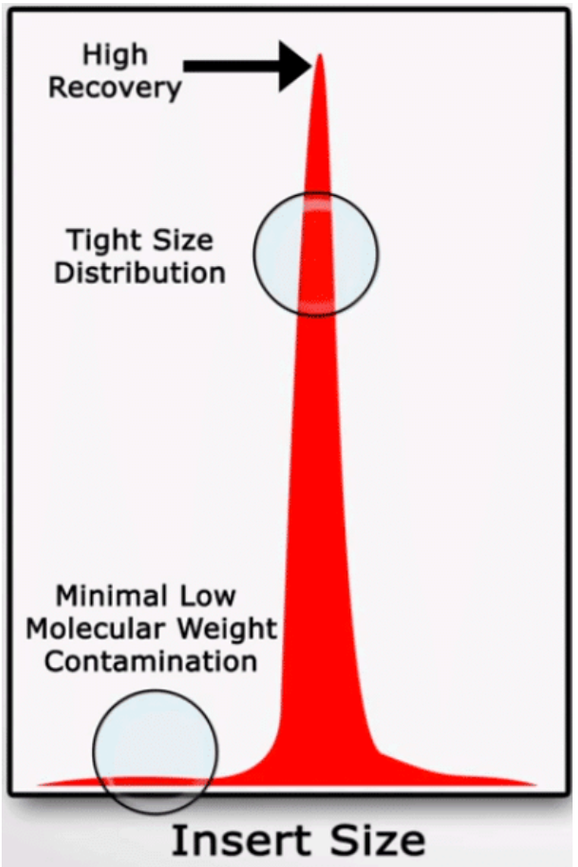
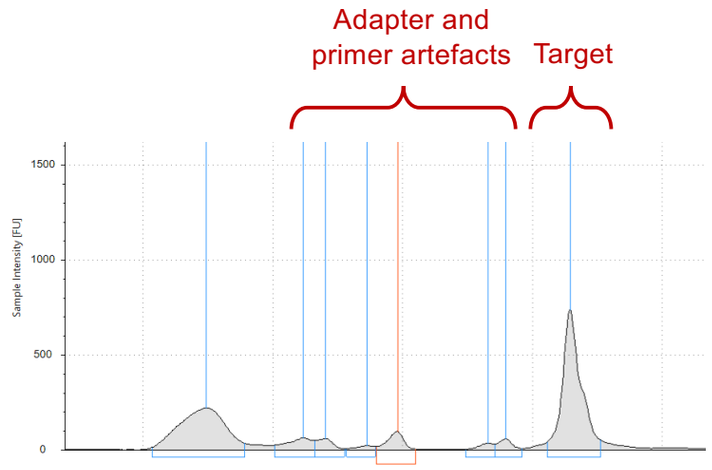
Library Size Selection



