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

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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.

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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

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

Protocol

Make beads stock solution

Step 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.

Make beads stock solution

Step 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

Make beads stock solution

Step 3.

Wash beads by removing supernatant and adding 1 ml Milli-Q water

Make beads stock solution

Step 4.

Take tube of the magnet, mix well, spin down in a microcentrifuge and put back on the magnet

Make beads stock solution

Step 5.

Wait for beads to assemble at the back of the tube

Make beads stock solution

Step 6.

Pipette off and discard supernatant

Make beads stock solution

Step 7.

Repeat washing (steps 3 - 6) 3 more times

Make beads stock solution

Step 8.

After pipetting of the supernatant the last time take off tube from the magnet and add 40 µl of the previous (step 1) prepared stock solution, mix well, spin down and pipette everything into the remaining stock solution in the 50 mL tube and mix

Make beads stock solution

Step 9.

Now the 2.2 ml 50% PEG can be added to the stock solution, which after vortexing very well is ready for use.

Be careful to actually pipette 2.2 ml as solution is very viscous, but the final concentration of PEG is crucial for the clean up to work properly.

Clean up

Step 10.

Bring you DNA sample in a 1.5 ml tube to comfortable pipetable volume (I usually do it with some volume between 20 - 200 µl) and if you know already that your sample contains a lot of contaminants and/or DNA (hence a really viscous solution) diluting it out and splitting into two tubes can make life easier.

(A lot of contaminants can also clump around the beads which makes binding to the magnet slower and sometimes pipetting off the supernatant really difficult)

Clean up

Step 11.

Make sure to know the exact volume of your sample and add 1 V of that (or 0.9 V for removal of more smaller fragments but also higher risk of less recovery) of well homogenized, room temperature beads solution and mix by flicking the tube

Clean up

Step 12.

Place the tube on a rotor (or mixer if you don't have one) and mix for 10 minutes

Clean up

Step 13.

Spin down tube in the microcentrifuge and place on the magnet

Clean up

Step 14.

Wait until beads have moved to the back of the tube and the solution becomes clear

(depending on viscosity of the solution that can take between 1 min to much longer like 1h, if after that the beads look like they are stuck in a big cloud and just don't properly bind to the magnet I add same amount of buffer and beads (the ratio always has to stay the same!) mix again and the put it back on the magnet - usually that solved the problem. But you probably will never have that problem, I just worked with really contaminated samples (plants))

Clean up

Step 15.

Remove and discard supernatant

Clean up

Step 16.

Wash beads with fresh 70% Ethanol by adding 1 - 1.5 mL to the opposite side than where the beads bind and wait 30 seconds

Clean up

Step 17.

Remove and discard Ethanol

Clean up

Step 18.

Repeat washing once more (steps 15 - 18)

(Don't remove tube from magnet during the washing steps until here)

Clean up

Step 19.

For the last removal of Ethanol make sure that all the Ethanol is removed, therefore take tube off the magnet , spin down for a second and place back onto the magnet, like that also the last drops of Ethanol can be pipetted off

Clean up

Step 20.

Let beads air dry for a maximum of 30 seconds or else elution will be difficult

Clean up

Step 21.

Add 50 ul (or in whatever final volume and concentration the sample is needed) of preheated to 50°C 10 mM Tris (or TE-Buffer)

Clean up

Step 22.

Make sure the beads are resuspend properly by flicking the tube gently and spinning it down - the solution will be homogeneous and brown

(I put it in a 50°C Thermoblock for about 20 seconds to encourage the elution reaction)

Clean up

Step 23.

Spin down the tube before placing it on the magnet again and wait until the beads have bound the the back of the tube (that can again take its time especially if the sample contains really long DNA fragments) Depending on the sample that will take between 1 minute to a few hours, I usually wait 5 - 10 minutes

Clean up

Step 24.

When the solution has cleared up completely, pipette the supernatant to a fresh tube and discard beads.

Quality control

Step 25.

Meassure the DNA concentration with a Qubit.

