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A method to prepare Sera-Mag SpeedBeads for purification and size selection of nucleic acids V.2

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We use this protocol and it's working

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Abstract

In this protocol, we describe a method to prepare Sera-Mag Speedbeads for purification and size selection of nucleic acids. We additionally describe a method to validate speedbead preparations and a general method for purification of nucleic acids using speedbeads. This protocol is based on previously described methods (DeAngelis et al., 1995; Rohland and Reich, 2012; Glenn et al., 2019; Jolivet and Foley, 2020; and Möller et al., 2023). We use homebrewed speedbeads as a cost-effective substitute for commercial solid-phase reversible immobilization (SPRI) products (e.g., Mag-Bind TotalPure NGS Beads, AMPure XP) during the preparation of samples for high-throughput sequencing and other applications. The amounts of polyethylene glycol (PEG) and sodium chloride (NaCl), which drive SPRI activity, have been optimized to meet our needs.

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Guidelines

Standard laboratory guidelines and practices should be followed when performing this protocol. To ensure accurate size selection, researchers should use careful and consistent pipetting when performing this protocol or applying prepared speedbeads in other protocols.




Materials

Reagents:

- Sera-Mag SpeedBeads, magnetic carboxylate modified particles (Cytiva 65152105050250, ThermoFisher Scientific 09-981-123)

Note

Sera-Mag Speedbeads are manufactured by Cytiva and can be sourced from various suppliers (e.g.,

 Sera-Mag Speedbeads, magnetic carboxylate modified particles (Cytiva 65152105050250) **ThermoFisher Scientific Catalog #09-981-123**

).

- DNA ladder (e.g., Quick-load purple 1 kb plus DNA ladder, NEB N0550L)
- Polyethylene glycol, average molecular weight 8000 (PEG-8000)
- Tween 20
- 100% ethanol
- Nuclease-free water
- MilliQ water

Solutions:

- 0.5 M EDTA (pH 8.0, sterile)
- 1 M Tris-HCl (pH 8.0, sterile)
- 10 mM Tris-HCl (pH 8.0, sterile)
- 5 M NaCl (sterile)
- 10% (v/v) Tween 20 (prepared as described in Steps 1-4)
- 80% ethanol (freshly prepared with MilliQ water)

Consumables:

- 50 ml conical tubes (nuclease-free)
- 1.5 ml microcentrifuge tubes (nuclease-free)
- 0.2 ml PCR tubes or strips (low-bind, nuclease-free)

Equipment:

- Magnetic separation rack for 1.5 ml microcentrifuge tubes (e.g., MagJET Separation Rack, ThermoFisher Scientific)
- Magnetic separation rack for 0.2 ml PCR tubes (e.g., NEBNext Magnetic Separation Rack, NEB)

Note

See Sergi Lab Supplies for economical and effective magnetic separation racks.

Troubleshooting

Safety warnings

- ⚠ Standard safe laboratory practices and procedures and institution-specific waste management programs should be followed when performing this protocol.

Preparation of 10% (v/v) Tween 20

- 1 Place a 50 ml conical tube on a balance and tare the balance.

Note

Preparation of 10% (v/v) Tween 20 is based on a method described by Jolivet and Foley, 2020.

- 2 Slowly add 5.475 g of Tween 20 to the conical tube using a serological pipette.
- 3 Remove the conical tube from the balance and add 45 ml of MilliQ water.
- 4 Thoroughly mix the solution with shaking until homogenous (approximately 20 min). Store 10% (v/v) Tween 20 at room temperature.

Preparation of speedbead DNA buffer

- 5 Prepare the following buffer in a 50 ml conical tube:

Speedbead DNA buffer

| A | B | C |
|-----------------------|----------|-------------|
| Reagent | Volume | Final conc. |
| 1 M Tris-HCl (pH 8.0) | 500 µl | 10 mM |
| 0.5 M EDTA (pH 8.0) | 100 µl | 1 mM |
| 10% (v/v) Tween 20 | 250 µl | 0.05% |
| Nuclease-free water | 49.15 ml | – |
| Total | 50 ml | – |

Note

Speedbead DNA buffer is used for speedbead preparation and in speedbead-based purification/size selection protocols. Speedbead DNA buffer can be prepared in advance and stored at 4°C for several months.

Preparation of speedbead DNA binding mix

- 6 Equilibrate Sera-Mag Speedbeads to room temperature and mix thoroughly by vortexing.

Note

Speedbead preparation is based on methods described by DeAngelis et al., 1995, Rohland and Reich, 2012, and Glenn et al., 2019 (see also Jolivet and Foley, 2020 and Möller et al., 2023).