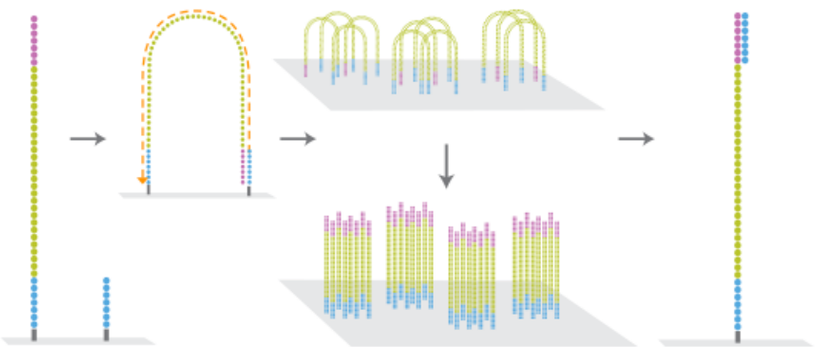
Library Size Selection

Why you might want to perform size selection after library construction…

1. Remove non-target molecules and adapters



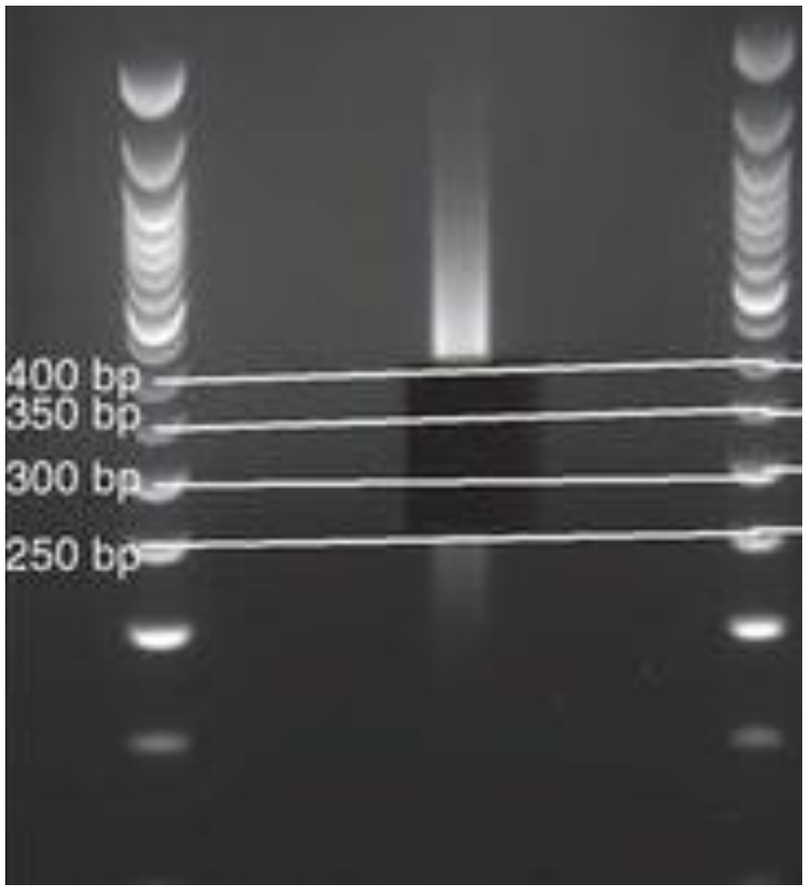
2. Isolate ideal size for flow cell clustering



