Formulating Magnefy™ Magnetic Particles for Nucleic Acid Purification by Solid-Phase Reversible Immobilization (SPRI)

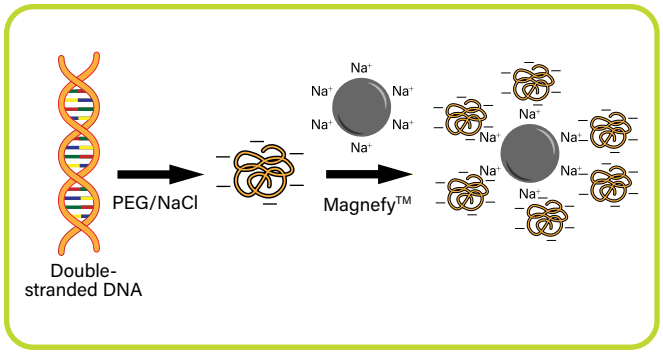

Figure 1. Double-stranded DNA in NaCl and PEG. In water, double-stranded DNA (dsDNA) has a looser, linear double-stranded helical coil structure. The addition of NaCl and PEG forces the negatively charged backbone to aggregate into globular structures that are attracted to the positively charged surface of magnetic particles.

INTRODUCTION

Prepared beads can be used for purifying nucleic acid fragments from solution. The ability to purify DNA molecules is dependent on the coil-to-globular transition, that is, the collapse of a linear expanded helical coil to a globular state, or vice versa. (Lerman *et al.* 1971) (Fig. 1). Manipulating the solution conditions drives the conformational change in nucleic acid structure. Solid-phase reversible immobilization (SPRI) purifies nucleic acids from crude cellular lysates by collapsing the nucleic acids onto magnetic particles, which are then easily separated and washed by placing the solution in a magnetic field. The final nucleic acid sample is then easily eluted from the beads by reversing the salt and PEG concentrations (DeAngelis *et al.* 1995). This concept was refined to isolate nucleic acid fragments of a specific length by utilizing PEG in the presence of NaCl (He *et al.* 2013). Adjusting the concentration of PEG ensures specific lengths of the DNA fragments are captured (Lis *et al.* 1975) while the NaCl concentration decreases the effective concentration needed to activate PEG for a given fragment length (Vasilevskaya *et al.* 1995 and Ramos *et al.* 2005). It is important to note that the concentration of nucleic acid was not a significant factor in driving this reaction (He *et al.* 2013). Therefore, the most important parameters are the PEG and salt concentration.

The following protocol is an adaption of "SPRI bead mix" by Jolivet and Foley. It can be used at a starting point to formulate Magnefy Magnetic Particles to bind either DNA or RNA for purification. In addition, a basic procedure with relevant references for establishing custom ratios for size selection is also presented.

MATERIALS

Beads

- Magnefy COOH (Bangs Labs catalog number MFY0002)

Chemicals (molecular biology grade)

- Sodium chloride (NaCl)
- Poly(ethylene glycol), avg. mol. wt. 8000 (PEG 8000)
- Polysorbate 20 (Tween® 20) (Bangs Labs catalog number AA016)
- Hydrochloric acid (HCl) concentrate
- Nuclease-free water

For DNA

- Tris(hydroxymethyl)aminomethane (Tris base)
- Disodium ethylenediaminetetraacetate dihydrate (EDTA)

For RNA

- Trisodium citrate dihydrate

Consumables

- 50 mL conical tubes
- 1.5 mL microcentrifuge tubes
- Disposable weighing vessels
- Disposable Pasteur pipettes
- Parafilm® M
- 0.22 µm syringe filters
- 10 mL disposable syringes
- 25 mL, 10 mL, 5 mL serological pipettes
- 1000 µL, 200 µL micropipette tips

Equipment

- Milligram-range balance
- Funnels
- Spatulas
- Heating plate
- Rotary mixer
- Microcentrifuge
- 25 mL graduated cylinder
- 50 mL volumetric flasks and stoppers
- 1000 µL, 200 µL adjustable-volume micropipettes
- Squirt bottle
- Magnetic separation block for 1.5 mL microcentrifuge tubes (Bangs Labs catalog number LS001)

STOCK SOLUTIONS

Common Solutions

1 N HCl
Prepare at least 10 mL 1 N HCl in a glass bottle.

5 M NaCl
For 50 mL final volume
Add 14.61 g of NaCl to 40 mL water. Mix thoroughly. Add water to a final volume of 50 mL and mix again.

