High molecular weight gDNA extraction after Mayjonade et al. optimised for Eucalyptus

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Abstract

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

Optimized for DNA extraction from eucalyptus grandis and also tested on wheat stripe rust and brachypodium distachyon.

Citation: Miriam Schalamun High molecular weight gDNA extraction after Mayjonade et al. optimised for Eucalyptus. **protocols.io**
dx.doi.org/10.17504/protocols.io.hcgb2tw
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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 optimising the protocol for *Eucalyptus grandis*.

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

Serapure beads solution (adapted from Nadin Rohland and David Reich, 2012):

2% Sera-Mag SpeedBead magnetic carboxylate modified particles (wash 4 times with water to remove sodium azide)

18% PEG 8000

1 M NaCl

10 mM Tris-HCl pH 8

1 mM EDTA pH 8

Note: AMPURE XP (Beckman Coulter, Brea, CA,USA) can be used but must be washed 4 times with water and resuspended in their initial buffer.

Binding buffer:

0.2% (w/v) of PEG8000

0.175 % (w/v) sodium chloride

in molecular biology grade water adjusted to desired volume

mix until solution becomes clear

Other solutions:

Potassium Acetate 5M pH 7.5

Filter-sterilized

Ethanol 70%

Enzymes

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

Proteinase K (800U/ml, NEB P81072)

AMPure beads from Beckman

Protocol

Prepare lysis buffer

Step 1.

Prepare lysis buffer:

1% PVP 40

1% PVP 10

500 mM NaCl

100 mM TRIS pH 8

500 mM EDTA

5 mM Dithiothreitol (DTT)

1.25% SDS

1% Sodium metabisulfite

Adjust with molecular biology grade Water to desired volume

Heat lysis buffer to 64 °C for 30 minutes.

Prepare tissue

Step 2.

Prepare 2 mL Eppendorf tubes with 1-2 metal beads and 100 mg of tissue frozen in liquid nitrogen and then kept at 80°C.

Step 3.

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

DURATION
00:00:40

Step 4.

Add 700 uL of preheated buffer and 4 uL of RNAsa A and immediatelly mix by inverting 20 times

Step 5.

DURATION
00:30:00

Extraction I

Step 6.

Add 200 uL (0.3 volumes of lysis buffer) of 5M Potassium Acetate and mix be inverting the tube 20 times and then immediatelly keep on ice at 4oC

Step 7.

Centrifuge at 5000g for 10 minutes at 4oC

DURATION
00:01:00

Extraction I

Step 8.

Transfer the supernatant (600 uL) to a new 2 mL tube without disturbing the pellet

Step 9.

Extraction I

Step 10.

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

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

DURATION
00:10:00

Extraction I

Step 11.

Spin down the tube for 1 second

Extraction I
Step 12.

Place the tube in a magnetic rack for 3 minutes (until beads are stuck to the wall of the tube and solution becomes clear)

Extraction I
Step 13.

Remove the supernatant without disturbing the pellet

Extraction I
Step 14.

Add 1 mL af fresh 70 % Ethanol, remove the tube from the magnetic rack and mix by inverting the tube

Extraction I
Step 15.

Spin down the tube for 1 second

Extraction I
Step 16.

Place the tube in the magnetic rack and wait for at least 30 seconds (until beads are stuck to the wall of the tube and solution becomes clear)

Extraction I
Step 17.

Remove supernatant without disturbing the pellet

Extraction I
Step 18.

Repeat steps 14-1

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 30-60 seconds, but not longer beaucse this would decrease elution

efficiency

Step 21.

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

Extraction I

Step 22.

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 23.

Transfer the supernatant (eluted DNA) into new tube

Step 24.