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HMW DNA extraction for Long Read Sequencing using CTAB

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We use this protocol and it's working

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Abstract

High Molecular Weight DNA extraction protocol for Long Read sequencing.
Extraction is performed from flash frozen leaves stored at -80°C.
The protocol is adapted for an extraction of 1g of leaves.
This protocol is based on the protocol provided by Oxford Nanopore Technologies, Oxford, UK (ONT), "High molecular weight gDNA extraction from plant leaves" provided by the ONT community in March 2019, with slighly modifications.
This protocol involves a conventional CTAB extraction followed by purification using commercial Qiagen Genomic tips (QIAGEN, MD, USA). DNA fragment size selection is performed using Short Read Eliminator (Circulomics, MD, USA).
This protocol is particularly adapted for plant leaves, but also works with many other organisms (microalgae, insects...).

Guidelines

Use only wide bore tips.
Work in a chemical hood when using 2-mercaptoethanol.
Allow the DNA to resuspend for at least of 24 hours before proceeding with QC.

Materials

Reagents :

- Tris HCl **P212121**
- EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G
- CTAB (Hexadecyltrimethylamm onium bromide) **BBI Biotech Catalog #**CB0108-100g
- Sodium chloride **P212121**
- PEG-8000 **Promega Catalog #**V30111
- Liquid nitrogen
- 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
- RNase A **Qiagen Catalog #**19101
- Chloroform
- Isopropanol
- Genomic tip 100G **Qiagen Catalog #**10223
- Buffer G2 **Qiagen Catalog #**1014636
- Buffer QBT **Qiagen Catalog #**19054
- Buffer QC **Qiagen Catalog #**19055
- Buffer QF **Qiagen Catalog #**19056
- Ethanol 70%

Consumables :

- MBP™ Wide Bore Pipette Tips **Thermo Fisher Catalog #**02707600
- Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific Catalog #**14-959-49A
- 15 ml conical tubes
- 1.5ml Eppendorf DNA LoBind tubes

Equipment :

- Porcelain Mortar, 145mL **Thermo Fisher Catalog #**CP1782004
- Eppendorf ThermoMixer C **pipette.com Catalog #**2231000667

Vortex Mixer

Glass pasteur pipettes

Protocol materials

- Buffer QF **Qiagen Catalog #**19056
- MBP™ Wide Bore Pipette Tips **Thermo Fisher Catalog #**02707600
- 1.5ml Eppendorf DNA LoBind tubes
- 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
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- Ethanol 70%
- 15 ml conical tubes
- CTAB (Hexadecyltrimethylamm onium bromide) **BBI Biotech Catalog #**CB0108-100g
- Porcelain Mortar, 145mL **Thermo Fisher Catalog #**CP1782004
- EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G
- Genomic tip 100G **Qiagen Catalog #**10223
- Vortex Mixer
- PEG-8000 **Promega Catalog #**V30111
- 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
- Liquid nitrogen
- RNase A **Qiagen Catalog #**19101
- Eppendorf ThermoMixer C **pipette.com Catalog #**2231000667
- Chloroform
- Isopropanol

Buffer G2 **Qiagen Catalog #**1014636

Buffer QBT **Qiagen Catalog #**19054

Genomic tip 100G **Qiagen Catalog #**10223

Buffer QC **Qiagen Catalog #**19055

Buffer QF **Qiagen Catalog #**19056

Buffer QF **Qiagen Catalog #**19056

Isopropanol

70% Ethanol

DNA LoBind Tube 1.5ml **Eppendorf Catalog #**022431021

Buffer TE 1x

Troubleshooting

preparation of reagents

1 **Extraction Buffer** : Prepare 20 mL of extraction buffer (per 1g of leaves) in a 50 mL tube

	Reagents	Final concentration
	Tris-HCl ph8	100 mM
	EDTA	20 mM
	CTAB	2%
	NaCl	1.4. M
	PEG 8000	1 %
	H2O	QSP 20 ml

DNA Extraction

3h

2 Add 50 µl of **2-Mercaptoethanol** to 20 ml of **extraction buffer** and preheat to 65 °C (approx. 15 min).

20 mL Extraction buffer

50 µL

2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250

65 °C

3 Put a **mortar in ice.**
Cool the mortar and pestle with **liquid nitrogen** until the bubbling stops.

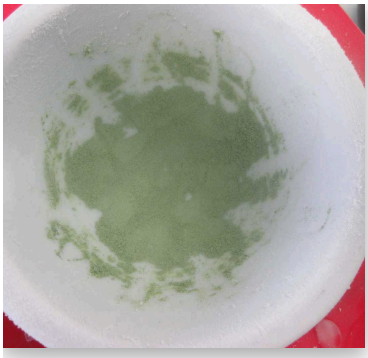
Liquid nitrogen

4 **Grind 1g of frozen sample** to a fine powder (approx. 2 min), without adding liquid nitrogen.

2m

1 g 00:02:00

Before grinding



After grinding

5 **Transfer the powder** to the pre-warmed extraction buffer

6 **Add 40 µl of RNase A** (100mg/ml) and vortex the tube 5 sec. 5s

40 µL | RNase A **Qiagen Catalog #**19101

00:00:05 Vortex

7 **Incubate 1h at 65°C** with intermittent agitation (300 rpm every 10 min) 1h

01:00:00 65°C

Eppendorf ThermoMixer C **pipette.com Catalog #**2231000667

8 Let the tube cool for 5 min at RT 5m

00:05:00 RT

9 **Add 1 volume (20ml) of chloroform** and vortex 2×5sec.

