Mega Shear" as the ratio (bead to sample volume) is altered. Our data demonstrates successful selections using 0.65x ratio for Left Side Selection, 0.7x ratio for Right Side Selection, and three ranges of Double Side Selections (0.9 L-0.5 R; 0.85 L-0.56 R; and 0.8 L – 0.61 R) on the Biomek 4000 platform.

Introduction

The Biomek 4000 Workstation and SPRIselect demonstrated method provide an excellent starting point for DNA fragment size selection. Under current deck setup, the estimated time to completion (ETC) for processing 96 samples is 1:09 for Left Side Selection, 1:40 for Right Side Selection and 1:38 for Double Side Selection. This demonstrated method is fast, simple, and can easily be adapted to test user's targeted ranges.

Using the Biomek 4000 Workstation and SPRIselect demonstrated method to generate data, we were able to process:

- Larger DNA fragments by Left Side Selection
- Smaller DNA fragments by Right Side Selection
- Certain ranges of DNA fragments by Double Side Selection

Materials and Methods

Reagents

Part No.	Manufacturer	Description
B23318	Beckman Coulter	SPRIselect Kit
5067-4626	Agilent Technologies	High Sensitivity DNA Reagents
5067-4626	Agilent Technologies	High Sensitivity DNA Chip
NA	Beckman Coulter	E. Coli Mega Shear, 20ng/µL, 2000bp
E7023-500mL	Sigma	Ethanol, 100% Proof
AB02123-00600	American Bioanalytical	Water, Nuclease-free/ Elution Buffer

Discovery *in motion.*

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Biomek 4000 Configuration (Tools, ALPs, Magnets, Reservoirs, and Consumables)

Type	Quantity	Description	Part No.
Instrument and Tools	1	Biomek 4000 Laboratory Automated Workstation	B22867
	1	Gripper Tool	987371
	1	MP20 Eight-Tip Pipette Tool	391900
	1	MP200 Eight-Tip Pipette Tool	986146
ALPs	1	Liquid Waste ALP	B21398
	1	Disposal ALP	609751
	7	Labware Holder	609120
	5	Tip Rack Holder	391910
	1	Gripper Tool Rack	609641
	1	Off Deck Gripper Rack	B21400
	1	Off Deck Tool Rack Mount	B21399
	1	Left Side Module	987264
	1	Right Side Module	987263
Magnet Plate	1	Agencourt SPRIPlate 96R – Ring Super Magnet Plate	A32782
Reservoirs	1	Reservoir Frame	372795
	3	Quarter Reservoir	372790
Consumables	5	Biomek AP96 P250 Tipboxes	717251
	5	96-Deep Square Well Plate	N/A

Biomek 4000 SPRIselect Workflow

Figure 1 illustrates the Biomek 4000 SPRIselect workflow. The entire workflow takes three major steps as follows, Step 1: Mega Shear Sample Preparation (Beckman Coulter Genomics, Danvers, MA): 10 µg E.Coli gDNA were sheared in 120 µL 1x TE per Covaris tube (Covaris E210, PN 600027, see Table 1 for shearing conditions). After shearing, pool each individual shear and dilute each shear to the desired concentration with 1x TE and mix the 3 diluted shears 1:1:1; Step 2: SPRIselect on Biomek 4000 Workstation (based on SPRIselect protocol version_B24965AA): 50 µL of Mega Shear sample was used per well. Each size selection was performed for one column of samples, i.e. one column of 0.65 Left, one column of 0.7 Right and three columns of three ranges of Double Size Selections. All samples were eluted into 50 µL water. Step 3: Data Analysis: 1 µL of 50 µL eluate were used on Agilent 2100 Bioanalyzer (Agilent Technologies, G2940CA) for the final selected size analysis.

