Preparation of 1.5 mg/mL Sera-mag carbolylate modified magnetic particles

Molly Miranda

Abstract

Preparation of 1.5 mg/mL Sera-mag magnetic particles for DNA purification, a low cost Agencourt Ampure XP reagent substitute

Adapted from B. Faircloth & T. Glenn, 11.19.11, Ecology and Evolutionary Biology, UCLA

https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf

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Guidelines

Materials:

Component	Vendor	part #	amt/mL		in 50 mL final	final [ ]	500	
Sera-mag SpeedBeads carboxylate modified magnetic particles, 5% solids, 50 mg/mL (Hydrophobic)	GE Healthcare Life Sciences	44152105050350	0.030	1.5	1.5 mL/75 mg	1.5 mg/mL	15	mL
PEG-8000	Amresco	159	0.22	11.0	11 g	22% w/v	110	g
NaCl, 5 M			0.2	10.0	10 mL	1.0 M	100	mL
TE, 100X	Sigma	T9285	0.01	0.5	500 µL	1 X	5	mL
Tween 20	Sigma	P9416	0.55	27.5	27.5 µL	0.055 % v/v	275	µL

50 bp ladder

1x (10 mM) Tris-HCl (pH 8.0 - 8.5)

Note: Sera-mag non-SpeedBeads and/or hydrophilic types can be used. If non-SpeedBeads are used, more

time will be needed during magnetic separations.

Equipment:

Magnetic stand for 1.5 mL Eppendorf tubes

Magnetic stir plate

Stir bars

Laboratory scale

Before start

Prepare 1x TE, filter sterilize (0.2 µM).

Materials

- Tween 20 170-6606-MSDS by Bio-rad Laboratories
- ✓ PEG-8000 by Contributed by users
- Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles 44152105050350 by Ge Healthcare
- ✓ Sodium Chloride, 5 M by Contributed by users
- ✓ Tris-EDTA, 100x by Contributed by users

Protocol

Step 1.

For a 500 mL final volume:

Sera-mag carboxylate modified magnetic particles, 5% solids, 50 mg/mL (Hydrophobic) - 15 mL

PEG-8000 - 110 g

NaCl, 5 M - 100 mL

TE, 100X - 5 mL

Tween 20 - 275 µL

REAGENTS

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles 44152105050350 by Ge Healthcare

LINK: https://docs.google.com/spreadsheets/d/1s03EnedfadGRvxUioFEUlg6J3P8ue5SiLEO4yKHjQrE/edit#gid=0

Step 2.

Weigh PEG, add to 1 L graduated cylinder, add NaCl solution, TE, and water to 95 % of final volume. If prep is bigger than 500 mL, be sure to use a graduated cylinder that is ≥ 2x the final volume.

Step 3.

Cover with parafilm. Mix and stir on a magnetic stir plate until PEG is in solution, 5 - 30 minutes.

NOTES

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Some inverting of graduated cylinder may be necessary to get PEG fully in solution.

Step 4.

Add tween 20 and stir or mix gently to avoid foaming.

NOTES

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Tween 20 is very viscous. Use a wide-bore pipet tip, or cut a tiny amount off the end of a regular pipet tip before pipetting the tween 20. Rinse tip in PEG solution after pipetting.

Step 5.

Wash Sera-mag beads in 1.5 mL aliquots in Eppendorf tubes, 2 times with 1.5 mLs of 1x TE. For each wash, resuspend beads by pipetting. For the final resuspension, use 1 mL of 1x TE for every 1.5 mL of beads washed.

Step 6.

Add washed beads to PEG solution, mix well.

Step 7.

Fill to 100% volume with water. Mix until suspension is homogeneous. Transfer to storage containers. Store @ 4˚C.

Check function

Step 8.

Test new bead preparation compared to old and/or to AMPure XP beads as follows.

Check function

Step 9.

Prepare ≥ 200 uL of 100 ng/µL 50 bp ladder in 1x Tris-HCl.

Check function

Step 10.

Aliquot 10 µL of 100 ng/µL ladder into tubes.

Check function

Step 11.

Add reference and test bead preparations to 50 bp ladder at varied ratios.

Check function

Step 12.

Mix gently by pipetting up and down 10 times, by inverting tubes, or by slight vortexing. If droplets are stuck to sides of container, centrifuge quickly and gently.

Check function

Step 13.

Continue with Magnetic Particle Based DNA Purification protocol from 1st incubation step. Elute in 10 µL.

Check function

Step 14.

Check fragment patterns on an Agilent 2100 Bioanalyzer with a DNA 1000 kit. Fragment pattern may not match commercial beads exactly at the lower (< 1.0) bead/sample ratios. Fragment patterns at 1.0 bead to sample ratio and above should match.