DNA size selection (>3-4kb) and purification of DNA using an improved homemade SPRI beads solution.

Forked from DNA size selection (>3-4kb) and purification of DNA using an improved homemade SPRI beads solution.

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Working

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private for test

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ABSTRACT

Most noncommercial tradition DNA extraction protocols result in a crude DNA preparation. If the DNA is intended to be used for a high-end application like Nanopore sequencing, it requires a thorough clean-up and size selection before it could be used for sequencing.
Solid Phase Reversible Immobilisation (SPRI) magnetic beads is a quick and convenient way of purifying and size selecting intact double-stranded DNA from crude DNA. Most commercially available SPRI beads based DNA purification mix is quite expensive so our lab endeavored to develop an inexpensive beads mix which is as good as the commercially available ones. In this effort, our lab has optimized a beads mix for purifying and size selecting crude DNA extracted from eucalyptus and posted on protocol.io, https://www.protocols.io/edit/high-purity-high-molecular-weight-dna-extraction-f-n5ydg7w?step=16.
However, this solution was not very effective in purifying crude DNA extracted from fungal material. DNA extracted from fungal material is highly viscous which is indicative of high levels of impurities in the DNA preparation. I tried to improve the beads mix for purifying rust DNA by adding 0.25 % (v/v) Tween-20 into the beads mix.
I tested beads mix with Tween-20 to see if adding tween into the solution makes any difference in the recovery, purity and size selection. Turns out that bead solutions with Tween-20 make big difference in the size selection and recovery of the DNA compared to the bead solutions without.

I calibrated/tested the bead solution with and without Tween-20 on the 1 kb DNA ladder to establish which DNA solution to beads volume ratio gives optimal recovery and size selection. We found that beads mix with 0.25 % Tween-20 works much better in size selection and recovery than beads mix without Tween-20. The best DNA to beads volume ratios were 1.0: 0.9 and 1.0: 1.0.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Beads protocol was originally adapted from Philippe Jolivet and Joseph W. Foley, 2015 - Solutions for purifying nucleic acids by solid-phase reversible immobilization (SPRI)
Link: http://www.openwetware.org/images/f/f8/SPRI_buffers_v2_2.pdf

Calibrating the newly prepared beads mix: Every time you prepare new a fresh beads mix, make sure you test it with either ladder DNA or DNA which you don't mind losing.

pH makes a huge difference in the solubility of DNA and beads. If not set properly, beads tend to clump at DNA elution step that could lose as much 60-70 percent of the DNA. So make sure that your elution buffer (e.g. 10 mM Tris or 0.1 x TE) has a pH of 8.

Homogeneous beads mix at room temperature before use.

Always use freshly prepared 70 % Ethanol
Preheat your elution buffer of choice (TE-Buffer, Tris 10 mM, Water..) to 37-42°.

We use same beads solution for the MinION sequencing library preparation.

MATERIALS

NAME	CATALOG #	VENDOR
0.5 M EDTA		Contributed by users
1 M Tris-HCl pH 8.0		Contributed by users
Ethanol 70%		Contributed by users
10 % Tween-20		Contributed by users
10 mM Tris-HCL pH 8.0		Contributed by users
50 % Polyethylene Glycol (PEG)		Contributed by users
5 M NaCl		Contributed by users

Make beads stock solution

1 For 10 mL beads stock solution:

Final	stock	Input
10 mM Tris-HCl	1 M	100 µl
1 mM EDTA pH 8	0.5 M	20 µl
1.6 M NaCl	5 M	3.2 ml
11% PEG 8000	50% (w/v)	2.2 ml
0.25 % Tween-20	10 % (v/v)	200
0.4% beads (v/v)	100%	40 µl
Milliq Water		4.24 ml
Total		10 ml

Frist combine only Water, Tris-HCl, EDTA and NaCl in a 50 mL tube.

2 Vortex Sera-Mag SpeedBeads® Carboxyl Magnetic Beads (GE Healthcare) very well and pipette 40 µl into a 1.5 ml tube, put it on the magnetic rack and wait until the solution has cleared up and all beads have bound to the back of the tube