High molecular weight gDNA extraction for after Mayjonade et al. optimised for eucalyptus for nanopore sequencing

Version 6

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Abstract

Extraction of high quality DNA for long read sequencing e.g. the Oxford Nanopore

Optimized for DNA extraction from eucalyptus grandis and eucalyptus pauciflora.

This protocol contains an optional Chloroform clean up step which is necessary for eucalyptus but might not be for other tissue.

For long DNA fragments don't vortex the DNA sample.

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Guidelines

Modified from the protocol of Baptiste Mayjonade, Jérome Gouzy, Cécile Donnadieu, Nicolas Pouilly, William Marande, Caroline Callot, Nicolas Langlade and Stéphane Munos, High molecular weight gDNA extraction, Bio Techniques, Vol. 61, No. 4, October 2016, pp. 203-205.

Link to webpage:
http://www.biotechniques.com/BiotechniquesJournal/2016/October/Extraction-of-high-molecular-weight-genomic-DNA-for-long-read-sequencing-of----single-molecules/biotechniques-365135.html

When citing please make sure to also mention the original Mayjonade et al. protocol as described above.

Thank you Benjamin Schwessinger for the constant support in the lab and outside!

Reagents required

Lysis buffer:

1% PVP 40

1% PVP 10

500 mM NaCl

100 mM TRIS pH 8

500 mM EDTA

1.25% SDS

1% Sodium metabisulfite

Adjust with molecular biology grade water to desired volume

Beads solution (adapted from Philippe Jolivet and Joseph W. Foley, 2015 - Solutions for purifying nucleic acids by solid-phase reversible immobilization (SPRI))

0.4% Sera-Mag SpeedBead magnetic carboxylate modified particles (wash 4 times with water to remove sodium azide)

11% PEG 8000

1.6 M NaCl

10 mM Tris-HCl pH 8

1 mM EDTA pH 8

adjuste with molecular biology grade water to desired volume

Other solutions:

Potassium Acetate 5M pH 7.5

Ethanol 70%

TE-Buffer (10 mM Tris, 1 mM EDTA)

Additional for Clean up step:

Chloroform:Isoamylalcohol (24:1)

Natriumacetate 3 M

Ethanol 100 %

Ethanol 70 %

TE-Buffer

Enzymes

RNAse A (1000 U/ml, Thermo Fisher EN0541)

Proteinase K (800U/ml, NEB P81072)

Materials:

TissueLyser II (Qiagen)

Thermomixer

Magnetic rack for Eppendorf tubes

Before start

Prepare stock of beads 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

For exact preparation of beads solution please look at:
https://www.protocols.io/view/dna-size-selection-1kb-and-clean-up-using-an-optim-ir3cd8n

Always use fresh lysis buffer and fresh 70 % Ethanol

Clean up steps 26 - 41 are optional and are only necessary in recalcitrant tissue like eucalyptus. The extraction and clean up step can also be done on seperate days if DNA is stored at 4°C.

Protocol

Prepare lysis buffer

Step 1.

For 10 mL Lysis Buffer:

Final	stock	Input
1% PVP 40	10%	1 mL
1% PVP 10	10%	1 mL
500 mM NaCl	5 M	1 mL
100 mM TRIS pH 8	1 M	1 mL
50 mM EDTA	0.5 M	1 mL
1.25% SDS	20%	625 µL
1% (w/v)! Sodium metabisulfite	190.1 g/mol	0.1 g
5 mM Dithiothreitol (DTT)	1 M	50 µL
Milliq Water		4.3 mL

Heat lysis buffer to 64 °C for 30 minutes.

After cooling down to room temperature add per 1 mL lysis buffer 1 uL RNase A (in this case 10 µL)

Prepare tissue

Step 2.

In the meantime prepare 2 mL Eppendorf tubes with 1-2 metal beads (4 mm) and 100 mg of tissue and transfer tubes into liquid nitrogen.

This is the easiest with fresh tissue because freeze thawing is avoided during cutting. But if there is no other way, tissue frozen in liquid nitrogen and making sure tissue stays frozen also works.

Grinding

Step 3.

Before ginding make sure tissue is completely frozen in liquid nitrogen and do the grinding steps as quickly as possible to avoid freeze thawing. The grinding reck can also be frozen to ensure that.

Grind tissue using an automated grinder (Qiagen TissueLyzer II) for 40 seconds (actual grinding time may differ from tissue to tissue)

Extraction I

Step 4.

Add 700 uL of preheated buffer and mix by inverting tube until no frozen clumps of tissue are left

(This can take up to a few minutes, but it's worth it)

Extraction I

Step 5.

Incubate at 37°C in a thermomixer shaking at 400 rpm (slowly) for 20 minutes

Extraction I

Step 6.

Add 10 uL Proteinase K per tube and incubate for another 20 - 30 min at 37°C in the thermomixer

NOTES

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Prepare box of ice for next step

Extraction I

Step 7.

Take the tubes out of the termomixer and cool down on ice for 5 minutes

Step 8.

Add 210 uL (0.3 volumes) of 5M Potassium Acetate and mix by inverting the tube 20 times and then immediatelly keep on ice at 4°C

Extraction I

Step 9.

