Gel-Free Size Selection Using SPRIselect For Next Generation Sequencing

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Introduction

Gel size selection for Next Generation Sequencing (NGS) fragment library preparation has been the standard process for achieving size selection. Aside from being tedious, gel size selection creates additional preparation time for scientists forcing them to purify the desired fragment sizes from the gel creating a process that can only be low throughput. To alleviate this problem, Beckman Coulter has recently launched the SPRIselect reagent kit that allows NGS size selection to become more stream-lined, high throughput, and automation-friendly. SPRIselect is a SPRI-based (Solid Phase Reversible Immobilization) paramagnetic bead technology that can be scaled for low to high throughput workflows and is tunable for various sequencing applications.

SPRIselect beads can selectively bind fragments based on the ratio of SPRIselect reagent to sample. Adjusting the ratio gives users the control to eliminate smaller or larger fragment sizes not within the target range. The workflow for the SPRIselect kit is adjustable and allows for users to perform right, left, or double size selection.

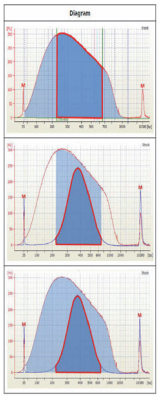
The different workflows are described in this poster along with guidelines for how to determine the ratios required for various size ranges. For these experiments, sheared E. coli DNA was used to demonstrate how SPRIselect beads work as the ratio is altered and how it could be used both manually and with automation to help NGS sample preparation become gel-free.

How SPRIselect Works

The SPRIselect beads can be used to start binding DNA fragments at a set fragment base pair (bp) size. When viewed on an Agilent Bioanalyzer High Sensitivity DNA chip, binding is shown to occur on a curve and not in a linear fashion. The fragment bp size at which SPRIselect beads start binding fragments is controlled by the volume ratio of SPRIselect beads to DNA solution. Altering the ratio gives the user the ability to selectively keep or discard undesired fragment sizes. The workflow used for Left and Right side selection can be optimized for different applications to increase yield or make a tighter selection range. Combining left and right selection can create a double size selection that removes smaller and larger fragments from either side of the targeted region.

To demonstrate the various workflows and how altering volume ratios affects SPRIselect beads binding ability, E. coli DNA was sheared using the Covaris by following the shearing method suggested in Illumina's TruSeq protocol. All experiments used 1µg input of total sheared DNA in a volume of 100 µl to allow for easily pipetted volumes and distinct bioanalyzer traces for comparison. Concentration and volume is user adjustable to fit individual applications.

The figure below demonstrates how size selection influences the percent recovery of the input sample in relation to Double Size selection.



Target bp Region's Percent of Shear: Dark blue region represents the maximum potential recovery of the stock sample for the targeted size selection range.

Recovered Percentage of bp Region: Dark blue region represents the actual percent recovery of the targeted region.

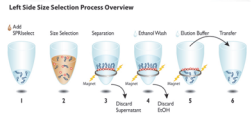
Recovered Percentage of the Shear: Dark blue region represents the percent recovery of the Shear sample.

Figure 1: How Double Size Selection influences % recovery of stock sample

Left Side Selection

To perform Left Side Selection, add the appropriate ratio volume of SPRIselect reagent to the sample. SPRIselect beads will bind the larger fragments to the right of the desired range that are to be washed and eluted. The supernatant now contains the smaller fragments to the left of the target range that can now be discarded or saved to be processed separately.

Figure 2 (Left): Description of the Left side selection process steps.



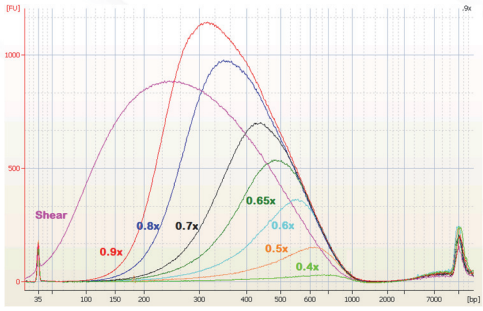


Figure 3 (Above): Bioanalyzer trace showing E. coli DNA that was sheared using the Covaris profile suggested within Illumina's TruSeq protocol. The sheared DNA was processed using several different ratios for Left Side selection.

