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Feb 10, 2021

Serapure Bead production

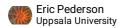
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Works for me

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methods



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ABSTRACT

Production of DNA size separation magnetic beads.

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PROTOCOL CITATION

Eric RA Pederson 2021. Serapure Bead production . protocols.io https://dx.doi.org/10.17504/protocols.io.bkguktww

KEYWORDS

speed beads

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41204

MATERIALS TEXT

MATERIALS

Sera-Mag Speed Beads Ge

Healthcare Catalog #65152105050250

Aldrich Catalog #81268

SAFETY WARNINGS

Wear gloves.

BEFORE STARTING

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

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Seranure	head	production
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- 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
 - $-100~\mu L~0.5~M~EDTA$

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.

	11	Test against AMPure XP using aliquots of ladder (Fermentas GeneRuler). I recommend the 50 bp ladder in place of the ultralow 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).		
		 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 34 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.
- Mix supernatant with 3 μ Lloading dye. Use the same ladder Electrophorese in 1.5 % agarose for 60 minutes at 100 V (or as you please)