

Dynabeads® MyOne Carboxylic Acid, CA-beads (Invitrogen, cat# 65011 or 65012)

preparation:

Take 500 μ l beads, wash with buffer EB for 3 times 10 min, resuspend in same volume of buffer EB

Polyethylene glycol (PEG):

PEG 6000 dissolved in MilliQ, 0.9M NaCl and 10mM Tris-HCL, pH6

70% Ethanol (prepare fresh, or not more than 1 week)

70% ethanol with 10mM Tris-HCL, pH6

dSPRI size selection:

1. Add 50 μ l DNA (reaction product adjust with water to 50 μ l) to each tube, then add 100 μ l 13 % PEG 6000, pipet up and down 10 times (or vortex, spin down)
2. Add 10 μ l CA-magnetic beads
3. Mix by pipet up and down 10 times or vortex gently, incubate at room temperature for 5 min, spin down briefly
4. Place on magnet plate for 5 min
5. Save 150 μ l supernatant and transfer to new tube
6. Add 100 μ l 13.5% PEG 6000 to supernatant, pipet up and down 10 times (or vortex, spin down)
7. Add 10 μ l CA-magnetic beads, mix by pipet up and down 10 times or vortex gently, incubate 5 min, spin down briefly
8. Place on magnet plate for 5 min
9. Discard supernatant. Add 200 μ l 70% ethanol and incubate for 30 s
10. Discard ethanol. Repeat ethanol wash.
11. Air dry for 5-10 min (Do not overdry the beads. The beads are dried when they turn from dark brown to light brown)
12. Off magnet plate, add 20 μ l buffer EB and pipet mix 10-20 times, incubate for 5 min
13. On magnet plate for 5 min
14. Transfer eluant to new tube

SPRI purification:

1. Add 50 μ l DNA (reaction product adjust with water to 50 μ l) to each tube, then add 100 μ l 16% PEG 6000, pipet up and down 10 times (or vortex, spin down)
2. Add 10 μ l CA-magnetic beads, mix by pipet up and down 10 times or vortex gently, incubate at room temperature for 5 min, then spin down briefly
3. Place on magnet plate for 5 min
4. Discard supernatant. Add 200 μ l 70% ethanol and incubate for 30 s
5. Discard ethanol. Repeat ethanol wash.
6. Air dry for 5-10 min (Do not overdry the beads. The beads are dried when they turn from dark brown to light brown)
7. Off magnet plate, add 15 μ l buffer EB and pipet mix 10 times, incubate for 5 min

8. On magnet plate for 5 min
9. Transfer eluant to new tube