How to Use Mag-Bind Beads to Remove Bacterial RNA from Plasmid DNA Minipreps

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NOTE: Carboxyl-coated paramagnetic beads such as AMPure, SPRISelect, and Omega Mag-Bind are frequently used in molecular biology to purify DNA from within reaction mixtures. Beads are typically supplied in a solution containing polyethylene glycol (PEG) and sodium chloride (NaCl). These components cause the negatively-charged DNA to condense and bind to the positively-charged beads. Once the supernatant is removed from the magnetized bead pellet, purified DNA is obtained by washing the beads in ethanol and eluting the DNA into elution buffer.

The nature of the reversible immobilization of DNA onto the beads is dependent upon the amount of PEG and NaCl in solution. Bead suppliers typically recommend a bead:sample ratio (v/v) of 1.8 to 1 (referred to as 1.8x) in order to recover DNA fragments above 100bp in length, at the exclusion of unincorporated dNTPs and primers. Using ratios lower than 1.8x means there is less PEG/NaCl in solution, which prevents small DNA fragments in the mixture from condensing and binding to beads in the solution. Thus, by using ratios other than the recommend 1.8x, DNA fragments of differing lengths will be preferentially bound, which permits the targeted the recovery of desired lengths of DNA. This behavior can be utilized to target a desired size cutoff, i.e. an approximate size division between the "small" and "large" fragments.

Protocol:

1. Shake or vortex the Mag-Bind® Total Pure NGS to resuspend any particles that may have settled. Allow Mag-Bind® Total Pure NGS to come to room temperature before use.
2. Concentrate the plasmid DNA (i.e., one miniprep) by ethanol precipitation, and dissolve it in 20µl ddH_2o and transfer to 200µl PCR tubes.
3. Add 8µl Mag-Bind beads into the DNA-containing 200µl PCR tubes. Pipet up and down 5-10 times or vortex for 30 seconds to mix well.
4. Let the tubes sit at room temperature for 5 minutes.
5. Spin it down briefly at low speed and put it on the magnetic separation device.
6. Aspirate and discard the clear supernatant. Do not disturb the Mag-Bind beads.
7. With the tubes are mounted on the magnet, add 200µl 70% ethanol.
8. Let the tubes sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® Total Pure NGS.
9. Aspirate and discard the clear supernatant. Do not disturb the Mag-Bind beads.
10. Repeat Steps 7-9 for a second 70% ethanol wash. Leave the tubes on the magnetic separation device for 2-3 minutes to air dry the Mag-Bind beads. Remove any residual liquid with a pipette.
11. Remove the tubes from magnetic separation device. Add 50-100µL ddH_2O to elute plasmid DNA.
12. Pipet up and down 20 times or vortex for 30 seconds. Let the tubes sit at room temperature for 5 minutes
13. Place the tubes on a magnetic separation device. Let the tubes sit at room temperature until the Mag-Bind beads are completely absorbed by the magnet, and cleared from solution.
14. Transfer the cleared supernatant containing purified DNA to new tubes for downstream uses (e.g., transfection, DNA sequencing, or NGS).