- 7 Immediately after mixing, transfer 1 ml of bead slurry to a 1.5 ml microcentrifuge tube. The speedbeads will settle quickly. The bead slurry must be thoroughly mixed immediately before transferring the 1 ml to a 1.5 ml microcentrifuge tube.
- 8 Place samples on a magnetic stand until solution is clear.
- 9 Carefully remove and discard cleared supernatant without disturbing the bead pellet.
- 10 Remove beads from magnetic stand. Add 1 ml of speedbead DNA buffer and vortex beads on setting 4 for 15 seconds. Very briefly centrifuge samples after mixing. Stop the centrifuge before beads settle.
- 11 Repeat Steps 8-10 twice. Keep bead slurry at room temperature.
- 12 Prepare the following mixture in a 50 ml conical tube as described in Steps 13-21:

Speedbead DNA binding mix

| | A | B | C |
|--|---------|--------|-------------|
| | Reagent | Volume | Final conc. |

| | A | B | C |
|--|-----------------------|-------------|-----------|
| | PEG-8000 | 9 g | 18% (w/v) |
| | 5 M NaCl | 20 ml | 2 M |
| | 1 M Tris-HCl (pH 8.0) | 500 µl | 10 mM |
| | 0.5 M EDTA (pH 8.0) | 100 µl | 1 mM |
| | 10% (v/v) Tween 20 | 250 µl | 0.05% |
| | Prepared speedbeads | 1 ml | – |
| | Nuclease-free water | up to total | – |
| | Total | 50 ml | – |

- 13 Add 9 g of PEG-8000 to a 50 ml conical tube.
- 14 Add 20 ml of 5 M NaCl to the conical tube.
- 15 Add 500 µl of 1.0 M Tris-HCl (pH 8.0) to the conical tube.
- 16 Add 100 µl 0.5 M EDTA (pH 8.0) to the conical tube.
- 17 Add nuclease-free water (up to 45 ml) to the 50 ml conical tube.
- 18 Mix the solution with shaking until PEG-8000 goes into solution (approximately 5-10 min).
- 19 Add 250 µl of 10% (v/v) Tween 20 to the conical tube and mix with gentle shaking until Tween 20 goes into solution (approximately 5 min).
- 20 Ensure beads (prepared in Steps 6-11) are fully resuspended in speedbead DNA buffer and add the bead suspension to the conical tube.

- 21 Add nuclease-free water up to 50 ml to the conical tube and mix the speedbead DNA binding mix thoroughly by vortexing. Nuclease-free water can be added up to 50 ml by eye using a 1000 µl pipet.
- 22 Wrap the speedbead DNA binding mix in foil and store at 4°C. Speedbead DNA binding mix can be stored at 4°C for several months.
- 23 Validate speedbeads after preparation to ensure they are working as expected (see Step 25).

Preparation of speedbead DNA binding buffer

- 24 Prepare the following buffer in a 50 ml conical tube:

Speedbead DNA binding buffer

| A | B | C |
|-----------------------|-------------|-------------|
| Reagent | Volume | Final conc. |
| PEG-8000 | 9 g | 18% (w/v) |
| 5 M NaCl | 20 ml | 2 M |
| 1 M Tris-HCl (pH 8.0) | 500 µl | 10 mM |
| 0.5 M EDTA (pH 8.0) | 100 µl | 1 mM |
| 10% (v/v) Tween 20 | 250 µl | 0.05% |
| Nuclease-free water | up to total | – |
| Total | 50 ml | – |

Note

Speedbead DNA binding buffer is used in speedbead-based purification/size selection protocols. Speedbead DNA binding buffer can be prepared as described under speedbead preparation by substituting 1 ml of prepared speedbeads with 1 ml of speedbead DNA buffer. Store 1 ml aliquots of speedbead DNA binding buffer in the dark at -20°C.