Right Side Selection

To perform Right Side selection, add the appropriate ratio of SPRIselect beads to the sample. This binds the larger fragments to the right of the target range that are to be discarded while the smaller fragments to the left of the target range are removed in the supernatant to a fresh tube. A re-bind step is used to exchange the buffer by adding SPRIselect beads back into the supernatant to bind all the fragments. Although the ratio for the re-bind step can be altered to fit the application, 1.8x, 1.2x and 1.0x are commonly used ratios.

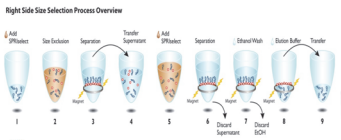


Figure 3: Description of the Right Side selection process steps.

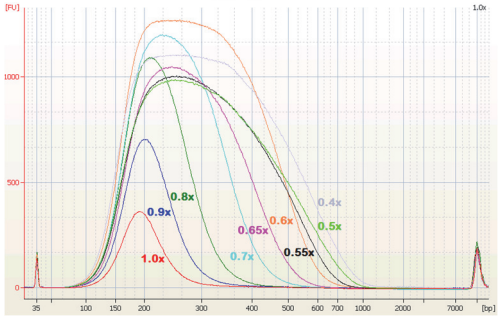


Figure 4: Bioanalyzer trace showing E. coli DNA that was sheared using the Covaris profile suggested within Illumina's TruSeq protocol and processed using several different ratios for Right Side selection and a 1.2x ratio for the re-bind step.

Double Size Selection

Double size selection can be performed in several different ways. The most common way uses less reagent and takes less processing time and is the same as the Right Side selection workflow except that it utilizes the re-bind step to perform Left Side selection. To calculate the volume of SPRIselect beads required to perform a Left Side selection re-bind, subtract the Right Side ratio from the Left Side ratio, and multiply that by the initial volume of sample. Care must be given to pipette the volume of beads accurately to achieve a consistent and accurate size selection.

When the re-bind volume is to small to easily pipette, a modified workflow using Left into Right selection allows for larger volumes of SPRIselect to be pipetted.

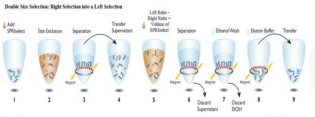


Figure 5 (Left): Process overview for Double Size Selection using the Right into Left method.

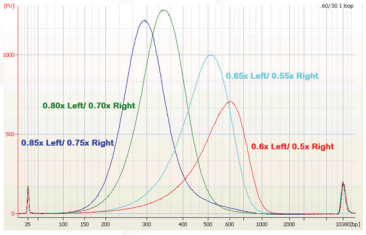


Figure 6 (Right): The sheared E. coli DNA has been processed using four different ratio sets for Double Size selection.

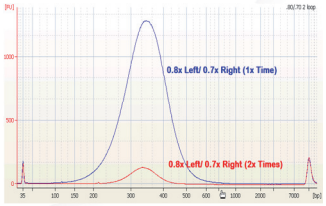
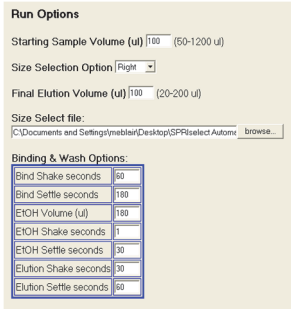


Figure 7 (Left): Double size selection can be repeated on the same sample to achieve a tighter selection range. This workflow does decrease yield and must be optimized to obtain the range and yield desired for the application. Using 0.80x Left/ 0.70x Right and processing twice gives a ~125bp delta and a 340bp average.

Automated Size Selection using Biomek

Using a .CSV file, users can define different size selection ratios for each sample well. The method's simple User Interface gives users control over selection type, sample input volume , binding and elution parameters.

Figure 8(Right): The User Interface for the Biomek method. The UI allows for many different options without changing the method.



Conclusions

- Gel-Free size selection frees up time by skipping difficult gel purification steps
- Reproducible results both manually and automated
- Automated size selection saves time and allows per well selection
- User Interface allows for quick customization of various options