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. $MgCl_2$
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. Phytagel (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 µm and 40 µm 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 µm and 40 µm nylon mesh sequentially.
4. Centrifuge the filtered homogenate at 1,500 *x 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 *x 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 *x 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 used immediately, they should be re-suspended in 400 µl NSB buffer, quickly frozen in liquid N_2, 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 $MgCl_2$ 0.25 ml (1 M)
   250 mM sucrose 12.5 ml (2 M)
   Add H_2O 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 $MgCl_2$ 0.25 ml (1 M)
   0.2% Triton X-100 0.2 ml
   Add H_2O 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 $MgCl_2$ 0.25 ml (1 M)
   Add H_2O 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 $MgCl_2$ 0.25 ml (1 M)
   Sucrose 15.1 g
   Add H_2O to 100 ml
   Add PI before use (1/100 dilution)
5. MS
   Sucrose 10 g
   Murashige and Skoog basal medium 4.4 g
   Phytagel 3 g
   Add H_2O to 1 L, adjust pH to 5.6-5.8 using KOH and autoclave for 30 min.

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References

1. Cheng, Y. T., Germain, H., Wiermer, M., Bi, D., Xu, F., Garcia, A. V., Wirthmueller, L., Despres, C., Parker, J. E., Zhang, Y. and Li, X. (2009). Nuclear pore complex component MOS7/Nup88 is required for innate immunity and nuclear accumulation of defense regulators in *Arabidopsis*. *Plant Cell* 21(8): 2503-2516.
2. Xu, F., Xu, S., Wiermer, M., Zhang, Y. and Li, X. (2012). The cyclin L homolog MOS12 and the MOS4-associated complex are required for the proper splicing of plant resistance genes. *Plant J* 70(6): 916-928.