20 mL | Chloroform

10 **Centrifuge** with low acceleration and deceleration 10m

5500 x g, 4°C, 00:10:00 , Acc/Dec : 6/3

Before centrifugation

After centrifugation

11 Gently **transfer the aqueous phase** to a new 50 ml tube.

12 Add **0.7 volume of isopropanol** and mix gently by inversion 10 times

Isopropanol

13 Put the tube at **-80°C for 15 minutes.** 15m

00:15:00 -80°C

14 ***If a DNA medusa appears after this step, recover the medusa using a Pasteur pipette (without breaking the tip of the pipette).***
Wash the medusa in 3 successive baths of 70% ethanol then resuspend the medusa in 100µl of 1X TE.
Incubate for 1 hour at 55°C then store the tube at 4°C before quality control.
Continue the protocol with the rest of the tube (without the medusa).
If no DNA medusa is visible continue the protocol.

15 **Centrifuge** the tube with low acceleration and deceleration 30m
5500 x g, 4°C, 00:30:00 , Acc/Dec : 6/3

16 Carrefully **remove the supernatant** without resuspending the pellet.
Remove the remaining liquid by turning the tube upside down on a paper towel (make sure the pellet does not come off).

17 Gently **resuspend with the pipette the DNA pellet with 9.5 ml of G2 buffer** (QIAGEN Genomic-tip 100/G).
Buffer G2 **Qiagen Catalog #**1014636

18 **Incubate 15 min at 50°C**. *The sample can be stored overnight at 4°C at this stage.* 15m
00:15:00 50°C

Génomic tip purification 1h

19 **Equilibrate a QIAGEN Genomic-tip 100/G** column with **4 ml of QBT buffer**.
Genomic tip 100G **Qiagen Catalog #**10223
Buffer QBT **Qiagen Catalog #**19054

20 **Preheat 5 ml of QF buffer** to 55°C.
Buffer QF **Qiagen Catalog #**19056

21 Allow all the QBT buffer to drain by gravity flow into a 50 ml tube.

22 **Add the sample** in G2 buffer (9.5ml) on the column and let it to enter the resin by gravity flow.

23 **Wash the column twice** with 7.5 ml of QC buffer.

Buffer QC **Qiagen Catalog #**19055

24 Put the column on a 15 ml tube.

25 **Elute the DNA** with 5 ml of QF buffer preheated to 55°C.
Buffer QF **Qiagen Catalog #**19056

DNA précipitation

3h

26 Add **0.7 volume of isopropanol** to the eluate.
Mix gently by inverting 20 times.
Isopropanol

27 Incubate 15 minutes at RT.
00:15:00 RT
15m

28 **Centrifuge** with low acceleration and deceleration
5500 x g, 4°C, 00:30:00 , Acc/Dec : 6/3
30m

29 Carefully discard the supernatant, gently resuspend the pellet with 1 ml of cold 70% ethanol.
70% Ethanol

30 **Transfer the resuspended DNA** to a 1.5 ml DNA LoBind tube.
DNA LoBind Tube 1.5ml **Eppendorf Catalog #**022431021

31 **Centrifuge** with low acceleration and deceleration
5000 x g, 4°C, 00:10:00 , Acc/Dec : 6/3
10m

32 Carefully discard the supernatant.
Air dry the pellet at RT for about 10 minutes.
00:10:00 RT
10m

33 **Resuspend the pellet** with 50-100 µl of 1X TE buffer.

Buffer TE 1x

34 **Allow the DNA to resuspend** for 2 hours at 55°C or ON at RT. 2h
02:00:00 55°C or Overnight RT

35 **Store the DNA at 4°C.**

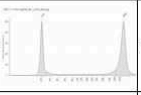
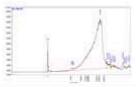
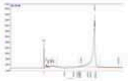
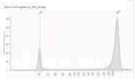
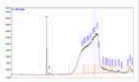
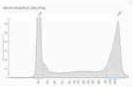
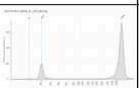
Sample quality control

36 Quantify your sample with a **Qubit DNA HS assay.**
Check the purity of the sample with a **Nanodrop** (measurements of 260/280 and 260/230 absorbance ratios).
Estimate the molecular weight of the sample with a **Tapestation** and/or a **Femto pulse** and/or a **Pippin Pulse.**

37 Depending on the DNA concentrations and DNA length profiles, deplete short DNA molecules using **SRE size selection kits** (SRE XS, SRE or SRE XL kits).

Results

38 **QC results** obtained on different species.

				DNA lenght (kb) estimation with		
species	DNA quantity (µg)	Extraction yield (µg DNA / g organism)	Nanodrop 260/280 260/230	Tapestation	Femto pulse	Nanopore N50 reads
Arabidopsis thaliana	14,8	24,7	1,97 1,73	> 60 kb 	72 kb 	30 kb
Lactuca sativa	38,5	38,5	1,96 2,30	> 60 kb 	149 kb 	43 kb
Brassica napus	169	181	1,88 1,92	> 60 kb 		45 kb
Fagus sylvatica	33,8	254	1,84 2,07	> 60 kb	100 kb 	30 kb
Quercus robur	98	183	1,98 1,64	> 60 kb 		48 kb
Ectocarpus sp.	3,2	70	1,88 1,81	> 60 kb 		22 kb
Tenebrio molitor	22,4	370	1,88 2,17	> 60 kb 		35 kb