10% (w/v) Tween 20 (Polysorbate 20)
For 50 mL final volume
1. Place a labeled 50 mL conical tube on the balance and tare it.
2. With a new disposable Pasteur pipette, aspirate approximately 0.5 mL of Tween 20 (Polysorbate 20)
3. Slowly dispense the Tween 20 (Polysorbate 20) into the 50 mL conical tube to reach 5.5 g.
4. Remove the tube from the balance and add 45 mL of water with a 25 mL serological pipette.
5. Cap the tube and mix thoroughly.

50% (w/v) PEG 8000
For 25 mL final volume
1. Place the 50 mL graduated cylinder on the balance and tare it.
2. Weigh 12.5 g of PEG 8000 powder directly into the cylinder. It is recommended to use latex gloves instead of nitrile to reduce static charges that make the powder fly off the spatula.
3. Add no more than 14 mL of nuclease-free water with a serological pipette on top of the PEG powder in the cylinder. The water level will reach over the 25 mL mark as the cylinder already contains about 20 mL of dry PEG powder.
4. Seal the cylinder with a double layer of Parafilm.
5. Shake vigorously to suspend the powder in the water until there are no more lumps of dry solid sticking to the cylinder wall. It will be very viscous and clumpy.
6. Let the suspension stand at room temperature for at least an hour to allow the solids to dissolve and the air bubbles to rise.
7. Remove the Parafilm and complete the volume with nuclease-free water to the 25 mL mark.
8. Seal the cylinder again and mix well by inverting. The solution is very viscous and homogenizing it can take a while.
9. Transfer the solution to a 50 mL conical tube for storage.

DNA Solutions

1 M Tris base
For 50 mL final volume
Mix 6.06 g Tris-base with 45 mL nuclease-free water. Add water to adjust volume to 50 mL.

0.1 M EDTA
For 50 mL final volume
Mix 1.86 g Na_2-EDTA•$2H_2O$ with 45 mL nuclease-free water. Adjust to final volume of 50 mL with water.

RNA Solutions

1 M Trisodium citrate
For 50 mL final volume
Mix 14.71 g Na_3-citrate•$2H_2O$ with 45 mL water. Adjust to final volume of 50 mL with water.
Note: Some gentle heating may be necessary. Ensure the solution comes back to room temperature before completing the volume to the mark on the flask. Store in 50 mL conical tubes.

Optional: Filter the stock solutions with syringes and filters to remove undissolved solids. It is strongly recommended to filter the solutions used for making RNA mix for sterilization.

PART I. PREPARING MAGNEFY™ MAGNETIC PARTICLES IN NUCLEIC ACID BINDING BUFFER

Wash/Storage Buffer Recipes

Nucleic acid storage and elution (SAE) buffers

DNA SAE Buffer (10 mM Tris-base, 1 mM EDTA, 0.05% Tween 20, pH 8.0 @ 25 °C)
Mix the following in a 50 mL conical tube:
45 mL Nuclease-free water
500 µL 1 M Tris-base
500 µL 0.1 M Disodium EDTA
250 µL 10% Tween 20
Add 1 N HCl until pH reaches 8.0
Bring final volume to 50 mL using nuclease-free water

RNA SAE Buffer (1 mM Trisodium citrate, 0.05% Tween 20, pH 6.4 @ 25 °C)
Mix the following in a 50 mL conical tube:
45 mL Nuclease-free water
50 µL 1 M Trisodium citrate
250 µL 10% Tween 20
Add 1 N HCl until pH reaches 6.4
Bring final volume to 50 mL using nuclease-free water

Note: These solutions are used for preparing the beads before adding them to the mix. They are also useful for DNA and RNA elution and storage.

Procedure

Wash Beads

1. Mix the Magnefy beads very well to resuspend.
2. Quickly transfer 1 mL to a 1.5 mL microcentrifuge tube to avoid settling.
3. Place the tube on a magnet stand until the supernatant is clear.
4. Remove and discard the supernatant.
5. Add 1 mL of DNA SAE Buffer or RNA SAE Buffer to the bead pellet and close the tube.
6. Remove the tube from the magnet and resuspend the beads by vortexing for at least 15 sec.
7. Put the tube back on the magnet until the beads collect on the magnet.
8. Remove and discard the supernatant.
9. Repeat steps 5 to 8 twice (for a total of 3 washes with DNA SAE Buffer or RNA SAE Buffer).

Note: Do not repeat step 8 for the final wash. Instead, leave the tube on the magnet without removing the supernatant.

