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Serapure Preparation and Testing

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rute.carvalho Carvalho[1]

[1]Hakai Institute

Better Biomolecular Ocea…

Andreas Novotny

University of British Columbia, Hakai Institute, Stockholm U...

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Draft!

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 weeklyto 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 publication:

CITATION

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

LINK

10.1101/gr.128124.111

Modified by B. Faircloth & T. Glenn November 19, 2011
Ecol. and Evol. Biology, UCLA

Protocol materials

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

TWEEN 20 for molecular biology viscous liquid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9416-50ML In 2 steps

NaCl (5 M), RNase-free **Thermo Fisher Catalog #**AM9759 In 3 steps

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

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

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #**44152105050350 In 2 steps

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

PREPARATIONS

1 **Materials:**

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 dH20.

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 amagnet 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 uL EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G to conical.

15 Fill conical to ~ 49 mL using sterile dH20. 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 dH20 (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.

22

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 dH20. 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

24

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 µL GeneRuler with 18 µL dH20.

27 Add 20 µL 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 µL 70% EtOH.

32 Incubate on stand for 1 min.

33 Remove supernatant.

34 Add 500 µL 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 µL dH20.

39 Place on magnet stand.

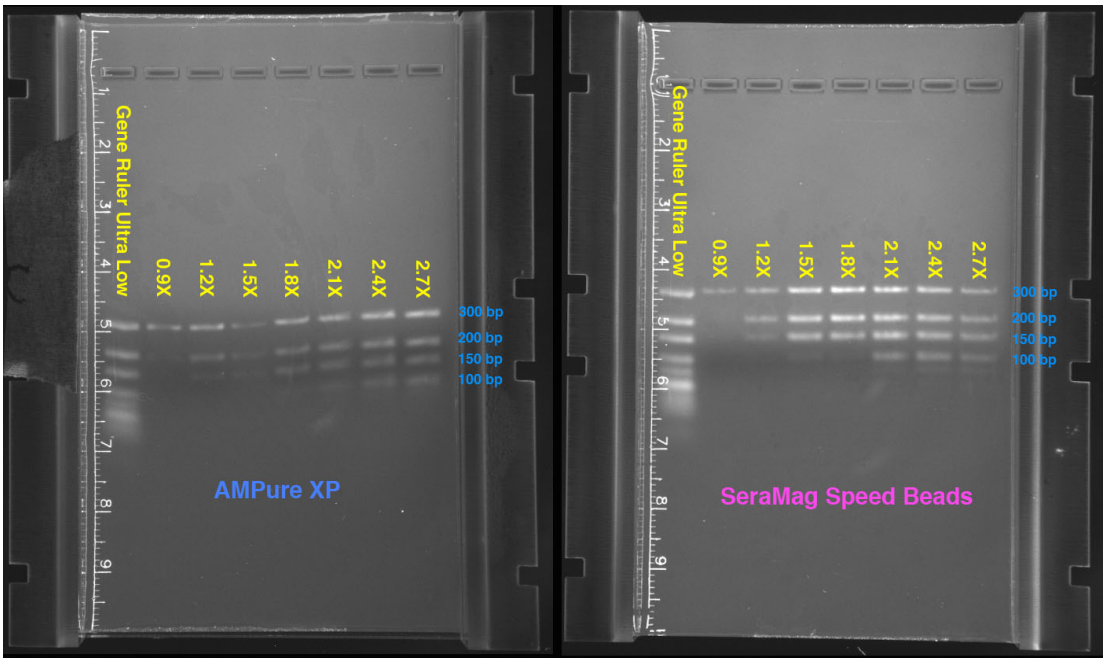
40 Transfer the supernatant to a new tube.

41 Mix supernatant with 1 µL loading dye.

42 Electrophorese in 1.5 % agarose for 1h at 100 V.

QUALITY CONTROL

43 The following image compares the results of “purifying” a mix of 2 µL Fermentas Ultra Low Range Ladder + 18 µL dH20 using several different amounts of AMPure or Serapure solution to DNA solution. AMPure is on the left, “Serapure” is on the right. After preparing 20 µL of ladder + water mix, we combined that with the volumes of AMPure or Serapure listed below and then purified using the standard protocol:



As you can see, the volume of AMPure or SeraPure controls the size of fragments recovered. More specifically, the ratio of PEG solution used to the volume of the DNA in the solution makes the difference, not the count of beads in the solution (provided they are above the minimum
level). This is what makes it possible to do “double-SPRI” size selection.

Citations

Rohland N, Reich D. . Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.
https://doi.org/10.1101/gr.128124.111