Validation of speedbeads



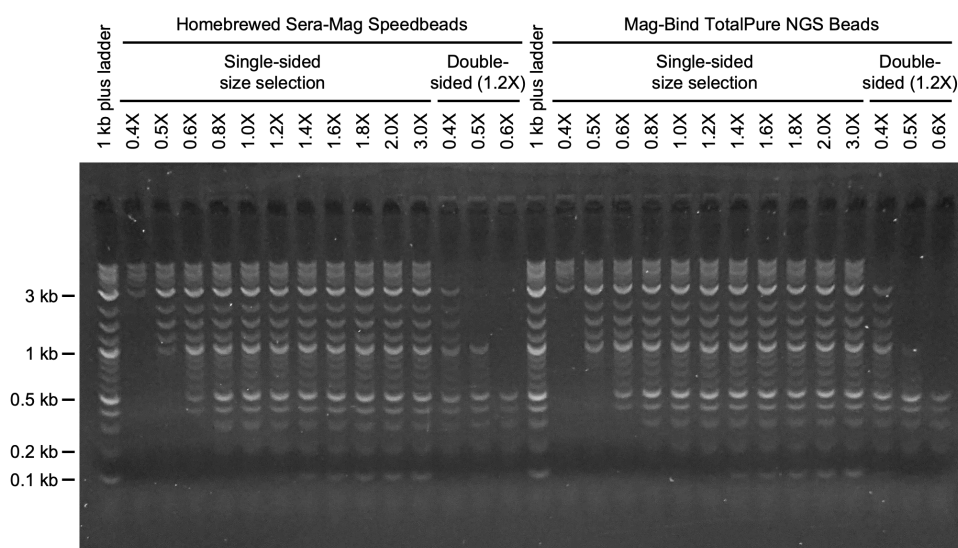
- 25 Prepare 10 aliquots of DNA ladder in 0.2 ml PCR tubes. Combine 3 μ l of a 1:10 dilution of DNA ladder and 22 μ l of 10 mM Tris-HCl (pH 8.0) for each aliquot if using Quick-load purple 1 kb plus DNA ladder.
- 26 Add 0.4X, 0.5X, 0.6X, 0.8X, 1.0X, 1.2X, 1.4X, 1.6X, 1.8X, and 2.0X volumes of speedbeads to the aliquots of DNA ladder. **Careful and consistent pipetting of speedbeads is essential to ensure accurate size selection.**

Note

Additional ratios of beads to sample can be tested as needed. If using speedbeads as a "drop-in" replacement for another SPRI product (e.g., Mag-Bind TotalPure NGS Beads, AMPure XP), that product should be included during validation to ensure speedbeads perform as intended.

- 27 Purify the aliquots of DNA ladder as described in the general method for speedbead purification.
- 28 Elute DNA in 21 μ l of 10 mM Tris-HCl (pH 8.0) prewarmed to 50°C.
- 29 Combine 4 μ l of 6X DNA loading dye with each purified sample (20 μ l). Run 8 μ l of the mixture on a 1.0% agarose gel at 150 V for 60 min. As a control, run 8 μ l of a mixture of DNA ladder (3 μ l of a 1:10 dilution), 10 mM Tris-HCl (pH 8.0) (17 μ l), and 6X DNA loading dye (4 μ l). Conditions for gel electrophoresis (e.g., percent agarose, voltage, time) can vary depending on lab preferences.

Expected result



Representative results of speed validation. An equal mass DNA ladder (Quick-load purple 1 kb plus DNA ladder) was purified using different ratios of Homebrewed Sera-Mag Speedbeads or commercial Mag-Bind TotalPure NGS Beads. Purification was performed as described in the general method for speedbead purification. Although not described in this protocol, speedbeads can be used for double-sided size selection.

General method for speedbead purification

- 30 Equilibrate speedbeads to room temperature and mix thoroughly.
- 31 Carefully add indicated amount of speedbeads to the sample.
- 32 Mix well by pipetting up and down a minimum of 10 times. Alternatively, vortex samples on setting 4 for 3-5 seconds. If samples require centrifugation after mixing, stop the centrifuge before beads settle.
- 33 Incubate samples at room temperature for 5 min. Prolonged incubation or incubation at low temperature will increase binding of small DNA fragments (e.g., adapter dimers).



- 34 Place samples on a magnetic stand for 5 min (or until solution is clear) to collect beads. Keep samples on the magnetic stand for Steps 34-38.
- 35 Carefully remove and discard cleared supernatant without disturbing bead pellet.
- 36 Add 200 μ l of 80% ethanol (freshly prepared with MilliQ water) and incubate for 30 s. Carefully remove ethanol and discard without disturbing bead pellet.
- 37 Repeat Step 36 once. Carefully inspect samples and remove remaining ethanol using a 10 μ l pipet.
- 38 Air dry samples for 2 min with lid open. Do not over-dry samples. Speedbeads (and other SPRI products) will clump and can not be fully resuspended during elution if samples are over-dried.
- 39 Remove samples from the magnetic stand. Add the indicated volume of appropriate solution for elution and mix well by pipetting or vortexing (see Step 32).
- 40 Incubate mixture at room temperature for 2-5 min.
- 41 Place samples on a magnetic stand for 2 min (or until solution is clear) to collect beads.
- 42 Transfer cleared supernatant (volume of solution added for elution less 1 μ l) to a fresh PCR tube.