Transfer Beads Into Incomplete Binding (IB) Buffer

10. In a 50 mL conical tube, mix the first three ingredients (nuclease-free water, NaCl, and HCl) using the correct amounts for DNA or RNA. For DNA, also add Tris and EDTA; for RNA, add the trisodium citrate. Cap and mix well. The 50 mL conical tube should have:
    a. For DNA IB Buffer (10 mM Tris base, 1 mM EDTA, 2.5 M NaCl, 20% PEG 8000, 0.05% Tween 20, pH 8.0 @ 25 °C):
       25 mL 5 M NaCl
       3.582 mL Nuclease-free water
       168 µL 1 N HCl
       500 µL 1 M Tris-base
       500 µL 0.1 M Disodium EDTA
    b. For RNA IB Buffer (1 mM Trisodium citrate, 2.5 M NaCl, 20% PEG 8000, 0.05% Tween 20, pH 6.4 @ 25 °C):
       25 mL 5 M NaCl
       4.672 mL Nuclease-free water
       28 µL 1 N HCl
       50 µL 1 M Trisodium citrate
11. Remove the DNA or RNA SAE Buffer supernatant from the bead tube still on the magnet (see step 9).
12. Add 1 mL of DNA or RNA IB Buffer (prepared at step 10) to the bead tube on the magnet.
13. Remove the bead tube from the magnet and resuspend by vortexing for 15 sec. Briefly spin down the liquid without pelleting the beads.
14. Transfer the 1 mL bead/DNA or RNA IB Buffer mixture from the 1.5 mL microcentrifuge tube to the remaining DNA or RNA IB Buffer in the 50 mL conical tube. Be sure that the beads transfer completely.

Complete Binding (CB) Buffer

a. For DNA CB Buffer (IB Buffer plus 20% PEG 8000, 0.05% Tween 20)

b. For RNA CB Buffer (IB Buffer plus 20% PEG 8000, 0.05% Tween 20)

15. With a serological pipette, add 20 mL of 50% PEG stock solution. Dispense slowly and allow the viscous liquid to slide down the inside walls of the pipette to ensure an accurate volume is added.
16. Add 25 µL 10% Tween 20, again ensuring the viscous liquid is fully dispensed.
17. Cap the tube and mix by inversion gently but thoroughly, until the color appears homogeneous.

The Magnefy beads are now ready for use in nucleic acid purification or isolation. Store at 4 °C.

Note: EDTA chelates Mg^{+2} and Mn^{+2} ions, which may interfere with downstream reactions.

Note: If Tween 20 causes foaming or is not compatible with downstream uses, its volume can be replaced with nuclease-free water.

PART II. PREPARING MAGNEFY™ BEADS FOR NUCLEIC ACID PURIFICATION AND/OR SIZE SELECTION

When developing a protocol for your specific application, it is important to remember that NaCl and PEG concentrations are the key factors for nucleic acid binding to magnetic beads. The purification/size selection strategy will ultimately combine beads in binding solution (as prepared in Part I), nucleic acid sample, and potentially, additional PEG/NaCl to drive the desired reaction. To collect a given size of nucleic acid fragments, you can try different salt concentrations as it has been shown that adjusting the NaCl concentration from 0.8M to 0.3M lowers the PEG concentration needed to precipitate a given fragment size of nucleic acid (He *et al.* 2014) while Vasilevskaya *et al*. went lower still in their investigation of NaCl on PEG concentrations.
We suggest stabilizing the salt concentration in your

reaction, then titrating different amounts of PEG to determine the best conditions that target your fragment size of interest. For guidance on fragment isolation using PEG and a designated NaCl concentration, see the references.

Procedure

1. Wash beads and reconstitute in binding solution using the procedure in Part I or similar.
2. Based on the nucleic acid fragment size of interest, determine concentrations of PEG and NaCl to be used in the reaction.
3. Adjust the ratios of bead solution and combine them with nucleic acid samples.

Note: It is crucial to adjust the volume ratios appropriately to target the fragment length of interest. The solutions above (both DNA and RNA) contain 5 M NaCl and 50% PEG. These will be diluted to the active concentration when adding your sample. Refer to the citations below for more detailed information regarding fragment length, concentration of NaCl, and concentration of PEG.

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TRADEMARKS

PRODUCT INFORMATION

CAT. #	DESCRIPTION
LS001	1.5mL Magnetic Separator
MFY0002	Magnefy™ COOH 1µm
AA016	Tween® 20 Nonionic Surfactant

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