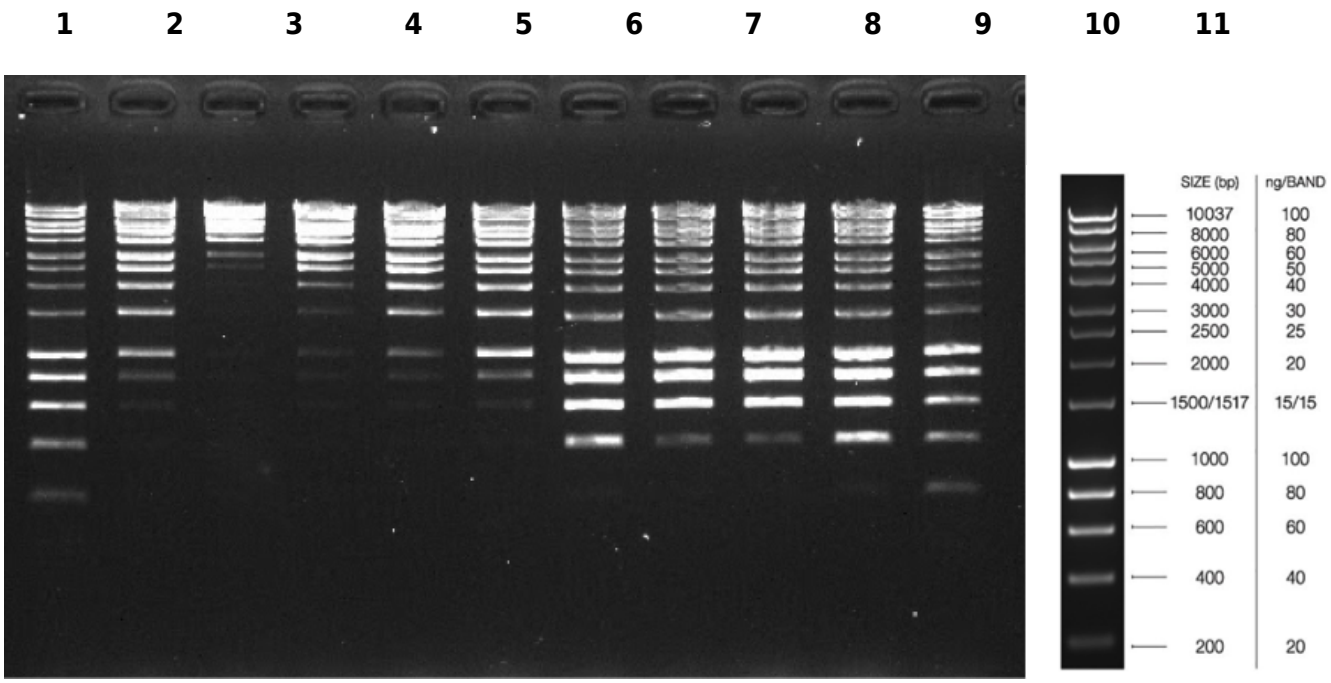
The recoveries for HMW DNA > 20kb should lie between 60 - 90%

Quality control

Step 26.

Run a 1.0% TBE agarose gel

80 ng DNA per sample, normalized to 10 ul per lane



Lanes:

Lane 1 and 11: Bioline 1kb hyperladder

Lane 2:0.45 AMPure XP Beads (Beckman Coulter). This is the standard dilution used in PacBio and Oxford Nanopore protocols.

Lane 3: 1.0:0.8 V our SPRI beads with Tween-20

Lane 4: 1.0:0.8 V our SPRI beads w/o Tween-20

Lane 5: 1.0:1.0 V our SPRI beads with Tween-20

Lane 6: 1.0:1.0 V our SPRI beads w/o Tween-20

Lane 7: 1.0:1.5 V our SPRI beads with Tween-20

Lane 8: 1.0:1.5 V our SPRI beads w/o Tween-20

Lane 9: 1.0:2.0 V our PRI beads with Tween-20

Lane 10: 1.0:2.0 V our SPRI beads w/o Tween-20