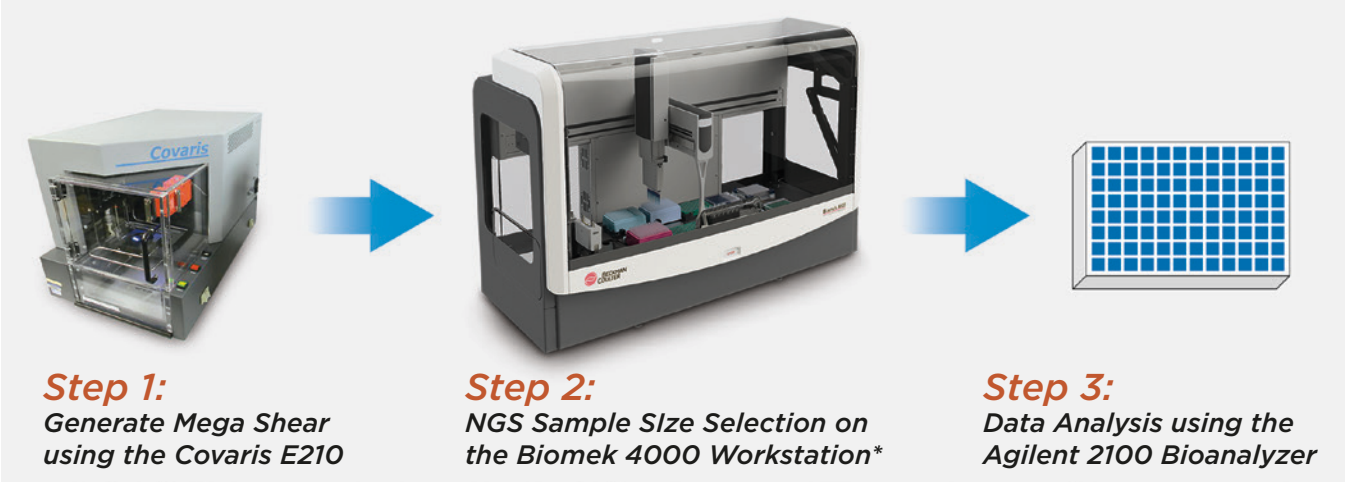


Figure 1: Workflow for Biomek 4000 SPRIselect. 96 samples from one 96-well plate can be made on Biomek 4000 Workstation.

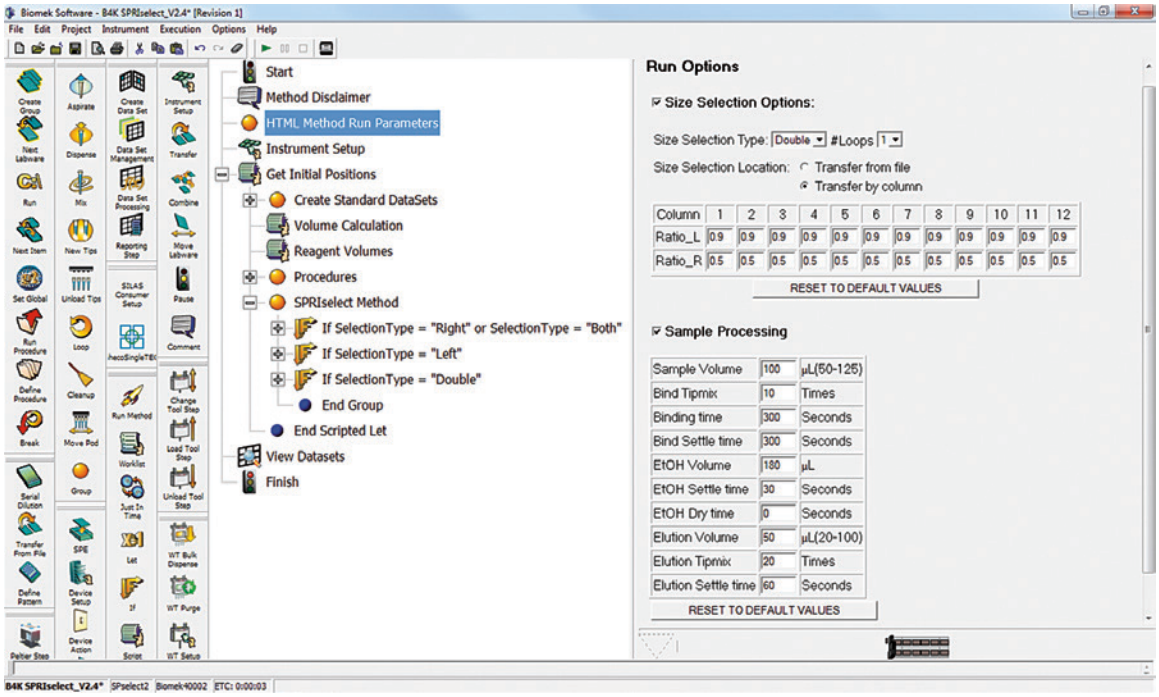
Table 1	200bp	TruSeq v2 (250bp)	600bp
Duty Cycles	10	10	5
Intensity	5	5	3
Cycles/Burst	200	200	200
Seconds	180	120	65

