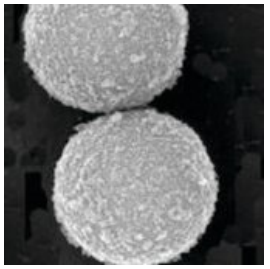
Nov 10, 2020

Home-Brew SPRI Beads



In 1 collection

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Protocol status: Working
This protocol works for DNA and RNA purification

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Keywords: library preparation for massive sequencing, sequencing library, genome biol, nucleic acids re, brew spri, genome research, massive sequencing, substitute for ampure xp, libraries for multiplexed target capture, pcr product, dna, bead, developers of this protocol

Abstract

The is a protocol to make a substitute for AMPure XP that is of equal effectiveness in comparison to the commercial product but far more cost-effective.

Credit for this goes to the developers of this protocol:

Ethan Ford;**https://ethanomics.wordpress.com/**
B. Faircloth & T. Glenn
November 19, 2011
Ecol. and Evol. Biology
Univ. of California – Los Angeles

This protocol is derived from the referenced protocol created by Nadin Rohland.

Suggested Reading

http://core-genomics.blogspot.com/2012/04/how-do-spri-beads-work.html

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

DeAngelis 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.

Guidelines

I list stock solutions that can be purchased pre--mixed and sterilized.This is in an attempt to minimize variation to the degree possible. You can certainly prepare your own stock solutions at appropriate pH.

I prepare this making 4 50ml conicals worth at one time to limit batch variability.

Materials

MATERIALS

- Magnetic stand for micrcentrifuge tubes **Life Technologies Catalog #**12321D
- Tris, 1 M, pH 8.0 **Ambion Catalog #**AM9855G
- nuclease free water
- Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL **GE Healthcare Catalog #**65152105050250
- 5M NaCl **Ambion Catalog #**AM9760G
- Tween™ 20 Surfact-Amps™ Detergent Solution **Thermo Fisher Catalog #**85113
- EDTA (0.5 M), pH 8.0, RNase-free **Thermo Fisher Catalog #**AM9260G
- Poly Ethylene Glycol (PEG) 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**89510-250G-F
- TE Solution

Any Tween 20 is acceptable. Make into a 10% stock solution for use in the protocol.

You can make many of these solutions in lab. If you do so be sure to make with Nuclease-free water.

5M NaCl is a saturated solution.Use care in making NaCl solution and heat may be required to fully dissolve the needed NaCl.Make at least 1 day in advance.When solution is fully dissolved and cool enough to handle aliquot in 10-30ml batches in 50ml conical tubes.

Troubleshooting

Before start

Make a 10% Tween20 stock solution if you do not have a diluted stock.

Make TE solution (10mM TrisHCl, 1mM EDTA) 5m

1 Make TE solution (10mM Tris-HCl, 1mM EDTA pH 8)

500 µL 1M Tris-HCl pH 8
100 µL 0.5M EDTA
49.6 mL Nuclease free water

Wash SpeedBeads 5m

2 Mix SpeedBead bottle thoroughly by shaking and vortexing. 2m

3 Transfer 1 mL of SpeedBeads to a 1.5ml microtube.

4 Place microtube on magnetic stand until the beads are drawn to magnet. 2m

5 Remove and discard supernatant.

6 Add 1 mL TE to the beads and remove from the magnet. 1m

7 Remove microtube from magnet and resuspend beads by mixing or vortexing.

8 go to step #4 Repeat steps 4-7 twice
This will be a total of 3 washes.

Leave washed beads on rack until needed in Step 16.

Make SPRI Buffer 30m

9 Add 9 g PEG-800 directly to a new sterile 50ml conical tube.

Do this in chemical room. Tear the balance to 0 with the tube on the balance in a holder. Then weight out 9g into the tube. Seal tube and bring to bench to finish buffer.

