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# Serapure Preparation and Testing

Forked from DNA EXTRACTION Protocol Template

This protocol is a draft, published without a DOI.

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**Protocol status:** In development
**We are still developing and optimizing this protocol**

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**Protocol Integer ID:** 99223

## Disclaimer

Draft!

## Abstract

A protocol template created through the BeBOP project for DNA Extraction.

## Guidelines

**MIOP: Minimum Information about an Omics Protocol**

| MIOP Term | Value |
|---|---|
| analyses | |
| audience | |
| broad-scale environmental context | |
| creator | |
| environmental medium | |
| geographic location | |
| hasVersion | |
| issued | |
| language | |
| license | |
| local environmental context | |
| materials required | |
| maturity level | |
| methodology category | |
| personnel required | |
| project | |
| publisher | |
| purpose | |
| skills required | |
| target | |
| time required | |

See https://github.com/BeBOP-OBON/miop/blob/main/model/schema/terms.yaml for list and definitions.

**AUTHORS**

| PREPARED BY All authors known to have contributed to the preparation of this protocol, including those who filled in the template. | AFFILIATION | ORCID (visit https://orcid.org/ to register) | DAT |
|---|---|---|---|
| Content Cell | Content Cell | Content Cell | yyyy-mm-dd |
| Content Cell | Content Cell | Content Cell | yyyy-mm-dd |

This protocol is derived from the referenced protocol created by Nadin Rohland. Please do not cite this protocol as being originally produced by me.

**RELATED PROTOCOLS**

| PROTOCOL NAME AND LINK | ISSUER / AUTHOR | RELEASE / ACCESS DATE |
|---|---|---|
| Content Cell | Content Cell | yyyy-mm-dd |
| Content Cell | Content Cell | yyyy-mm-dd |

This is a list of other protocols which should be known to users of this protocol. Please include the link to each related protocol.

Suggested Reading

Rohland N, Reich D. **Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.** Genome Research. Early Online Access. Doi: **10.1101/gr.128124.111**

DeAngeliss MM, Wang DG, Hawkins TL: **Solid-phase reversible immobilization for the isolation of PCR products.** *Nucleic Acids Res* 1995, **23**:4742–4743.

Fisher S, et al.: **A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries.** *Genome Biol* 2011, **12**:R1.

Lundin S, Stranneheim H, Pettersson E, Klevebring D, Lundeberg J: **Increased throughput by parallelization of library preparation for massive sequencing.** *PLoS One* 2010, **5**:e10029.

**ACRONYMS AND ABBREVIATIONS**

| ACRONYM / ABBREVIATION | DEFINITION |
|---|---|
| Content Cell | Content Cell |

**GLOSSARY**

| | SPECIALISED TERM | DEFINITION |
|---|---|---|
| | Content Cell | Content Cell |
| | Content Cell | Content Cell |

**BACKGROUND**

This document describes the required protocol to conduct insert name of the method/protocol.

*Summary*
The goal here is to create a substitute for AMPure XP that is of equal effectiveness in
comparison to the commercial product but far more cost-effective ($19/mL versus $0.46/mL).

Insert a short description of the background for the method/protocol (e.g. why and for which purpose do you perform water sampling).
Please provide a brief summary of your method including, as appropriate, a brief description of what techniques your best practice is about, which ocean environments or regions it targets, the primary sensors covered, what type of data/measurements/observing platform it covers, limits to its applicability.
*Method description and rationale*

Insert a short description of the functioning principal of the methodology used in the protocol (i.e. how does the method work?). Please note that this is different from the step-by-step description of the protocol procedure.
Insert a short statement explaining why the specific methodology used in the protocol has been selected (e.g. it is highly reproducible, highly accurate, procedures are easy to execute etc.…).

*Spatial coverage and environment(s) of relevance*

If applicable, please specify the region where the protocol is applied. For regional term guidance see here. If applicable, please indicate here the environment(s) of relevance for the protocol, e.g. Abyssal plain. Select from the ENVO terminology.

*Personnel Required*
Insert the number of technicians, data managers, and scientists required for the good execution of the procedure

*Safety*

Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure

*Training requirements*

Specify technical training required for the good execution of the procedure.

*Time needed to execute the procedure*

Specify how much time is necessary to execute the procedure.

