Protocol: Prepare protoplast and isolate nuclei from Arabidopsis

Material: 3-4 weeks old plants with 1-2cm leaves before flowering (plants grown on soil are better)

Procedure:

**Prepare protoplast**

1. Weigh 1g cut leaves without petiole and cut 0.5-1mm strips with fresh razor blades without wounding.
   
   Note: The leaves pieces don’t need very small. More small piece more dirty solution. It is very easy that cut leaves on a flat glass.

2. Wash the pieces with WI and remove by pipet, then add 5ml fresh Enzyme Solution (5ml/gram). Keeping in darkness and incubate 3 hours in room temperature. It is not necessary to shake during incubating. It can be shake gently just before step 3.

3. Filter through a 120um mesh filter on glass funnel into a new tube and wash the tissue with 2ml WI.

4. Filter the filtrate of step 3 through 20um mesh filter. The protoplasts stay on filter. Wash them with 2ml WI two times. Overturn the filter and wash down the protoplasts in a glass dish with WI. Remove the protoplast into a new tube.
   
   (The following step was operated on ice.)

5. Adjust the volume to 5ml, then centrifuge at 100×g for 5 min at 4°C. Decant the supernatant.

**Isolate nuclei**

6. Resuspend pellet at 10^6/ml of cold NIB in 2ml eppendorf tube or bigger tubes. Put on ice for 7 min.

7. Fill a large syringes with protoplasts and pass it 4times through a 25G5/8 gauge needle.

8. Filter the lysate through 20um filter into a new tube. Centrifuge at 400×g for 10min. Discard supernatant, resuspend in NIB+glycerol or SDS solution for SDS gel electrophoresis.
Enzyme Solution
1.5% cellulose R10
0.4% macerozyme R10
0.4M mannitol
20mM KCl
20mM MES, pH5.5
(Heat to 55°C for 10min, then cool to room temperature before adding)
10mM CaCl2
0.1% BSA

Washing and Incubation solution (WI)
0.5M mannitol
4mM MES-KOH, pH5.5
20mM KCl

Nuclei Isolation Solution (NIB)
0.1mM Spermidine
10mM MES-KOH, pH5.5
2.5mM EDTA
10mM NaCl
10mM KCl
0.2M Sucrose
0.15% Triton X-100
2.5 mM DTT (Add fresh just before using)