3. Isolate desired size for read length and to create/eliminate paired-end overlap

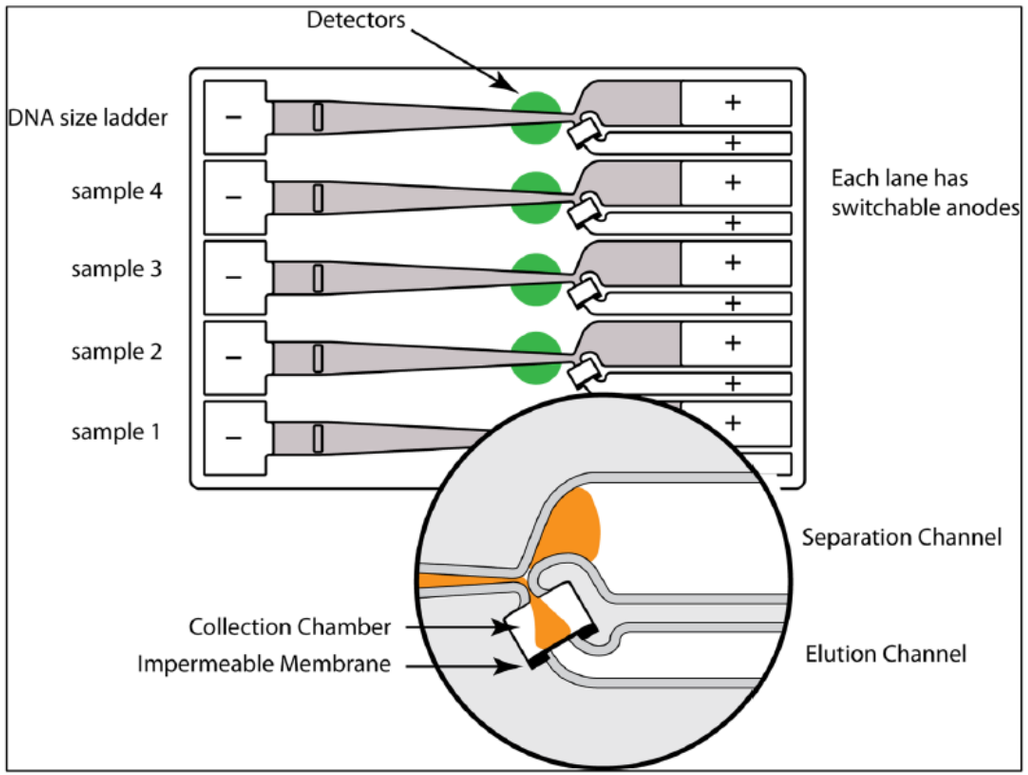
Gel Cut Method

Desired size fractions can be cut out of a gel followed by standard gel extraction protocols.



Pippin Size Selection

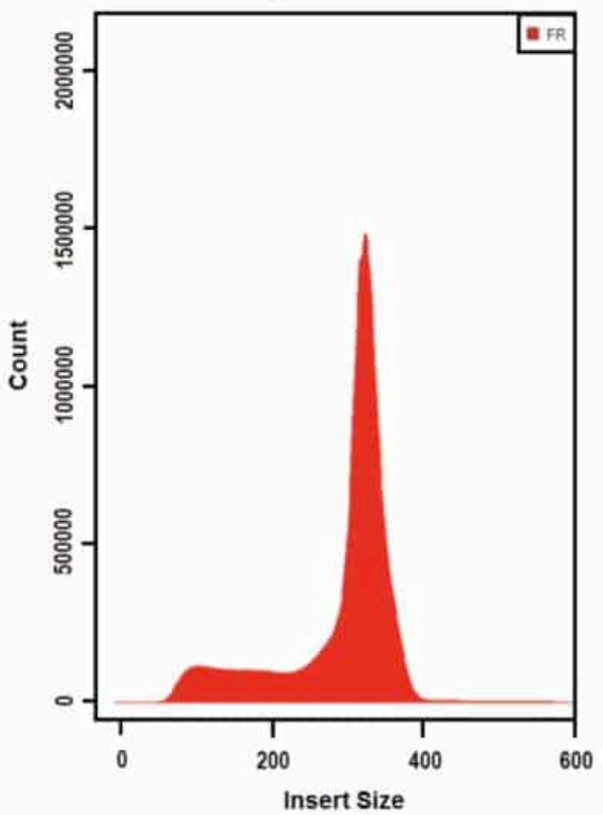
Pippin Prep instruments (Sage Science) use electrophoresis to separate DNA samples by size without the need for a gel cut.