## Materials

| | DESCRIPTION e.g. filter | PRODUCT NAME AND MODEL Provide the official name of the product | MANUFACTURER Provide the name of the manufacturer of the product. | QUANTITY Provide qua |
|---|---|---|---|---|
| | Durable equipment | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Consumable equipment | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Chemicals | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |

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

**Note**

These are the same beads used by Beckman, per Orapure product sheet and http://bit.ly/vmiDzU

- PEG-8000 (Amresco 0159)
- 0.5 M EDTA, pH 8.0 (Amresco E177)
- 1.0 M Tris, pH 8.0 (Amresco E199)
- Tween 20 (Amresco 0777)
- 5 M NaCL

GeneRuler Ultra Low Range DNA Ladder **Thermo Fisher Catalog #**SM1211

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

Optional
• Agencourt SPRIPlate Super Magnet Plate (Beckman Coulter A32782)

## Before start

Read background information, MIOP and BePOP-OBON information under the "Guidelines" tab.

## STANDARD OPERATING PROCEDURE

1 In the following SOP, please use the exact names of equipment as noted in the table above.

Provide a step-by-step description of the protocol. The identification of difficult steps in the protocol and the provision of recommendations for the execution of those steps are encouraged.

## STEPS

2 In a 50 mL conical using sterile stock solutions, prepare TE ( [M] 10 millimolar (mM) TrisNHCl, [M] 1 millimolar (mM) EDTA = 500 µL [M] 1 Molarity (M) Tris pH8 + 100 µL [M] 0.5 Molarity (M) EDTA, fill conical to 50 mL mark with dH20).

3 Mix SeraNmag SpeedBeads and transfer 1 mL to a 1.5 mL microtube.

4 Place SpeedBeads on 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 magnet, mix, return to magnet.

7 Remove supernatant with P200 or P1000 pipetter.

8 Add 1 mL TE to beads, remove from magnet, mix, return to 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 rack (i.e. not on magnet stand).

11 Add 9 g PEGN8000 to a new 50 mL, sterile conical.

12 Add 10 mL [M] 5 Molarity (M) NaCL (or 2.92 g ) to conical.

13 Add 500 µL [M] 1 Molarity (M) TrisNHCL to conical.

14 Add 100 µL [M] 0.5 Molarity (M) EDTA 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 3N5 minutes until PEG goes into solution (solution, upon sitting, should be clear).

17 Add 27.5 µL Tween 20 to conical and mix gently.

18 Mix 1 mL 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 ultraNIow 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 SeraNmag 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 [M] 5 Molarity (M) NaCL (or 7.3 g ) 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 [M] 70 Mass / % volume 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 00:05:00 at Room temperature . 5m

29 Place on magnet stand.

30 Remove supernatant.

31 Add 500 µL [M] 70 Mass / % volume EtOH.

32 0 µL Incubate on stand for 00:01:00 . 1m

33 Remove supernatant.

34 Add 500 µL [M] 70 Mass / % volume EtOH.

35 Incubate on stand for 00:01:00 . 1m

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.

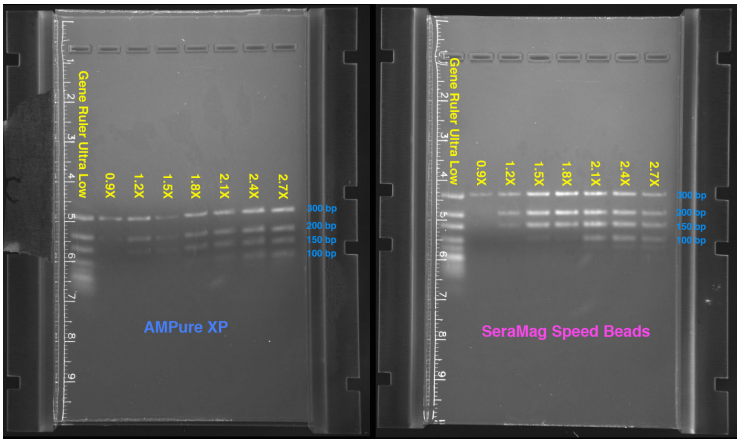
40 Transfer supernatant to new tube.

41 Mix supernatant with 1 µL loading dye.

42 Electrophorese in 1.5 % agarose for 01:00:00 at 100 V. 1h

## 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, it is the ratio of PEG solution used to the volume of the DNA in solution which makes the difference, not the count of beads in solution (provided they are above the minimum level). This is what makes it possible to do “doubleNSPRI” size selection.

## BASIC TROUBLESHOOTING GUIDE

44 Identify known issues associated with the procedure, if any.

Provide troubleshooting guidelines when available.

## APPENDIX A: DATASHEETS

45 Link templates (e.g. preformatted spreadsheets) used to record measurements and report on the quality of the data as well as any documents such as manufacturer specifications, images, etc that support this protocol. Please include a short note describing the document's relevance.

## Protocol references

Insert all references cited in the document.

Please insert full DOI address when available, e.g. http://doi.dx.org/10.1007/s11258-014-0404-1