

Nuclear Extraction from *Arabidopsis thaliana*

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[Abstract] This protocol is to isolate nuclei from *Arabidopsis* cells. They can be further used for other experiments, such as nuclear protein detection, nuclear protein immunoprecipitation and so on.

Materials and Reagents

1. Tris-HCl (pH 7.4)
2. Glycerol
3. KCl
4. EDTA (pH 7.5)
5. MgCl₂
6. Sucrose
7. Triton X-100
8. Murashige and Skoog basal medium (Sigma-Aldrich, catalog number: M0404-10L)
9. Phenylmethanesulfonylfluoride (PMSF)
10. Dithiothreitol (DTT)
11. Proteinase inhibitor (PI) (complete EDTA-free) (Roche Diagnostics, catalog number: 04693132001)
12. Phytigel (Sigma-Aldrich, catalog number: P8169-1KG)
13. Liquid nitrogen
14. Lysis buffer (LB) (see Recipes)
15. Nuclei resuspension buffer with 0.2% Triton X-100 (NRBT) (see Recipes)
16. Nuclei resuspension buffer (NRB) (see Recipes)
17. MS (see Recipes)
18. Nuclei storage buffer (NSB) (see Recipes)

Equipment

1. Centrifuges (e.g. Eppendorf centrifuge 5810 R that can be refrigerated and will hold 50 ml tubes)
2. Mortar and pestle
3. 100 μ m and 40 μ m nylon mesh (BD Biosciences, Falcon[®], catalog number: REF352360, REF352340)
4. 50 ml conical tube

Procedure

Grow *Arabidopsis* seeds on MS for 2 weeks or on soil for 4 weeks. Collect approximately 1 g of *Arabidopsis* tissue (seedlings of about 50 plate-grown plants, leaves of approximately 20 soil-grown plants), freeze in liquid nitrogen, and then follow the steps listed below.

Note: Always keep the sample on ice.

1. Grind the tissue to a fine powder in liquid nitrogen using a cold mortar and pestle. Collect the powder into a 50 ml conical tube.
2. Add 2 ml cold Lysis buffer into the powder and homogenize the mixture by gentle shaking or pipetting.

Note: If the sample is frozen from excess liquid nitrogen, wait until it is thawed enough that it can be homogenized.

3. Filter the homogenate through a 100 μ m and 40 μ m nylon mesh sequentially.
4. Centrifuge the filtered homogenate at 1,500 \times g at 4 °C for 10 min to pellet the nuclei.
5. Discard the supernatant and add 3 ml NRBT to the pellet. Re-suspend the nuclei by pipetting.
6. Centrifuge the sample at 1,500 \times g at 4 °C for 10 min. Repeat step 5 and 6 twice more.
7. Discard the supernatant and add 3 ml NRB to the pellet. Re-suspend the nuclei by pipetting.
8. Centrifuge at 1,500 \times g at 4 °C for 10 min to pellet the nuclei, and discard the supernatant. The purpose of this step is to remove the Triton X-100. The nuclei can now be used for any purpose. For example, they can be used to detect nuclear protein using a western blot. If the nuclei cannot be used immediately, they should be re-suspended in 400 μ l NSB buffer, quickly frozen in liquid N₂, and stored at -80 °C. The nuclei can last for at least half a year.

Recipes

1. Lysis buffer (LB)
 - 20 mM Tris-HCl (pH 7.4) 2 ml (2 M)
 - 25% Glycerol 25 ml
 - 20 mM KCl 1 ml (2 M)
 - 2 mM EDTA 0.4 ml (0.5 M)
 - 2.5 mM MgCl₂ 0.25 ml (1 M)
 - 250 mM sucrose 12.5 ml (2 M)
 - Add H₂O to 100 ml
 - Add DTT and PMSF to a final concentration of 1 mM respectively, immediately before use.
2. Nuclei resuspension buffer with 0.2% Triton X-100 (NRBT)
 - 20 mM Tris-HCl (pH 7.4) 2 ml (1 M)
 - 25% glycerol 25 ml
 - 2.5 mM MgCl₂ 0.25 ml (1 M)
 - 0.2% Triton X-100 0.2 ml
 - Add H₂O to 100 ml
3. Nuclei resuspension buffer (NRB)
 - 20 mM Tris-HCl (pH 7.4) 2 ml (1 M)
 - 25% Glycerol 25 ml
 - 2.5 mM MgCl₂ 0.25 ml (1 M)
 - Add H₂O to 100 ml
4. Nuclei storage buffer (NSB)
 - 20 mM Tris-HCl (pH 7.4) 2 ml (1 M)
 - 25% Glycerol 25 ml
 - 2.5 mM MgCl₂ 0.25 ml (1 M)
 - Sucrose 15.1 g
 - Add H₂O to 100 ml
 - Add PI before use (1/100 dilution)
5. MS
 - Sucrose 10 g
 - Murashige and Skoog basal medium 4.4 g
 - Phytigel 3 g
 - Add H₂O to 1 L, adjust pH to 5.6-5.8 using KOH and autoclave for 30 min.

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References

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