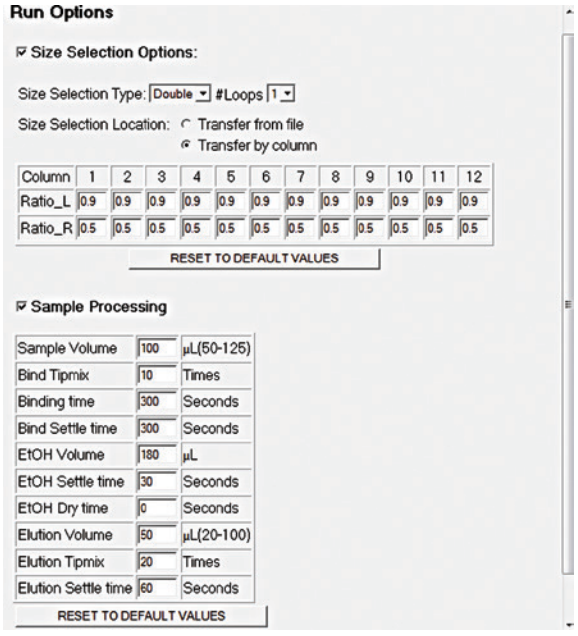
Table 1: Covaris Running Condition

* Shown with optional enclosure.

Biomek 4000 SPRIselect Demonstrated Method Configuration

Biomek 4000 SPRIselect demonstration method offers great flexibility to fit users' dynamic needs. Figure 2 illustrates the various steps and user interfaces used to configure a method. **2a. Method Layout:** This method consists of three main parts: User Interface, Reagent Calculator, and Running Procedures using Biomek. Data set track all samples' in-and-out liquid transfer activities. **2b. User Interface:** this method offers (1) four different size selection options: Left Size Selection, Right Size Selection, Both Side Selection (data not shown), and Double Size Selection with one or two Loops; (2) Samples can be tracked by plate or transfer from file via .csv file; (3) Each bead-to-samples ratio is entered by column, and can vary from column to column; (4) ten other variables including: sample volume, bind tipmix, binding time, bind settle time, ethanol volume, ethanol settle time, dry time, elution volume, elution tipmix and elution settle time. **2c. Deck Layout:** Deck requires five ML positions for tip boxes, five P positions for processing labware, two Off Deck tool racks for MP200, Gripper and P200L (optional, used for transfer from file), one Off Deck Liquid Waste Station position and one Tip Disposal position. **2d. Instrument Setup:** The maximum labware needed for Double Size Selection with two Loops are: five P250 tip boxes, four half-height process plates (AB-1127), one Super Magnetic Plate and one Quarter Reservoir for reagents.





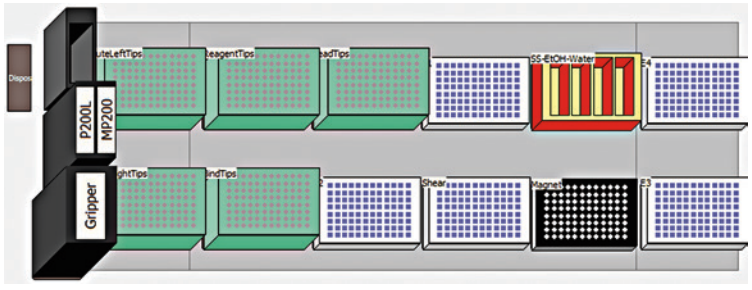


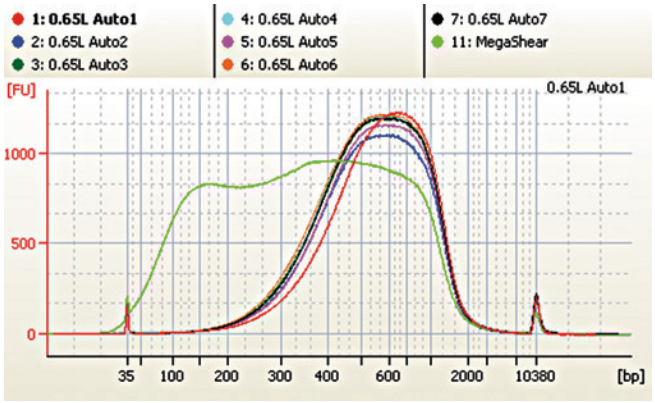
Figure 2: Biomek 4000 SPRIselect demonstrated method configuration

