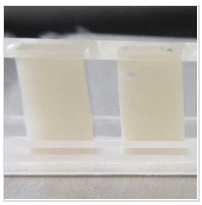

Apr 06, 2022

isolation and extraction of plant nuclei in plug

Karine Labadie[1], Benoît Vacherie[1]

[1]Genoscope/CEA

1

dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

Benoit

Benoît Vacherie
Genoscope/CEA

Method for isolation and extraction of plant cell nuclei.
Protocol for obtaining UHMW DNA (> 150kb) allowing the production of optical cards with Bionano technology.

DOI

dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

Karine Labadie, Benoît Vacherie 2022. isolation and extraction of plant nuclei in plug. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

dna extraction, HMW, UHMW, Plug, optic card, bionano, extraction, plants, nuclei

protocol ,

Feb 03, 2022

Apr 06, 2022

57758

Never use a vortex in order to maintain a good molecule size.
Use only wide bore tips.
Allow the DNA to resuspend for a minimum of 48 hours before proceeding with QC.
The nuclei isolation part should be done in a chemical hood because of the use of 2-mercaptoethanol

Reagents :

Tris HCl **P212121**

EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G**

KCl **Contributed by users**

Sucrose **P212121**

PVP **Sigma**

Spermine **Contributed by users**

Spermidine **Contributed by users**

Triton X-100 **Sigma Aldrich Catalog #T8787-50ML**

2-Mercaptoethanol **Sigma Aldrich**

N-lauryl sarcosine **Sigma Aldrich Catalog #L5125-50G**

NaCl **Contributed by users**

UltraPure™ Low Melting Point Agarose **Contributed by users Catalog #16520-100** Step 20

Agarase (0.5 U/µL) **Thermo Fisher Catalog #EO0461**

Proteinase K **Contributed by users**

RNase A **Contributed by users**

Dialysis membrane, 44mm **Bio Basic Inc. Catalog #TX0112.SIZE.2m**

Consumables :

MBP™ Wide Bore Pipette Tips **Thermo Fisher Catalog #02707600**

CHEF Disposable Plug Molds **BioRad Sciences Catalog ##1703713** Step 21

Certified Cheesecloth **Thermo Fisher Catalog #22055053** Step 10

Miracloth **Merck Millipore Catalog #475855** Step 10

Citation: Karine Labadie, BenoÃÂ®t Vacherie isolation and extraction of plant nuclei in plug https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

Falcon 40 µm Cell Strainer **Corning Catalog #352340** Step 10

Petri Dish **P212121 Catalog #LI-PD01100**

Nunc™ Cell Factory™ System Accessories, vent cap **Thermo Fisher Catalog #146008** Step 30

Equipment :

ThermoMixer® C

Eppendorf Catalog No. 2231000680

C1 Platform Shaker

Platform Shaker

New Brunswick Scientific SKU unknown

preparation of reagents

1 **NIB Buffer : 200 ml :** freshly prepared

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Reagent	Final concentration
Tris ph8	10 mM
EDTA	10 mM
KCl	80 mM
Sucrose	0.5 M
PVP 40	2 %
Spermine	1 mM
Spermidine	1 mM
H2O	qsp 200 ml

Adjust the Ph to 9.4 then filter at 0.22 µm

2 **NIBT Buffer : 160 ml**

A	B
NIB Buffer	160 ml
Triton X100	0.5%

3 **NIBTM Buffer : 40 ml**

A	B
NIBT Buffer	40 ml
2-Mercaptoethanol	0.75 %

4 **Cell suspension Buffer :** Can be stored for 1 year at 4°C.

A	B
Tris ph8	10 mM
EDTA	50 mM
NaCl	2 mM
H2O	Qsp 100ml

5 **Lysis Buffer :** Can be stored for 1 year at RT.

A	B
EDTA 0.5M	100 ml
N-Lauroylsarcosine	1 %

Nuclei isolation 3h

Putting a **mortar in ice**

Citation: Karine Labadie, BenoÃÂ®t Vacherie isolation and extraction of plant nuclei in plug
https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

6 Cool a mortar/pestle with liquid nitrogen until the bubbling stops.

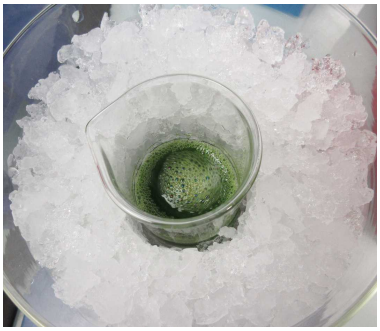
7 Place a beaker in ice and add a magnetic stirrer.
Add **20 ml of NIBTM** (10 ml/g of leaves) and stir gently
20 mL NIBTM

8 Grind 2g of frozen sample for without adding liquid nitrogen, until a fine powder is obtained (approx. 2 min) 2m
2 g **00:02:00**

