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Serapure Beads Preparation and Testing

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is used to prepare low-cost SPRI beads used for Illumina Library preparations. As part of the Hakai Institute Ocean Observing Program, from 0 m to near bottom (260 m), biomolecular samples have been collected weekly to genetically characterize plankton communities in the Northern Salish Sea since 2015. These SPRI beads have been used to clean up PCR products of 16S, 18S, COI, and 12S amplicons, and implemented as part of a standard procedure for eDNA analysis.

This protocol is a modification from the following protocol:

*B. Faircloth and T. Glenn. **Serapure v2.2**. Ecol. and Evol. Biology, UCLA, November 19, 2011*

Which was based on the following article:

CITATION

Rohland N, Reich D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.. Genome Res. 2012.

LINK

<https://doi.org/10.1101/gr.128124.111>

Protocol materials

⊗ GeneRuler 100 bp DNA Ladder **Thermo Fisher Scientific Catalog #SM0241** Step 1

⊗ 6-Tube Magnetic Separation Rack **New England Biolabs Catalog #S1506S** Step 1

⊗ UltraPure™ 1M Tris-HCl pH 8.0 **Thermo Fisher Scientific Catalog #15568025** In 3 steps

⊗ EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G** In 3 steps

⊗ Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

In 2 steps

⊗ PEG-8000 **Promega Catalog #V3011** In 2 steps

⊗ NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9759** In 2 steps

⊗ TWEEN 20 for molecular biology viscous liquid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416-50ML**

In 2 steps



PREPARATIONS

1 **Materials** (or equivalent):



Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**



PEG-8000 **Promega Catalog #V3011**



UltraPure™ 1M Tris-HCl pH 8.0 **Thermo Fisher Scientific Catalog #15568025**



EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G**



TWEEN 20 for molecular biology viscous liquid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416-50ML**



NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9759**



GeneRuler 100 bp DNA Ladder **Thermo Fisher Scientific Catalog #SM0241**



6-Tube Magnetic Separation Rack **New England Biolabs Catalog #S1506S**


STEPS

2 In a 50 mL conical using sterile stock solutions, **prepare TE** (10 mM Tris-HCl, 1 mM EDTA) **by adding:**

- 500 µL



UltraPure™ 1M Tris-HCl pH 8.0 **Thermo Fisher Scientific Catalog #15568025**

- 100 µL  EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G**

- Fill conical to 50 mL mark with dH₂O.

3 Mix the container of








Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

and transfer 1 mL to a 1.5 mL microtube.

4 Place SpeedBeads on a magnet stand until beads are drawn to magnet.

5 Remove supernatant with P200 or P1000 pipetter.



- 6 Add 1 mL **TE** to beads, remove from the magnet, mix, and return to the magnet.
- 7 Remove supernatant with P200 or P1000 pipetter.
- 8 Add 1 mL **TE** to beads, remove from the magnet, mix, and return to the magnet.
- 9 Remove supernatant with P200 or P1000 pipetter.
- 10 Add 1 mL **TE** to beads and remove from magnet. Fully resuspend and set microtube in the rack (i.e. not on magnet stand).
- 11 Add 9 g  **PEG-8000 Promega Catalog #V3011** to a new 50 mL, sterile conical.
- 12 Add 10 mL  **NaCl (5 M), RNase-free Thermo Fisher Catalog #AM9759** (or 2.92 g NaCl) to conical.
- 13 Add  **UltraPure™ 1M Tris-HCl pH 8.0 Thermo Fisher Scientific Catalog #15568025** to conical.
- 14 Add 100 µL  **EDTA (0.5 M), pH 8.0 Life Technologies Catalog #AM9260G** to conical.
- 15 Fill conical to ~ 49 mL using sterile dH2O. You can do this by eye, just go slowly.
- 16 Mix conical for about 3-5 minutes until PEG goes into solution (solution, upon sitting, should be clear).
- 17 Add 27.5 µL  **TWEEN 20 for molecular biology viscous liquid Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416-50ML** to conical and mix gently.



- 18 Mix the 1mL **SpeedBead + TE solution** and transfer to 50 mL conical.
- 19 Fill conical to 50 mL mark with dH2O (if not already there) and gently mix 50 mL conical until brown.
- 20 Test against AMPure XP using aliquots of ladder (Fermentas GeneRuler). I recommend the 50 bp ladder in place of the ultraNlow range ladder.
- 21 Wrap in tinfoil (or place in dark container) and store at 4°C.


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Note

You may also wish to prep an extra 50 mL of PEG solution that lacks Sera-mag SpeedBeads so that you can use it in a bead-inclusive library preparation protocol, derived from Fisher (2011).

In that case, just:

1. Add 10 g PEGN8000 to a new 50 mL, sterile conical.

2. Add 25 mL  NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9759** (or 7.3 g NaCL) to conical.

3. Fill conical to ~ 49 mL using sterile dH2O. You can do this by eye, just go slowly.

4. Mix conical for about 3-5 minutes until PEG goes into solution (solution, upon sitting, should be clear).

- 23 Test monthly.

TESTING

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Note

You should test the Serapure mixture to ensure that it is working as expected. You can do this using DNA ladder (Fermentas GeneRuler – NEB ladders may cause problems).



- 25 Prep fresh aliquots of 70% EtOH.
- 26 Mix 2 μ L GeneRuler with 18 μ L dH₂O.
- 27 Add 20 μ L GeneRuler mixture to a volume of Serapure and/or AMPure (the specific volume depends on whether you are trying exclude small fragments or not; see the figure on the next page).
- 28 Incubate mixture for 5 min at room temperature.
- 29 Place on magnet stand.
- 30 Remove supernatant.
- 31 Add 500 μ L 70% EtOH.
- 32 Incubate on stand for 1 min.
- 33 Remove supernatant.
- 34 Add 500 μ L 70% EtOH.
- 35 Incubate on stand for 1 min.
- 36 Remove supernatant.
- 37 Place beads on 37°C heat block for 3-4 min. until dry.



- 38 Rehydrate with 20 μ L dH₂O.
- 39 Place on magnet stand.
- 40 Transfer the supernatant to a new tube.
- 41 Mix supernatant with 1 μ L loading dye.
- 42 Electrophorese in 1.5 % agarose for 1h at 100 V.

Citations

Rohland N, Reich D. . Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.
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