

DNA size selection (>1kb) and clean up using an optimized SPRI beads mixture

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Abstract

This protocol describes a clean up and size selection method for nucleic acids (tested on DNA) to deplete and remove fragments below 1 - 2 kb.

The success of this depends on the cleanliness of your sample (it doesn't have to be super clean but a whole lot of contaminants make working with the beads more difficult, diluting the sample out before usage can help with that).

The concentrations of PEG and NaCl and the volume of the beads solution are crucial for recovery and proper removal of small fragments.

As a basic guideline it can be said: more PEG and NaCl - higher recovery but hence less removal of small fragments and the other way round. I found for my samples (eucalyptus) that with 1 volume of the beads solution respectively to DNA sample I'm on the safe side recovery wise, but if I want to make sure to get rid of more smaller fragments I use 0.8 volumes.

So in numbers that means:

Final reaction concentration of PEG8000:

1 V: 5.5%

0.8 V: 4.8%

Final reaction concentration of NaCl:

1V: 0.8 M

0.8V: 0.7 M

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Guidelines

Beads solution 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

There really accurate and helpfull recipies for making up every ingredient of the beads solution can be found, I just adapted the concentrations for my needs.

Thank you Benjamin Schwessinger for supporting and encouraging me to try every single concentration combination out there to find a simple and well working one.

I also use the same beads solution for the MinION sequencing library preparation.

Before start

Before doing this clean up on your whole (maybe very precious) sample I would recommend testing it out on a smaller amount (300 - 500ng) to make sure the beads work for you.

- For making the beads solution stock take Sera-Mag SpeedBeads ([GE Healthcare](#), PN 24152105050250) out of the fridge and let warm up to room temperature

- For further clean ups always take the beads stock solution out of the fridge at least 15 minutes before usage to let it warm up to room temperature and mix (vortex) very well until soluion looks homogeneous and no bead clumps are visible anymore (that is very important)

- Always make fresh 70 % Ethanol

- Preheat your elution buffer of choice (TE-Buffer, Tris 10 mM, Water..) to 50° until usage

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
0.4% beads (v/v)	100%	40 µl
Milliq Water		4.44 ml

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

■ ANNOTATIONS

Wai Yee Wong 25 Jan 2018

Would like to ask what is the pH value of 10mM Tris-HCl used in here? Thanks

Make beads stock solution

Step 2.

Vortex Ser-Mag SpeedBeads (GE Healthcare, PN 24152105050250) very well and pipette 40 µl into a 1.5 ml tube, put it on the magnetic rack and wait until solution has cleared up and all beads have bound to the back of the tube

■ ANNOTATIONS

Benjamin Schwessinger 03 Jul 2017

Apologies we got the product number wrong. This should be SpeedBeads magnetic carboxylate modified particles GE Healthcare, 65152105050250.

Make beads stock solution

Step 3.

Wash beads by removing supernatant and adding 1.5 ml milliq 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 your DNA sample in a 1.5 ml tube to comfortable pipettable 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 0.8 V for removal of more smaller fragments but also higher risk of less recovery) of well homogenised, room temperature beads solution and mix by flicking the tube

Clean up

Step 12.

Place 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 µl (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.

Measure the DNA concentration with a Qubit.

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

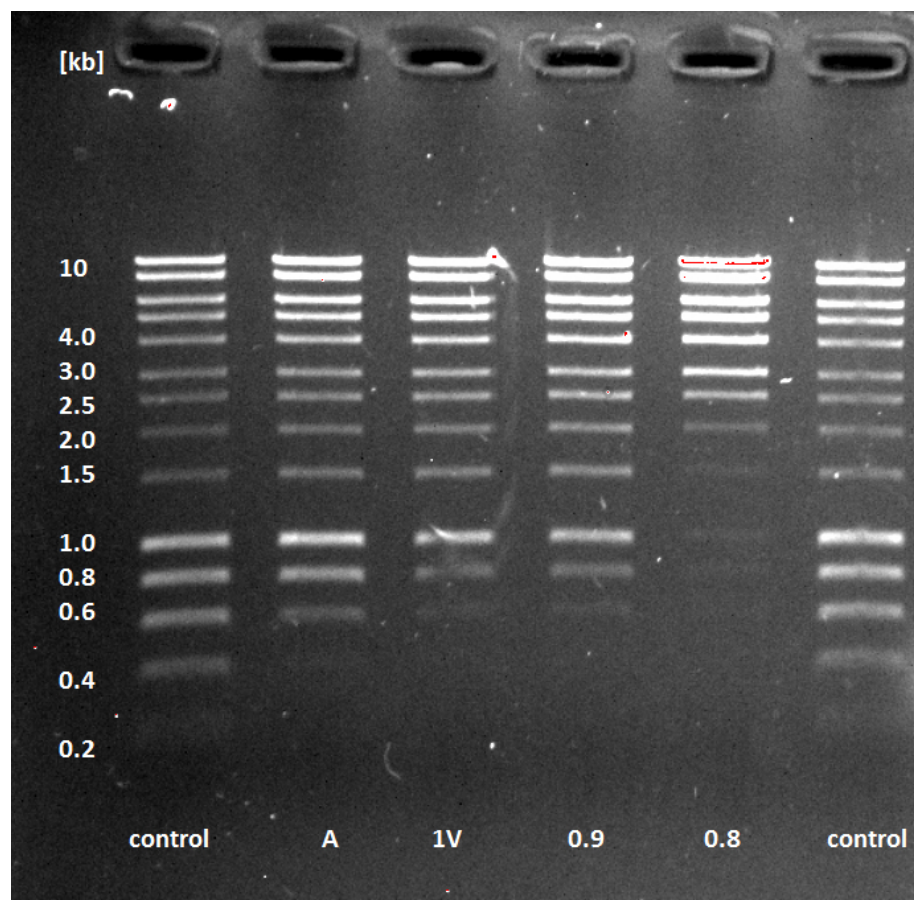
Quality control

Step 26.

Run a 0.8% agarose gel

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

run for 45 min in 1 x TBE buffer



Lanes:

control: untreated 10kb hyperladder

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

1: 1 volume of beads solution from this protocol

0.9: 0.9 volumes

0.8: 0.8 volumes