Before grinding

After grinding

9 Transfer the powder to the beaker and **shake gently for 10 minutes** in ice. 10m
00:10:00



10 **Filter the mixture** into a 50ml tube through autoclaved filters (2 cheese cloth + 2 Mira cloth) on a funnel (squeeze the filters at the end of filtration to recover more of the solution containing the nuclei).
Filter through a 40µm cell stariner into a new 50ml tube.
Certified Cheesecloth **Thermo**
Fisher Catalog #22055053

Citation: Karine Labadie, BenoÃÂ®t Vacherie isolation and extraction of plant nuclei in plug
https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

Miracloth **Merck**

Millipore Catalog #475855

Falcon 40 µm Cell

Strainer **Corning Catalog #352340**

11 **Pelleting the homogenate** by centrifugation.(acceleration and deceleration at level 3) 20m

800 x g, 4°C, 00:20:00 , Acc 3 / Dec 3

12 Remove the supernatant and gently **resuspend the pellet** in ice (use a brush if the pellet does not recover).

Add **20ml of cold NIBTM**

20 mL NIBTM

13 **Pelleting the homogenate** by centrifugation to remove residues and unlysed cells. 2m

60 x g, 4°C, 00:02:00 , Acc 3 / Dec 3

14 **Filter the supernatant** through a 40 µm cell sieve into a new 50 ml tube.

15 **Pellet the nuclei** by centrifugation. 15m

800 x g, 4°C, 00:15:00 , Acc 3 / Dec 3

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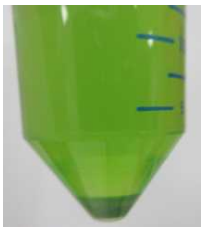
https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1



16 **Wash the pellet** 3 times in NIBT buffer : 15m

- Remove the supernatant
- Gently resuspend the pellet
- Add 40 ml of cold NIBT buffer **40 mL NIBT**
- Centrifuge **800 x g, 4°C, 00:15:00 , Acc 3 / Dec 3**

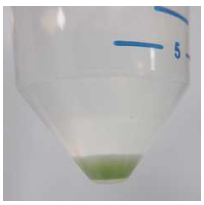
1st wash

2nd wash

3rd wash

17 Make a final wash in 30 ml of cold NIB buffer. 15m

800 x g, 4°C, 00:15:00 , Acc 3 / Dec 3



Final wash

18 Resuspend the last pellet in the residual buffer (approx. 200 µl) and **transfer the homogenate to a 1.5 ml tube.** 15m

Centrifuge.

800 x g, 4°C, 00:15:00 , Acc3 / Dec 3

Citation: Karine Labadie, BenoÃÂ®t Vacherie isolation and extraction of plant nuclei in plug
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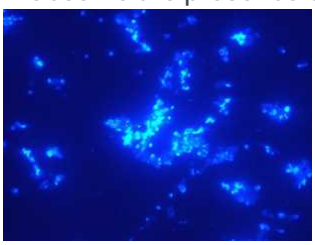
19 Remove the supernatant with a pipette and resuspend the pellet in an appropriate volume of **cell suspension buffer :**
60µl / plug
Adjust the number of plugs to be made according to the size of the pellet.

19.1 optional : microscopic observation
- Take an aliquot of 100 µl of suspension
- Stain with DAPI
- Observe the presence of nuclei



Embedding in agarose 30m

20

Nb plugs	1	2	3
Nuclei suspension	60 µl	120 µl	180 µl
Agarose 2%	40 µl	80 µl	120 µl

Use **Low Melting agarose** for a final agarose concentration of 0.8 %
☒ UltraPure™ Low Melting Point Agarose **Contributed by users Catalog #16520-100**

21 Put a **CHEF Disposable Plug Molds** on ice
☒ CHEF Disposable Plug Molds **BioRad Sciences Catalog ##1703713**

22 **Melt agarose** at 70°C for 5 min then equilibrate at 43°C for 5 min 10m
00:05:00 **70 °C**
00:05:00 **43 °C**

23 **Preheat the nuclei suspension** to 43°C for 3min and then add the appropriate amount of 2% agarose (see table). 3m
Mix gently with a wide-bore tip, avoiding bubbles.
00:03:00 **43 °C**

24 Immediately **dispense 100µl** of mixture per well using wide-bore tips. 15m
Allow to polymerise for 15 minutes on ice.

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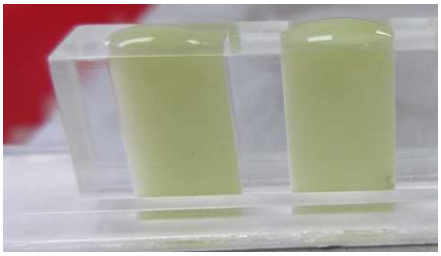
https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

00:15:00 On ice



Proteinase K digestion 18h

25 Prepare a fresh proteinase K digestion solution by mixing **200 µl of proteinase K** enzyme (20mg/ml) with **2.5 ml of lysis buffer** in a 50 ml tube.

200 µL Prot. K **2.5 mL Lysis Buffer**

26 **Transfer plugs** to the 50ml tube containing Proteinase K digestion solution.

