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SPRI Bead Cleanup

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SPRIselect is a SPRI-based chemistry that speeds and simplifies nucleic acid size selection for fragment library preparation for Next Generation sequencing. In this process, size selection is required to produce a uniform distribution of fragments around an average size. Using SPRIselect, the size distribution can be adjusted to suit the application and platform used. The process can be scaled for low to high throughput workflows.

The guidance provided below can be used to optimize the desired size selection range. Used manually or automated on a liquid handling system such as the Biomek Laboratory Automation Workstation, SPRIselect will provide rapid and consistent size selection suitable for most applications.

SPRIselect User Guide.pdf

https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/size-selection

George Testo 2022. SPRI Bead Cleanup. **protocols.io**
https://protocols.io/view/spri-bead-cleanup-cafksbkw

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Reagents

- 1 x Bottle of SPRI Beads
- 1x Bottle of Elution Buffer (from extraction kit)

Supplies

- Sample or Pool (to be purified)
- Extra 1.5mL Tubes
- Magnetic Stand

Equipment

- Serological pipette & 10mL serological pipette tip
- 200uL pipette & tips
- 20uL pipette & tips

Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

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- Samples should be fragmented double-stranded DNA.
- Samples should be dissolved in molecular biology grade water or standard buffer solution such as Tris or TE. See Effects of Common Laboratory Reagents on Size Selection at end of user guide for more information.
- Sample volume should be ≥ 50 µL. A lower volume will decrease pipetting accuracy of SPRIselect, therefore increasing selection point variability.
- DNA fragments may be size selected in a range no smaller than 150 bp and no larger than 800 bp.
- To maximize recovery for a Left Side Size Selection, the majority of the sample's size distribution should be larger than the selection point.
- To maximize recovery for a Right Side Size Selection, the majority of the sample's size distribution should be smaller than the selection point.

- To maximize recovery for a Double Size Selection, the size distribution should be centered between the selection points.

Preparing for Bead Cleanup 19m

1 Make **300 µL 80% Ethanol** (200-proof) (make fresh each time). Gently shake the SPRI beads bottle to resuspend any magnetic particles that may have settled. Take aliquot into a tube for working solution.

2 Pipette PCR product (single or pooled) and transfer to single 0.2mL tube(s).

3 Add **1.2X** the volume of **SPRI beads** to the samples.

3.1 Total volume of your sample or pool x **1.2** = required volume of SPRI beads

4 

Mix reagent and PCR product thoroughly by pipette mixing.

Performing Bead Cleanup 19m

5 Incubate mixed samples for **00:10:00** at room temperature for maximum recovery. 10m
Making sure to tap gently every 2-3 minutes.

> **Note:** This step binds PCR products 150bp or greater to the magnetic beads. The color of the mixture should appear homogenous after mixing.

6 Place the tube onto the magnetic stand for **00:02:00** to separate beads from the solution. 2m

> **Note:** Wait for the solution to clear before proceeding to the next step. At this point, there

will be a brown ring around the side of the tube. These are the SPRI beads containing the PCR product.

7 Remove supernatant and discard.

Note: This step must be performed while the tube(s) are situated on the magnetic stand. Do NOT disturb the cluster of separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

8 Add **120 µL of 80% Ethanol** to the 1.5mL tube on the stand and incubate for **00:01:00** at room temperature. Pipette off the ethanol and discard. Repeat for a total 2 washes. 1m

Note: It is important to perform these steps with the tube(s) situated on the magnetic stand. Do NOT disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the tube as it is known as a PCR inhibitor.

9 5m

Open the tube lid(s) to allow evaporation of excess ethanol **00:05:00** .

Note: Take care not to over dry the beads (bead cluster appears cracked) as this will significantly decrease elution efficiency.

Retrieving Desired PCR Product 19m

10 Remove the tube(s) from the stand. Add **40 µL of elution buffer** (from the extraction kit) and mix by pipetting. This will separate the beads from the PCR product.

Note: The liquid level needs to be high enough to contact the magnetic beads. A greater

volume of elution buffer can be used but using a low volume might require extra mixing and may not fully elute the entire product. Elution is quite rapid, and it is not necessary for the beads to go back into solution for it to occur.

11 Place the tube(s) back onto the magnetic stand for **00:01:00** to separate the beads from the solution. Transfer the eluent to a new tube. This contains the purified PCR product. 1m