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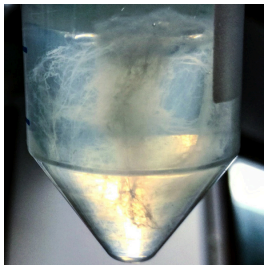
Plant DNA extraction and preparation for ONT sequencing



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High molecular weight DNA extraction from all kingdoms
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Protocol status: Working
We use this protocol and it's working

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Abstract

Plant DNA extraction and preparation for ONT sequencing

This CTAB-based protocol is suitable for extraction of genomic DNA from a wide range of plant species. The DNA quality is sufficient for ONT sequencing after SRE enrichment of long fragments and removal of short fragments. Quality control steps are described as part of this protocol.

Successful application for the genome sequencing of the following plant species:
Arabidopsis species, *Beta vulgaris* (sugar beet), *Brassica napus* (rapeseed/canola), *Dioscorea dumetorum* (yams), *Helichrysum umbraculigerum,* mosses, *Vitis vinifera* (grapevine)

References:

Siadjeu C.*, Pucker B.*, Viehoever P., Albach D. and Weisshaar B. (2020). High contiguity de novo genome sequence assembly of Trifoliate yam (Dioscorea dumetorum) using long read sequencing. Genes. doi:10.3390/genes11030274.

Pucker B, Rückert C, Stracke R, Viehöver P, Kalinowski J, Weisshaar B. Twenty-Five Years of Propagation in Suspension Cell Culture Results in Substantial Alterations of the Arabidopsis Thaliana Genome. Genes. 2019. doi:10.3390/genes10090671.

Photo provided by Hanna Schilbert (@HSchilbert).

Materials

Materials: morta & pestle, water bath, centrifuge for 50 ml tubes, NanoDrop, Qubit, magnetic rack, MinION/GridION

CTAB1
2 % CTAB
100 mM Tris-HCl, pH 8.0
20 mM EDTA
1.4 M NaCl
0.25 % PVP (optional)

CTAB2
1.0 % CTAB
50 mM Tris-HCl, pH 8.0
10 mM EDTA
0.125 % PVP (optional)

CTAB-TE
10 mM Tris, pH 8.0
0.1 mM EDTA pH 8.0
7.5 µg/ml RNAse (needs to be completely DNase free)

1M NaCl

70% ethanol

Isopropanol

N2

SRE (XS, normal, XL)

LSK109 components (AMPureXP beads) & sequencing components

Nuclease flush materials (ONT wash kit / nuclease flush buffer + DNaseI)

References

Composition of these CTAB buffers is based on GABI-Kat experiences:
https://www.gabi-kat.de/methods/dna-preparation.html

Rosso, Mario G., et al. "An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics." *Plant molecular biology* 53.1-2 (2003): 247-259.

Safety warnings

ß-ME is a neurotoxin. Use gloves when handling it.
Liquid nitrogen is dangerous. Wear protective glasses when using it.
Dichloromethane is dangerous. Use gloves and protective glasses when handling it.

Please check the instructions provided by suppliers of the materials for additional safety warnings.

CTAB-based DNA extraction

1 Preheat 5 ml CTAB1 + 300 µL ß-ME in 50 ml tube to 75 C
(The amount of ß-ME is working, but was not optimized)

2 Homogenize fresh material with morta and pestle in liquid N2
(It is important to use fresh material which was not frozen previously. Less than 1g of young leaves is usually sufficient. Keeping plants in the dark for a few days prior to DNA extraction might reduce the amount of starch and the number of plastids.)

3 Add homogenized material (powder) to the preheated CTAB1 buffer and resuspend carefully
(It is crucial to completely resuspend the powder. Avoid transfer of ice.)

4 Incubation for at least 30 minutes (up to 2 hours possible). Invert tubes frequently to carefully mix the solution.

5 Re-cool to room temperature

6 Add 5 ml dichloromethane and mix gently by inverting the tube.

7 Centrifugation for 30 minutes at >10,000 x g at room temperature

8 Transfer upper phase carefully to new 50 ml tube.
(Do not disturb interphase. Upper phase should be colorless.)

9 Add 10 ml CTAB2 and mix gently by inverting the tube.

10 Centrifugation for 30 minutes at >10,000 x g at room temperature

11 Discard supernatant.

12 Add 2 ml 1M NaCl and re-dissolve pellet.

13 Add 2 ml Isopropanol and mix gently.

14 Centrifugation for 30 minutes at >10,000 x g at room temperature

15 Discard supernatant.

16 Wash with 2 ml 70% ethanol.

17 Centrifugation for 10 minutes at >10,000 x g at room temperature

18 Discard supernatant.

19 Brief centrifugation to collect droplets.

20 Remove droplets.

21 Dissolve sediment in 100 µl CTAB-TE via incubation over night at room temperature.
(RNase is active during this time)

Quality control

22 NanoDrop measurement with 1 µl of DNA solution
(expecation: several µg/µl)

23 Agarose gel: 1 µl DNA + 3 µl loading buffer + 3 µl water
(1kb DNA ladder from NEB as size control; any large DNA fragment will do the job)

24 Qubit measurement with 1 µl DNA

SRE

25 Removal of short fragments following the SRE (Circulomics) handbook: https://www.n-genetics.com/products/1317/1023/17106.pdf

ONT sequencing

26 LSK109 works best for plant genome sequencing (in my hands).

Two libraries may be prepared for the cost of one by starting with 2 µg of DNA (as recommended for R10 flow cells) even when using R9 flow cells. This comes with the risk that not all pores will be occupied depending on the library quality. The volumen of the final step (Elution Buffer) is increased to 25 µl. Half of the library (12 µl) is used for sequencing right away and the rest of the library can be stored at 4 C in a fridge until needed after a nuclease flush. There might be a small decrease in average read length due to storage of the library.

27 Nuclease flush might be necessary after some time (e.g. 12-24 hours) depending on the DNA quality. Incubation with DNaseI on the flow cell for 2 hours works well in my hands.