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Serapure Bead production

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1 Works for me dx.doi.org/10.17504/protocols.io.bkguktww

methods

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SUBMIT TO PLOS ONE

ABSTRACT

Production of DNA size separation magnetic beads.

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KEYWORDS

speed beads

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MATERIALS TEXT

MATERIALS

Sera-Mag Speed Beads **Ge**
Healthcare Catalog #65152105050250 Step 3

PEG 8000 **Sigma**
Aldrich Catalog #81268

SAFETY WARNINGS

Wear gloves.

BEFORE STARTING

Just make sure all the components have been purchased. It is not a difficult protocol

Serapure bead production

1 In a 50 mL conical using sterile stock solutions, prepare TE (10 mM Tris--HCl and 1 mM EDTA);
- 500 µL 1 M Tris pH8
- 100 µL 0.5 M EDTA

Fill conical to 50 mL mark with dH20.

2 To a to a new 50 mL, sterile conical add;
- 9 g PEG--8000
- 10 mL 5 M NaCL (or 2.92 g)
- 500 µL1 M Tris--HCL
- 100 µL0.5 M EDTA
- 0.250 ul 10% tween-20
- Fill to ~ 49 mL using sterile dH20.

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

-Fill to ~ 49 mL using sterile dH20. gently.

3 Mix Sera--mag SpeedBeads and transfer 1 mL to a 1.5 mL microtube.

Sera-Mag Speed Beads **Ge**
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4 Place SpeedBeads on magnet stand until beads are drawn to magnet.
Discard supernatant

5 Add 1 mL TE to beads, remove from magnet, mix, return to magnet.
Discard supernatant

6 Add 1 mL TE to beads, remove from magnet, mix, return to magnet.
Discard supernatant

7 Add 1 mL TE to beads and remove from magnet. Fully resuspend and set microtube in rack (i.e. not on magnet stand).

8 Mix 1 mL SpeedBead + TE solution and transfer to 50 mL conical.

9 Fill conical to 50 mL mark with dH20 (if not already there) and gently mix 50 mL conical until brown.

10 Wrap in tinfoil (or place in dark container) and store at 4°C.

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11 Test against AMPure XP using aliquots of ladder (Fermentas GeneRuler).I recommend the 50 bp ladder in place of the ultra--low rangeladder.

12 Mix 2 µLGeneRuler with 18 µL dH20.

13 Add 20 µLGeneRuler 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).

13.1 Use 20ul of the ladder/water mix and add to these amounts of the serapure beads and the ampure beads.
0.9X sample - 18 ul
1.2X sample- 24 ul
1.5X sample - 30 ul
1.8X sample- 36 ul
2.1X sample - 42 ul
2.5X sample - 50 ul
2.7X sample - 54 ul

14 Incubate mixture 5 min.at room temperature.

15 Place on magnet stand.
Discard supernatant.

16 Add 500 µL70 % EtOH.

17 Incubate on stand for 1 min.
Discard supernatant.

18 Add 500 µL 70% EtOH.

19 Incubate on stand for 1 min.
Remove supernatant.

20 Place beads on 37°C heat block for 3--4 min.until dry.

21 Re-hydrate with 20 µLdH20 for 5 minutes.

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22 Place on magnet stand.
Transfer supernatant to new tube.

23 Mix supernatant with 3 µLloading dye.
Use the same ladder
Electrophorese in 1.5 % agarose for 60 minutes at 100 V (or as you please)

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