Results

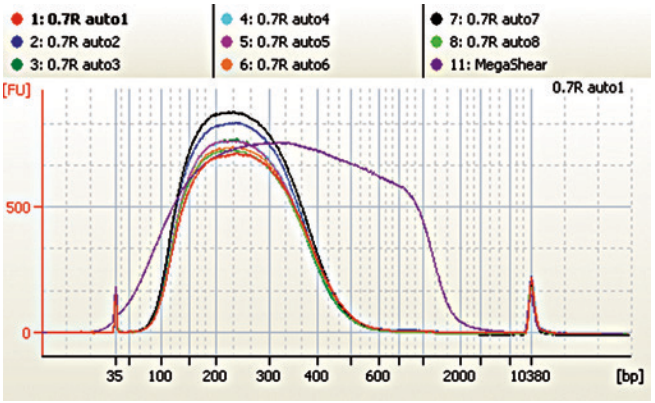
1. Left Side Selection (Bead to Sample Ratio = 0.65x): One column of Mega Sheared E.Coli gDNA (1ug/50 µL / well) was processed on the Biomek 4000 Workstation.

2. Right Side Selection (Bead to Sample Ratio = 0.7x): One column of Mega Sheared E.Coli gDNA (500ng/100 µL / well) was processed on the Biomek 4000 Workstation.

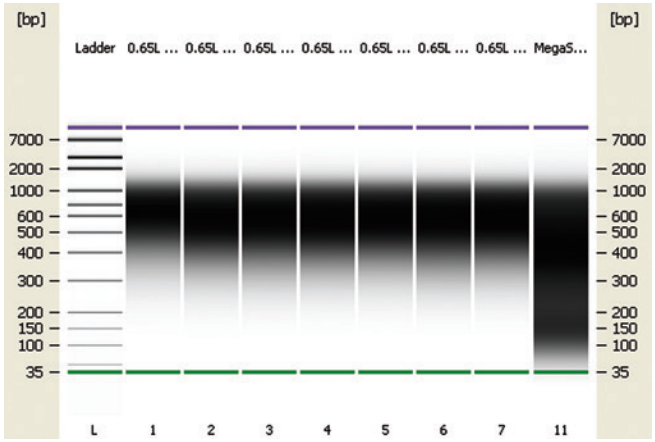
3a



4a



3b



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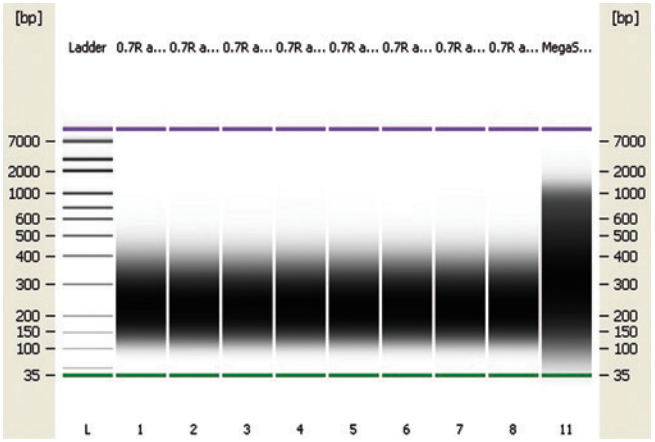


3c

0.65 Left Side Selection	Region (500-1000bp) Average Size (bp)
0.65 L auto 1	675
0.65 L auto 2	669
0.65 L auto 3	670
0.65 L auto 4	670
0.65 L auto 5	670
0.65 L auto 6	671
0.65 L auto 7	670
Average	671
MegaShear	665

4c

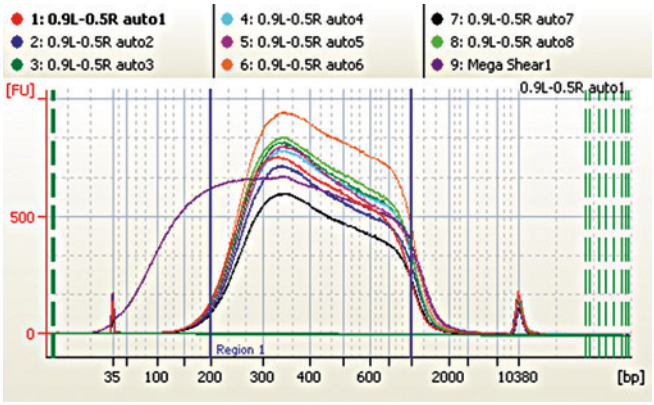
0.7 Right Side Selection	Region (150-350bp) Average Size (bp)
0.7 R auto 1	245
0.7 R auto 2	244
0.7 R auto 3	244
0.7 R auto 4	244
0.7 R auto 5	244
0.7 R auto 6	245
0.7 R auto 7	244
0.7 R auto 8	244
Average	244
Mega Shear	250

Figure 3: Agilent High Sensitivity DNA Chip Data from Biomek 4000 SPRIselect Left Side Selection (Bead to Sample Ratio = 0.65x). **3a:** Seven automation samples and one original Mega Shear gDNA sample. **3b:** The gel image of all samples. **3c:** Between 500-1000bp region, automated samples (0.65x Auto#1-7) had average size = 671bp.