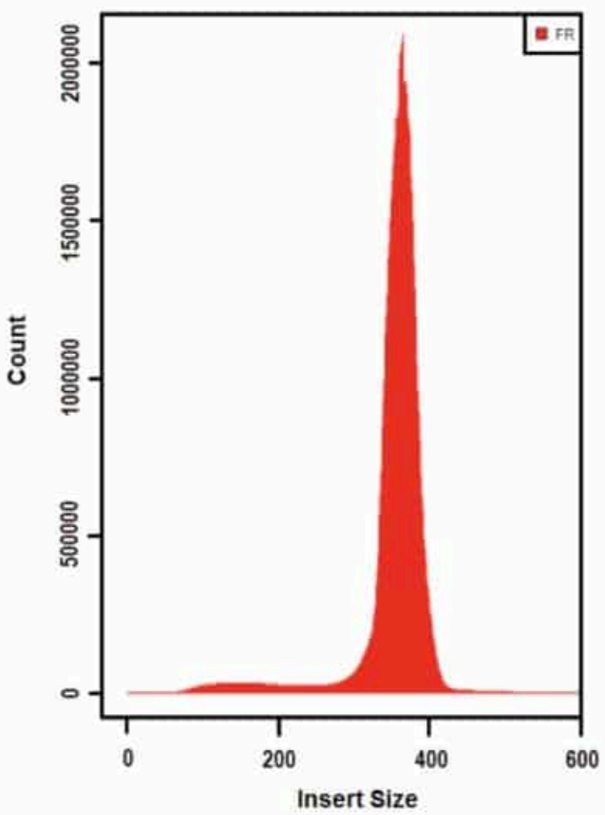
Pippin Size Selection

Pippin instruments and reagents are much more expensive than traditional gel purifications, but they can produce more repeatable and precise results.

Manual Gel Purification

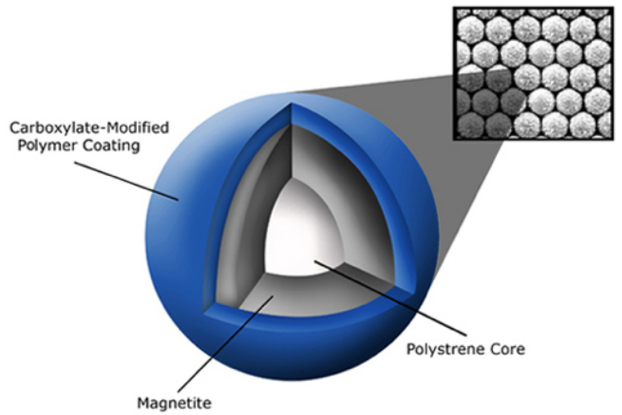


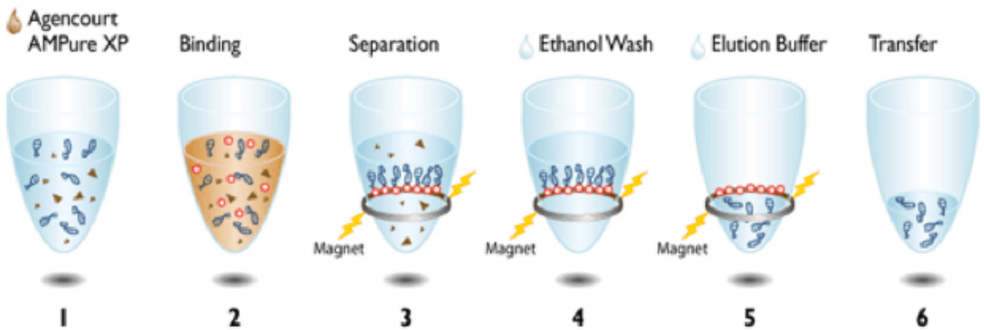
Pippin Size Selection



SPRI Beads

Solid-phase reversible immobilization (SPRI) beads can bind DNA and are used as a general tool for DNA clean-up procedures.





Commercially available as Ampure XP beads from Beckman-Coulter, but can be made (much) more cheaply with homebrew protocols.

https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

Images: CoreGenomics Blog

SPRI Beads and Size Selection

The DNA-binding affinity of SPRI beads depends on the concentration of polyethylene glycol (PEG) and salt (NaCl) in the buffer. Reducing these concentrations will prevent binding of small DNA fragments in a dose-dependent fashion.

