Preparation of SpeedBeads for DNA Cleanup

June 2021
KNP & MAH

Reagents

PEG-8000
NaCl
Tris pH 8
0.5M EDTA
Water, Molecular Biology Grade
TLE Buffer
SpeedBead Magnetic Carboxylate (Cytiva, 65152105050250)

Consumables

50 mL conical tubes, DNA/RNAse free
1.5 mL tubes, DNA/RNAse free
Micropipette tips (p200, p1000)

Equipment

Analytical balance
Micropipettes (p200, p1000)
Vortex mixer
Magnetic separation rack for 1.5-2.0 mL tubes

Materials and detailed instructions needed for cleanup and gel electrophoresis steps can be found in both library prep protocols.

1. Weigh out **9.00 g of PEG** and add to a 50 mL conical tube.
2. Weigh out **2.92 g of NaCl** and add to the same conical tube.
3. Add **500 µl of Tris** to the tube.
4. Add **100 µl of EDTA** to the tube.
5. Fill tube to the 45 mL line with **molecular grade water** and mix (shake and vortex) until clear.
   Note: This will take quite a while.
6. Thoroughly mix the SpeedBead stock (shake, vortex, and check for clumps with pipette tip). Aliquot **1 mL of beads** into a 1.5 mL tube.
   Note: SpeedBead stock should remain refrigerated until needed and immediately returned to the refrigerator after use.
7. Place tube on magnetic stand and wash three times with **1 mL of TLE buffer**, then re-elute in **1 mL TLE**.
   Note: Take care not to remove any beads during the wash steps.
8. Mix re-eluted beads well, then add to the solution in the 50 mL conical tube.
9. Fill the conical tube to 50 mL with **molecular grade water** and mix.
   Note: When pulled into a pipette tip, mixture should look something like the color of chocolate milk.

SpeedBead Performance Testing

10. Using the freshly prepared SpeedBeads, clean three aliquots of standard DNA ladder and three aliquots of ultra-low range DNA ladder (3uL ladder in 27uL dH_20 for each). This should be done in three ratios of DNA to SpeedBeads (0.8x, 1x, and 2x) for each ladder type.

    Note: See library prep protocols for instructions and materials needed to perform the cleanup.

11. Run on a 2% TBE agarose gel against the un-cleaned ladder (100V for 40 minutes). You should see results like those shown in the image and described in the table below.

    Note: See library prep protocols for instructions and materials needed to prepare the gel electrophoresis run.

	Ladder	**Ratio**	**SpeedBeads**	**Expected Result**
1	3 uL regular ladder, 27uL dH_20	0.8	24 uL	No bands below 400bp
2	3 uL regular ladder, 27uL dH_20	1.0	30 uL	No bands below 300bp
3	3 uL regular ladder, 27uL dH_20	2.0	60 uL	No bands below 200bp
4	3 uL ultra low-range, 27uL dH_20	0.8	24 uL	No bands below 400bp (300 may be faint)
5	3 uL ultra low-range, 27uL dH_20	1.0	30 uL	No bands below 300bp
6	3 uL ultra low-range, 27uL dH_20	2.0	60 uL	No bands below 200bp