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SPRI beads, variable PEG concentration V.1

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MPI EVA Ancient DNA C...

Ancient DNA Core Unit
MPI EVA

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Abstract

This is a protocol for the preparation of beads suspension for the purification of nucleic acids using the Solid Phase Reversible Immobilization (SPRI) method (DeAngelis et al. 1995). Preparations of SPRI beads are used in various steps of sample preparation by the Ancient DNA Core Unit of the MPI-EVA as an alternative to commercially available products, such as AMPure XP beads (Beckman Coulter).

The size selectivity of SPRI beads depends primarily on the concentration of polyethylene glycol (PEG) in the reaction, which can be modulated by adjusting the PEG concentration in the bead suspension or by changing the volume ratio between SPRI bead suspension and sample. We here provide protocols for the preparation of bead suspensions containing 18%, 33% and 38% PEG-8000 (w/v) in three different volumes (50 ml, 100 ml and 250 ml). SPRI bead suspension containing 18% PEG performs similar to AMPure XP beads, which are often used in a 1.8x beads suspension/sample ratio. SPRI bead suspensions containing 33% or 38% PEG are useful if smaller volume ratios are desired, for example due to constraints in pipetting or reaction volumes. Instructions for DNA purification using these SPRI bead suspensions are provided in the protocols citing this document.

References

DeAngelis, M. M., Wang, D. G., Hawkins, T. L. (1995) Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Res. 23(22):4742-3. doi: 10.1093/nar/23.22.4742

Troubleshooting

Note

SPRI bead suspension with 33% PEG also contains a slightly higher concentration of carboxyl coated beads to maximize the yield of DNA in the purification of indexing PCRs.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Sera-Mag SpeedBeads,15 ml, carboxylate-modified microparticles, 50 mg/ml	ThermoScientific	6515-2105-050250
	PEG 8000, Molecular Biology Grade	Promega	V3011
	5 M NaCl	Sigma Aldrich/Merck	S5150-1L
	0.5 M EDTA, pH 8.0	AppliChem	A4577,1000
	1 M Tris-HCl, pH 8.0	AppliChem	A4892,1000
	Tween-20	Thermo Fisher Scientific	11417160
	TE buffer*	Self	
	UltraPure DNA/RNAse-free water	Sigma Aldrich/Merch	1153332500
	Consumables		
	Square media bottle 100 ml	xxx	xxxx
	Square media bottle 250 ml	VWR	391-0629
	Falcon tube, 50 ml, skirted	Greiner	GB 227270
	50 ml serological pipette	Corning BV	357550

* See document in the Appendix for preparation of TE buffer.

Equipment

- Serological pipette controller (e.g. battery powered pipetting aid ROTILABO, cat. no. TC16.1)
- Laboratory balance with 0.1g readability
- Magnetic rack (model depending on tube type)

Protocol for the preparation of SPRI bead suspension

1. Weigh PEG powder into a 50 ml Falcon tube, 100 ml or 250 ml square media bottle (depending on the volume of SPRI suspension desired) and add the following components as detailed in the table below. Fill up with water to the indicated volume using the graduation of the tube/bottle.

	Reagent	Mass/volume for 50 mL	Mass/volume for 100 mL	Mass/volume for 250 mL	Final concentration
	PEG 8000				
	- for 18% PEG	9 g	18 g	45 g	18% (w/v)
	- for 33% PEG	17.5 g	33 g	82.5 g	33% (w/v)
	- for 38% PEG	19 g	38 g	95 g	38% (w/v)
	0.5 M EDTA, pH 8.0	100 µl	200 µl	500 µl	1 mM
	1 M Tris-HCl, pH 8.0	0.5 ml	1 ml	2.5 ml	10 mM
	5 M NaCl	10 ml	20 ml	50 ml	1 M
	Water	to ~45 ml	to ~95 ml	to ~240 ml	

