Double SPRI for Second Generation Sequencing

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Abstract

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Protocol

Step 1.
Add 65 µl SPRI Ampure beads to 50µl of sample

Step 2.
Mix and incubate for 20 minutes

DURATION
00:20:00

Step 3.
Separate on magnet (6 min.)

DURATION
00:06:00

Step 4.
Transfer all supernatant (about 115 µl) into a new well (off magnet)

Step 5.
Add 100 µl of SPRI beads

Step 6.
Mix and incubate for 15 minutes

DURATION
00:15:00

Step 7.
Separate on magnet (6min), discard supernatant

DURATION
00:06:00

Step 8.
Wash beads with 60 µl of 70% EtOH

Step 9.
Move well off magnet

Step 10.
Dry beads of EtOH (10min)

DURATION
00:10:00

Step 11.

Add 40 µl EB

Step 12.
Mix and incubate for 3 minutes

DURATION
00:03:00

Step 13.
Separate on magnet and transfer eluted product into destination plate

Warnings

Please note that depending on your application, it may or may not be necessary to eliminate high MW fragments assuming your shearing profile is relatively tight. And just eliminating the small material will help to increase yields. Make sure when you add the beads you mix well as this has dramatic effects on the yield.