Centrifuge at 8000g for 12 minutes at 4°C

Extraction I

Step 10.

Transfer the supernatant (600 uL) to a new 1.5 mL tube without disturbing the pellet

Extraction I

Step 11.

Add 1 volume (600 uL) of beads solution previously prepared (make sure beads are at room temperature and well homogenized via vortexing for approximatelly 30 seconds)

Extraction I

Step 12.

Mix by inversion and then incubate on a rotor for 10 minutes at RT

(In the meantime put TE buffer into waterbath at 50°C so that its preheated later)

Extraction I

Step 13.

Spin down the tube for 1 second

Extraction I

Step 14.

Place the tube in a magnetic rack for 5 minutes (until beads are stuck to the wall of the tube and solution becomes clear)

Extraction I

Step 15.

Remove the supernatant without disturbing the beads

Extraction I

Step 16.

Add 1 mL af fresh 70 % Ethanol and wait for 30 seconds

Extraction I

Step 17.

Remove supernatant without disturbing the beads

Extraction I

Step 18.

Repeat the washing steps 15 - 16 once more

Extraction I

Step 19.

Spin down the tube for 1 second and place the tube on the magnetic rack to remove the ramaining Ethanol

Extraction I

Step 20.

Let the beads air-dry for 30 seconds, but not longer beaucse this would decrease elution efficiency

Extraction I

Step 21.

Add 80 uL of TE buffer preheted to 50°C and resuspend the beads by flicking the tube (make sure they are not aggregated anymore)

Step 22.

Incubate the resuspended beads for 10 minutes at room temperature

(to make sure the DNA can go back into elution)

Extraction I

Step 23.

Spin down the tube for 1 second and place the tube in the magnetic rack and incubate for 10 minutes (until solution becomes clear)

Extraction I

Step 24.

Transfer the supernatant (eluted DNA) into new tube

QC I

Step 25.

Meassure DNA concentration with a Qubit and absorbance with a NanoDrop.

Aiming for:

Qubit/Nanodrop: 0.5 - 1.0

260:280: 1.8 - 2.0

260:230: 2 - 2.2

For tissue with less secondary metabolites and oils, the purity of the DNA sample most likely should be good and similar to the values described above.

For recalcitrant tissue though, like Eucalyptus the following clean up step is recommended.

Clean up
Step 26.

Transfer the DNA solutions into one tube (not exceeding 500 µL)

If the extraction is done with multiple tubes at a time (which usually is the case), then after the elution step all the eluted DNA can just be pipettet into one tube.

Clean up
Step 27.

Fill up to approximatelly 500 µL with TE-buffer

Clean up
Step 28.

Add 500 µL Chloroform:Isoamylalcohol (24:1) and invert tube about 100 times (2 minutes at least)

Clean up
Step 29.

Centrifuge tube to separate the phases at 8000g at 4°C for 10 minutes

Clean up
Step 30.

Transfer the upper phase (DNA) into a new tube and discard the chloroform phase

(depending on how good the transfer worked out one round is usually enough but if some of the intermediate phase has been transferred, steps 28 -29 can be repeated)

Clean up

Step 31.

Add 50 µL (0.1 V) 3 M Sodium Acetate (NaAc) and mix by inverting tube

Clean up

Step 32.

Add 500 µL (1 V) of 100% Ethanol and mix by inverting tube carefully a few times and then let incubate at 4°C for 5 - 10 minutes.

Depending on DNA concentration (for me at 100ng/µL) DNA starts to precipitate but if sample is very clean, which is desireable, this will be seethrough. So only is recognisable by a thickening of the solution and probably airbubbles bound to precipitate (at least that what it looks like to me).

Clean up

Step 33.

Centrifuge at 4°C and 5000g for 2 minutes.

(If nothing precipiates out of solution after that, centrifuge for 10 minutes at 10000g)

But the short centrifugation time and lower speed are used to select for longer fragments in the beginning.

Clean up

Step 34.

Pipette supernatant off and make sure to do it on the opposite side than the pellet is supposed to be.

If the the pellet was seethrough I would recommend pipetting the supernatant into a new tube just to make sure that if nothing precipitated out and no pellet is seen the supernatant can be centrifuged again at a higher speed and longer.

Sometimes the "pellet" for me is just a seethrough smear along the tube (due to the low speed I guess)

Clean up

Step 35.

Add 1 mL 70 % Ethanol to pellet to wash off salt

Clean up

Step 36.

Centrifuge at 4°C and 8000 g for 5 minutes (here the higher speed shouldn't matter anymore because DNA is pelleted already)

Clean up

Step 37.

Remove supernatant

Clean up

Step 38.

Repeat steps 35 - 37 once more and make sure all Ethanol is pipetted off

Clean up

Step 39.

Let air dry (for last removal of Ethanol) for 2 - 5 minutes

(here the drying time is not as critical as in the beads drying step as the DNA pellet should easier dissolve than the beads)

Clean up

Step 40.

Add 50 µL of preheated (to 50°C) 10 mM Tris (pH 8) or TE-Buffer and elute DNA as long as necessary for the whole pellet to dissolve.

If it's a large pellet I let it dissolve over night at room temperature

QC II

Step 41.

Meassure Qubit and Nanodrop values again (as in step 25)