2h

27 **Incubate** in thermomixer for 2 hours at 50 °C with intermittent mixing
Mixing cycle: 10 seconds at 450 rpm followed by 10 minutes at 0 rpm

02:00:00 **50 °C**

28 Screw a sieve caps onto the tube and empty the solution. Change the proteinase K Solution bath as before.
Incubate in thermomixer overnight at 50 °C with intermittent mixing
Mixing cycle: 10 seconds at 450 rpm followed by 10 minutes at 0 rpm

Overnight **50 °C**

RNase Digestion 1h 30m

29 Prepare the wash solutions :

TE 10:50 (Wash Buffer)

Reagent	For 500ml	Final concentration
Tris 1M ph8	5 ml	10 mM
EDTA 0.5M	50 ml	50 mM
H2O qsp 500ml	445 ml	

TE 10:5 (For Rnase)

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Reagent	For 500ml	Final concentration
Tris 1M ph8	5 ml	10 mM
EDTA 0.5M	5 ml	5 mM
H2O qsp 500ml	490 ml	

30 Empty the tube using a vent cap.

Nunc™ Cell Factory™ System Accessories, vent cap **Thermo Fisher Catalog #146008**

Rinse the plugs 3 times with 10ml of wash buffer.
Wash 2 times with 10ml wash buffer for 15 min at RT with gentle agitation (15 rpm) on a horizontal platform mixer.

15 rpm, Room temperature , 00:15:00

1h

31 **Rinse the plugs** 3 times with 10ml of TE 10:5

RNase

Add 2.5ml of TE 10:5 and 50 µl of **Rnase** Solution A **Qiagen Catalog #19101**

Incubate **1hour at 37°C** with intermittent mixing

01:00:00 **37 °C**

32 **Rinse the plugs** 3 times with 10ml of Wash Buffer.
NB : The plugs can be stored at 4°C in a wash buffer at this stage

Agarase treatment 2h

33 **Wash 4 times** with 10ml wash buffer for 15 min at RT with gentle agitation (15 rpm) on a horizontal platform mixer.

15 rpm, Room temperature , 00:15:00

34 **Transfer the plug** to a 1.5 ml tube with a sterile spatula

2m

35 **Melt the plug** in a water bath at 70°C for 2 minutes

00:02:00 **70 °C**

5m

36 **Transfer the tube** to a water bath at 43°C for 5 minutes

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https://dx.doi.org/10.17504/protocols.io.6qpvr6632vmk/v1

00:05:00 43 °C

37 **Add 2µl of agarase** and mix gently by rotating with the tip. 45m
Incubate 45 minutes at 43 °C
00:45:00 43 °C

Dialysis 1h

38 Place **10 ml of 1x TE Buffer** in a 6 cm Petri dish. 15m
Float a 0.1 µm dialysis membrane on the surface of the 1x TE Buffer. Place a cover on the Petri dish and let the membrane equilibrates for 15 minutes.
00:15:00

39 **Deposit the entire sample** in the centre of the membrane using a wide-bore tip. 45m
Place cover on the Petri dish and let the sample dialyze for **45 minutes at room temperature.**
00:45:00 Room temperature

40 **Transfer DNA** to a 1.5 ml microfuge tube with a Wide Bore Tip.

41 Allow the **DNA to resuspend overnight** at RT then 2 days at 4°C before performing quality control.
Overnight Room temperature

Sample QC

42 Quantify your sample with a **Qubit HS**.
NB : Before quantification, sonicate the DNA aliquot for 10 min to obtain a more reliable result

Visualise 1 µL of sample to estimate the molecular weight. (**Tapestation** or/and **pipin pulse** or/and **Femto pulse**)

Résults

43 QC results obtained on different plant species, using different technologies to estimate the size of the molecules.

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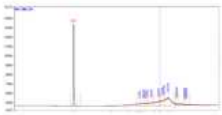
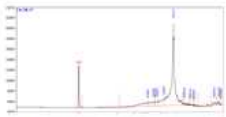
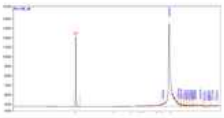
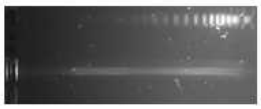
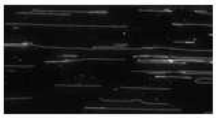
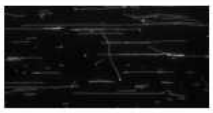
plant species	[c] ng/µl	yield µg/g	Size	profiles	Technology
Arabidopsis halleri	28,6	5,4	160 kb		Femto pulse
lactuca sativa	190	5,6	144 kb		
Silene latifolia	127	18	149 kb		
Musa acuminata	156	19,6	50-200 kb		Pippin pulse
Musa textilis	38,5	4,3	50-200 kb		
Fagus slvatica	93,6	6,2	50-200 kb		
Fallopia multiflora	155	24	50-250 kb		Qcard ARGUS
Citrus australasica	107,5	13	50-200 kb		
Spartina maritima	207	18,2	50-150 kb		

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