10 Add 10 mL 5M NaCl to the 50ml conical.

11 Add 500 µL 1M Tris-HCl pH8 to the 50ml conical.

12 Add 100 µL 0.5M EDTA pH8 to the 50ml conical.

13 Fill the 50ml conical to ~ 48 mL using nuclease free dH2O. You can do this by eye, just go slowly.

I add 2 mL of water to get to about 20 mL then add 28.5 mL of water.

14 Mix conical for about 5-20 minutes until PEG goes into solution (solution, upon sitting, should be clear). 20m

The exact time of mixing is not critical. You can leave it longer without issue.

15 Add 270 µL 10% Tween20 to the 50ml conical and mix gently.

Mix Beads & Buffer

16 Resusped the SpeedBead TE solution from Step 8 then add it to the 50ml conical with the SRPI buffer.

> Note
>
> I try to add the beads to the bottom of the 50ml conical.

17 Take buffer from the top of the 50ml conical tube and rinse out the eppendorf tube which contained the SpeedBeads to recover all the beads.

18 Fill conical to 50ml mark with dH2O (if not already there) and gently mix 50ml conical until a uniform brown color.

Storage

19 Wrap conical in tinfoil and store at 4C.

Note

I usually prep 4 x 50ml conicals at one time. Then pool them into an empty nuclease free water bottle for storage.

Validate home-brew SPRI

20 Test Home-Brew SPRI agains Ampure XP. I use total RNA and fragmented RNA to perform a head-to-head. You can also test using a cDNA library. Using RNA as the input serves as validation that there is no RNase activity in the solution.

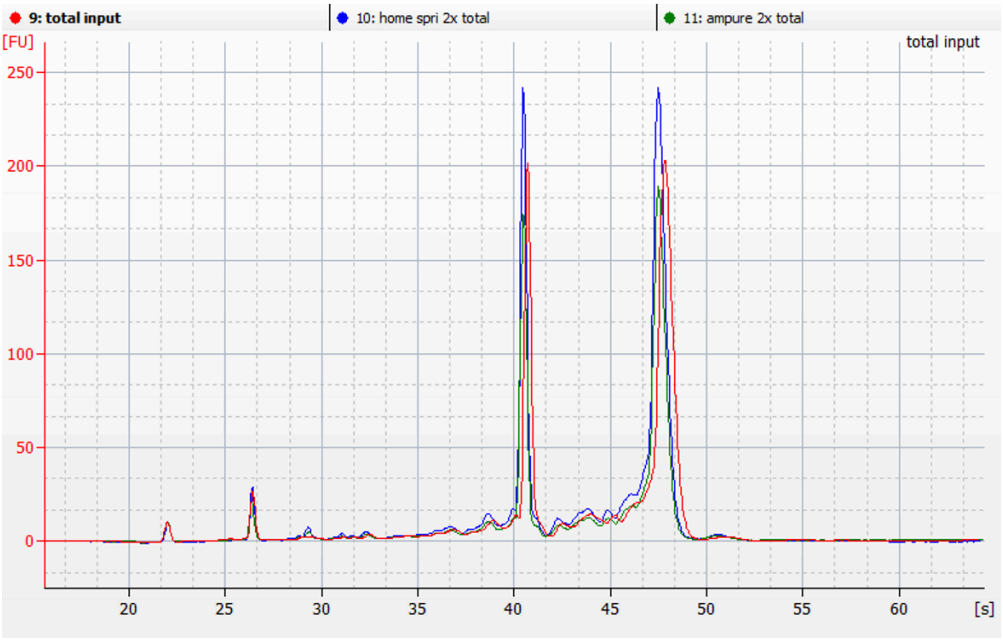
21 Prepare fresh 80% ethanol.

22 Purify Total RNA and Fragmented RNA at 1x and 2x SPRI ratio. You can test whatever ratios you need to confirm are working for your assay. These are good tests for my use.

Run purified sample on BioAnalyzer.

22.1 Total RNA 2x SPRI purification test results - 100ng/ul input

Expected result



22.2 Fragmented RNA 1x SPRI purification test results - 15ng/ul input

Expected result