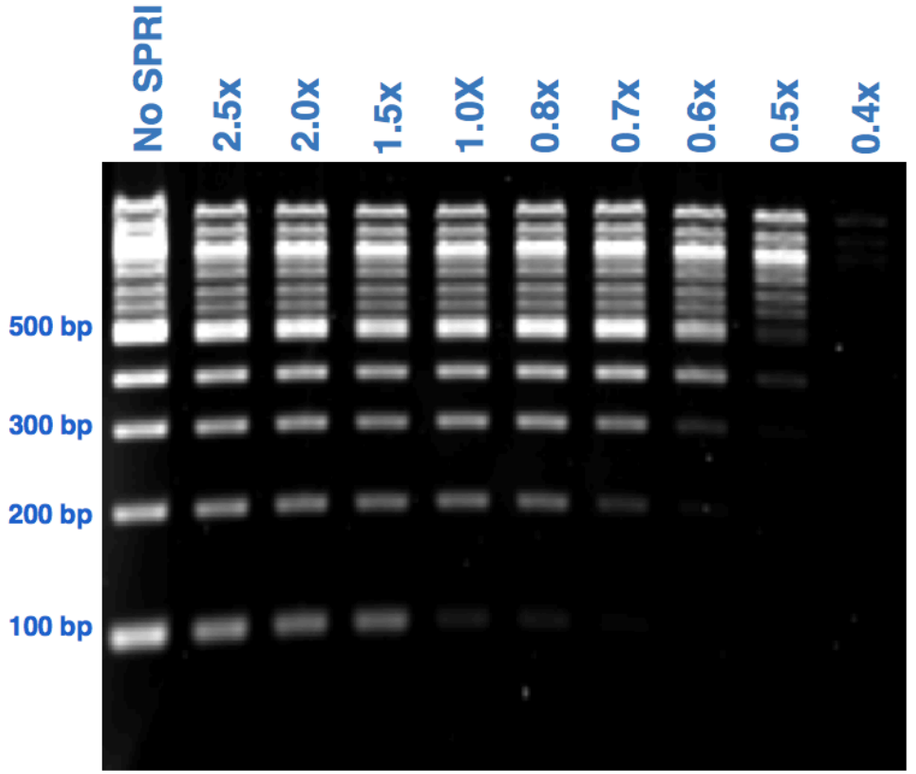


Image: CoreGenomics Blog

Double-SPRI Method

Double-sided size selection can be performed without a gel cut by using two rounds of SPRI-bead purification.

- In round 1, a low concentration of bead solution is added, so only large DNA molecules bind to the beads. The supernatant is saved and the beads are discarded.
- In round 2, more beads and solution are added so that medium-sized DNA molecules now bind to the beads but small molecules still stay in the supernatant. The DNA on the beads is then washed and eluted.
- This method is easier but less precise than a gel cut.

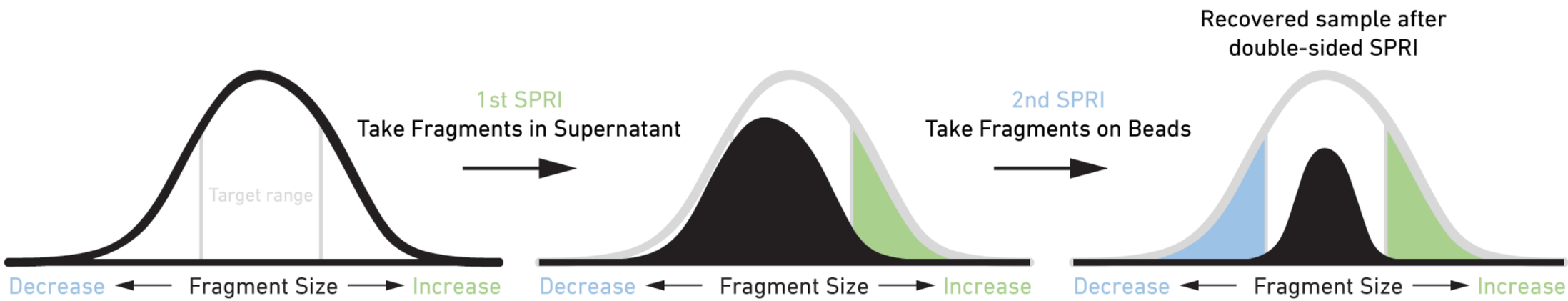


Image: 10X Genomics