2. Shake the tube or bottle until all PEG has dissolved.

3. Add the volume of Tween-20 indicated in the table below.

	Reagent	Volume for 50 mL	Volume for 100 mL	Volume for 250 mL	Final concentration
	Tween-20	25 µl	50 µl	125 µl	0.05% (v/v)

4. Mix by inverting or shaking the tube/bottle until the Tween-20 has completely dissolved.

5. Prepare Sera-Mag bead suspension:

5.1. Re-suspend stock solution of Sera-Mag beads by intense vortexing.

5.2. Transfer the volume of stock bead suspension indicated below to a 15 ml or 50 ml Falcon tube.

	Reagent	Volume for 50 mL	Volume for 100 mL	Volume for 250 mL	Final concentration
	Sera-Mag stock bead suspension (50 mg / ml)				
	- for 18% PEG	3 ml	6 ml	15 ml	3 mg/ml
	- for 33% PEG	3.55 ml	7.1 ml	17.75 ml	3.55 mg/ml
	- for 38% PEG	3 ml	6 ml	15 ml	3 mg/ml

5.3. Pellet the beads in a magnetic rack. Pipette off and discard supernatant.

5.4. Add 5 ml TE buffer and resuspend the beads by vortexing.

5.5. Pellet the beads in a magnetic rack. Pipette off and discard supernatant.

5.6. Repeat wash steps (5.4 and 5.5) once for a total of two washes.

5.7. Add 3 ml TE buffer and resuspend the beads by vortexing.

6. Add the complete volume of bead suspension to the tube/bottle from step 4. Fill up to 50, 100 or 250 ml with water using the graduation of the tube/bottle.

7. Mix bead suspension thoroughly by inverting the tube/bottle several times.

Note

[Labeling]

Label the bead suspension with "SPRI beads (XX% PEG)", date of production and the initials of the person who prepared the bead suspension.

Attention: Every single bottle prepared at the same day gets a new batch ID. Name the batches with Roman numerals (e.g. batch I, batch II, etc.)

8. Wrap the tube/bottle in aluminum foil (prevents light exposure) and store in the fridge for up to 1 year.

Note

[Note]

When preparing SPRI beads for the first time, use a DNA ladder to determine whether the size cutoff and DNA recovery are as expected (see Figure 1).

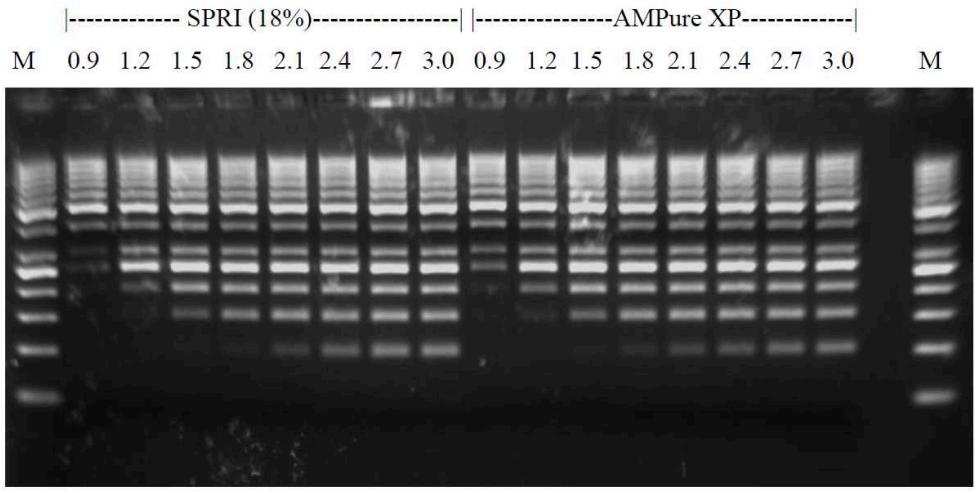

Fig. 1: Recovery of a DNA size marker (M: 50 bp ladder) using self-made SPRI bead suspension vs. a commercial product at different sample/bead suspension ratios.

Appendix

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

Preview