Populus nuclear DNA purification using the Qiagen Genomic-tip 100/G[1]

Reagents

EDTA, 0.5 M, pH 8.0
Ethanol, 100%
β-mercaptoethanol
2-(N-Morpholino) ethanesulfonic Acid (MES)
NaCl, 5 M
NP-40
Percoll
Proteinase K
Qiagen Genomic DNA buffers G2, QBT, QC and QF (see Qiagen Genomic DNA Handbook for ordering information or recipes).
RNAse A (100 mg/ml)
Spermidine trihydrochloride
Spermine tetrahydrochloride
Sucrose
TE buffer, pH 8.0 (10 mM Tris-HCl/1 mM EDTA)

Equipment and supplies

Autoclaved 200-500 ml beakers and stir bars
Centrifuge tubes, 50 ml polypropylene
Centrifuges (benchtop and ultra) and rotors (swinging bucket and SW41) at 4 ºC.
Cheesecloth, 2 layers inside 1 layer Miracloth (8x8 in squares, autoclaved)
Dounce homogenizers
Funnels
Ice
Mortar and pestle
13.5 ml Polyallomer tubes
Transfer pipets

[1] Adapted from Weising K, H Nybom, K Wolff and W Meyer. 1995. Chapter 4: Methodology. *In DNA Fingerprinting in Plants and Fungi*. Boca Raton, FL: CRC Press. Pp. 57-61; and the Qiagen Genomic DNA Handbook August 2001.

Solutions

Nuclear Isolation Buffer (NIB)[2]:

Reagent	weight	5X	1X (final)
Sucrose	85.6 g	1.25 M	250 mM
NaCl	2.0 ml 5M	50 mM	10 mM
MES	2.12 g	50 mM	10 mM
EDTA	10.0 ml 0.5 M	25 mM	5 mM
Spermine (mw=348)	52.0 mg	0.75 mM	0.15 mM
Spermidine (mw=255)	128.0 mg	2.5 mM	0.5 mM
β-mercaptoethanlol	n/a	n/a	2.0 %

Add sterile Type I water to 200 ml.
Sterilize by filtration and store at 4 ºC.

Before use:

Dilute 150 ml 5X NIB to 500 ml 1X with cold sterile R.O. distilled water.
Add NP-40 to 0.6% (4.5 g).
Add β-mercaptoethanol to 2.0% (15.0 ml).
Adjust final volume to 750 ml.
Chill thoroughly before use.

Floating Buffer

Reagent	weight
β-mercaptoethanol	1.0 g
5X NIB	6.0 g
Percoll	45.0 g
NP-40	0.3 g

Prepare immediately before use.

[2] The recipes are sufficient for four samples.

Protocol

1. Powder 3-5 g fresh young leaves in mortar and pestle using liquid nitrogen. Add to 150 ml ice cold 1X NIB and mix by stirring 10 min on ice.
2. Filter through 4 layers cheesecloth + 1 layer Miracloth into 4 x 50 ml centrifuge tubes.
3. Centrifuge at ~2,000 x g (4K rpm) for 10 min at 4˚ C in Sorvall Legend or equivalent swinging bucket rotor to obtain crude nuclear pellet.
4. Discard supernatant. Resuspend pellets in 4 x 10 ml 1X NIB, and combine into 1 tube for each genotype. Repeat #3.
5. Discard supernatant. Resuspend each pellet in 10+ ml Floating Buffer using Dounce homogenizer. Aliquot into 13.5 ml pollyallomer tubes and centrifuge at 5000 x g (6400 rpm) 30 min at 4˚ C in SW41 rotor.
6. Remove floating nuclei with transfer or Pasteur pipette and resuspend in 10.0 ml 1X NIB with Dounce homogenizer.
7. Centrifuge at ~2,000 x g (4K rpm) for 10 min at 4˚ C in to pellet nuclei.

From this point forward follow the Qiagen Genomic DNA Handbook (08/2001) protocol for yeast p. 37, step 8. Note modifications for plant tissue.

8. Resuspend nuclei in 5 ml Qiagen buffer G2 containing 10 ul RNAse A (100 mg/ml) and vortex briefly.
9. Add 100 ul Proteinase K stock and incubate with gentle shaking (50 rpm) at 50 ºC overnight to lyse.
10. Pellet the nuclear debris by centrifuging at 5000 x g 4˚C for 10 min. Promptly load the supernatant on the Genomic tip.

Genomic Tip Protocol Genomic DNA Handbook (08/2001), p. 44.

1. Equilibrate a QIAGEN Genomic-tip 100/G with 4 ml of Buffer QBT, and allow it to empty by gravity flow.
2. Transfer clarified lysate to the buffer QBT-equilibrated QIAGEN Genomic-tip.
3. Wash with Buffer QC 2 x 7.5 ml.
4. Elute with 5 ml Buffer QF.
5. Precipitate DNA by adding 3.5 ml (0.7) volumes of room-temperature isopropanol. Mix and centrifuge immediately at >5,000 x g for 20 min at 4˚C to pellet DNA, or spool out DNA on a glass rod.
6. Wash with 2 ml ice-cold 70% ethanol. Air-dry and resuspend in appropriate volume TE, pH 8.0. Dissolve DNA overnight or at 55 ˚C 1-2 h.