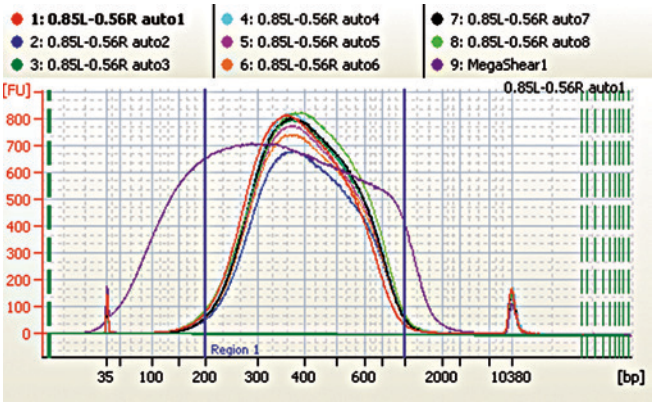
Figure 4: Biomek 4000 SPRIselect Right Side Selection (Bead to Sample Ratio = 0.7x). **4a:** Automation samples and one original Mega Shear gDNA sample. **4b:** The gel image of all samples. **4c:** Between 150-350bp region, automated Right Side Selection samples (0.7x Auto#1-8) had average size = 244bp.

3. Double Side Selection: Three Double Side Selection ranges (0.9 L – 0.5 R; 0.85 L – 0.56 R; 0.8 L – 0.61 R) were processed on the Biomek 4000 Workstation (500ng/100 µL/well).

5a



5b



5c

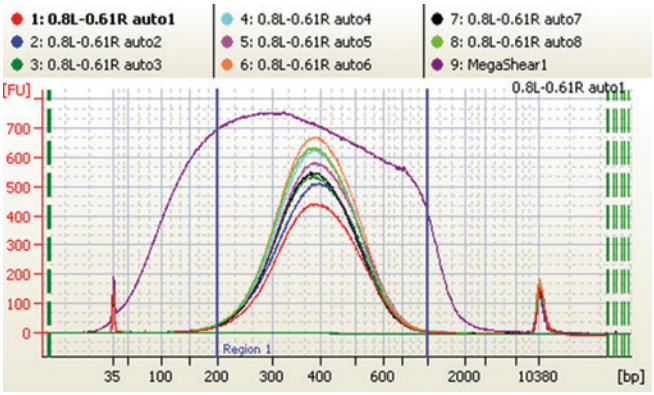


Figure 5: Biomek 4000 SPRIselect Double Side Selection Agilent High Sensitivity DNA chip data with bead to sample ratios of 0.9 L – 0.5 R (5a); 0.85 L – 0.56 R (5b); and 0.8 L – 0.61 R (5c). Between 200-1000bp regions, the average sizes for those three ranges are 450bp, 430bp and 407bp respectively (5d).

5d

Double Side Selections	Three Ranges Average (bp) in 200-1000bp region		
	0.9 L - 0.5 R	0.85 L - 0.56 R	0.8 L - 0.61 R
Auto 1	439	414	410
Auto 2	449	435	408
Auto 3	450	433	405
Auto 4	453	431	407
Auto 5	450	432	411
Auto 6	458	431	407
Auto 7	448	431	402
Auto 8	450	436	403
Auto Sample Average (bp)	450	430	407
Mega Shear Average (bp)	431	430	429

Summary

The Biomek 4000 SPRIselect demonstrated method can deliver 96 samples NGS DNA fragment size selection in the 96-well plate format. This method is fast, easy and extremely flexible. It can process sample fragments size selection from Left Side Selection, Right Side Selection and Double Side selection while providing the flexibility that allows users to easily adapt this method to their specific targeting conditions through a simple HTML user interface.

For more information, please contact your local Beckman Coulter representative or visit our Biomek 4000 Workstation website: **www.biomek4k.com**

For Beckman Coulter's worldwide office locations and phone numbers, please visit www.beckmancoulter.com/contact

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