

Isolation of Guard-cell Enriched Tissue for RNA Extraction

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[Abstract] This is a protocol for isolation of guard cell enriched samples from *Arabidopsis thaliana* plants for RNA extraction. Leaves are blended in ice-water and filtered through nylon mesh to obtain guard cell enriched fragments. With guard cell enriched samples, gene expression analysis can be done, e.g., comparing different gene expression levels in guard cells versus whole leaf to determine if a gene of interest is predominantly expressed in guard cells. It can also be used to study the effect of treatments or different genetic backgrounds in the regulation of the guard cell expressed genes.

Keywords: *Arabidopsis thaliana*, Guard cell isolation, RNA

[Background] Isolation of guard cells for RNA extraction has traditionally relied on guard cell protoplast extraction (Leonhardt *et al.*, 2004) or epidermal peels (Pandey *et al.*, 2010). Manual dissection of guard cells from freeze-dried leaves has also been used (Bates *et al.*, 2012). The protoplast procedure may introduce unwanted changes in gene expression from wounding effects and other methods are time consuming, require special equipment or expensive reagents (e.g., transcriptional inhibitors). Hence there is a need for a fast and simple protocol to isolate guard cells for gene expression analysis. Here we further describe a quick ice blender method for isolation of guard cell enriched tissue (Bauer *et al.*, 2013), in the form of epidermal fragments without mesophyll and other vascular cells. This method uses a nylon mesh to collect epidermis from blended leaf tissue. The adequately large pore size of the nylon mesh allows mesophyll and other vascular cells to pass through while retaining the epidermal fragments. We propose that the critical factor in this protocol is the type of blender used to process the samples.

Materials and Reagents

1. Razor blade
2. 210 µm nylon mesh (A. Hartenstein, laborversand.de, catalog number: PAS1)
3. Plastic embroidery hoop from a handicraft store (Figure 1)

Note: Nylon mesh is placed in the plastic embroidery hoop to provide a little tension to the mesh, to make it easier to pour the blended water and guard cell enriched fragments through the mesh.



Figure 1. Nylon mesh with a plastic embroidery hoop

4. Paper towel
5. Small medical spatula or plastic spoon
6. 1.5 ml tube or aluminum foil
7. *Arabidopsis* plants
8. Milli-Q or distilled water kept at 4 °C
9. Crushed ice
10. Liquid nitrogen

Equipment

1. Blender (Braun JB 3060 or other 800 W blender) (Braun Household, model: JB 3060)
Note: A blender with lower effect has been tried, but failed to isolate proper fragments, probably due to insufficient power to fully crush the ice.
2. Microscope (ZEISS, model: SteREO Discovery.v20)

Procedure

Collect leaves from 4-7 week old *Arabidopsis* plants grown in soil at 12 h/12 h photoperiod, use 17-18 plants and 4-5 leaves per plant. If plants are smaller in size, use more leaves per plant.

1. Remove the central vein (midrib) by razor blade. Collect about 1.0-1.5 g of leaves without midribs for the following procedures (Figure 2).

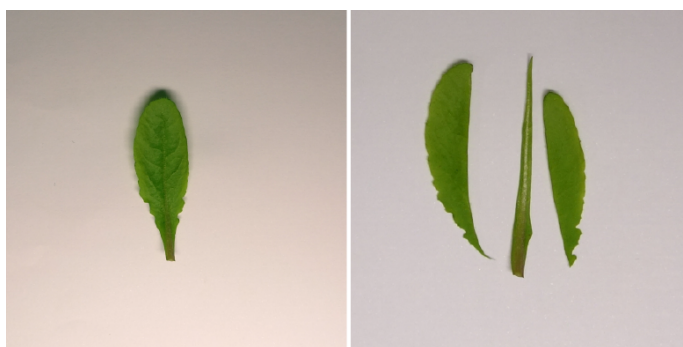


Figure 2. Midrib is cut and removed with the razor blade

2. Put the cut leaf blades in blender with 250 ml cold Milli-Q water and a handful of crushed ice. Blend 1 min (program 2 for the Braun blender), pour through mesh placed in a plastic embroidery hoop.
Note: Pouring through mesh should be done slowly, so that the guard cell enriched fragments are collected in the middle of the mesh. This is especially important at step 7 after which the sample is collected.
3. Wash the mesh with cold Milli-Q water above the blender cup so that the guard cell enriched fragments are transferred inside the blender cup. Add cold Milli-Q water up to 250 ml and handful of crushed ice.
4. Blend 1 min (program 2), pour through mesh placed in a plastic embroidery hoop.
5. Wash the mesh with cold Milli-Q water above the blender cup so that the guard cell enriched fragments are transferred inside the blender cup. Add cold Milli-Q water up to 250 ml and handful of crushed ice.
6. Blend 1 min (program 2), pour through mesh placed in a plastic embroidery hoop.
7. Remove the remaining dark green tissue fragments (small leaf pieces that have not been blended) from the light green epidermal fraction.
8. Dry the underside of the mesh from excess water with paper towel about 10-15 sec and use a small spatula or plastic spoon to carefully scrape the sample into a 1.5 ml tube or aluminum foil (Figure 3).



Figure 3. Guard cell enriched sample that is collected into a 1.5 ml tube

- Freeze the sample in liquid nitrogen and keep at -80 °C. Alternatively, the sample can be immediately ground in mortar with liquid nitrogen and the powder used for RNA isolation.

Note: If an estimation of guard cell enrichment is required, one easy control is to harvest a whole leaf sample from the same plants used for guard cell enrichment and freeze in liquid nitrogen and store at -80 °C. RNA can then be extracted in parallel with the guard cell samples. The level of guard cell enrichment can be estimated by real time quantitative PCR using guard cell preferentially expressed genes e.g., HT1 (AT1G62400), OST1 (AT4G33950) or GORK (AT5G37500).

Data analysis

Isolated guard cell enriched fragments can be visualized with a stereo microscope (Zeiss SteREO Discovery.v20), and no mesophyll fragments (green tissue) should be present (Figure 4A). While testing different blenders, successful isolation of guard cell enriched fragments was achieved with the Braun (800 W) blender but not with a blender with lower effect (Figure 4).

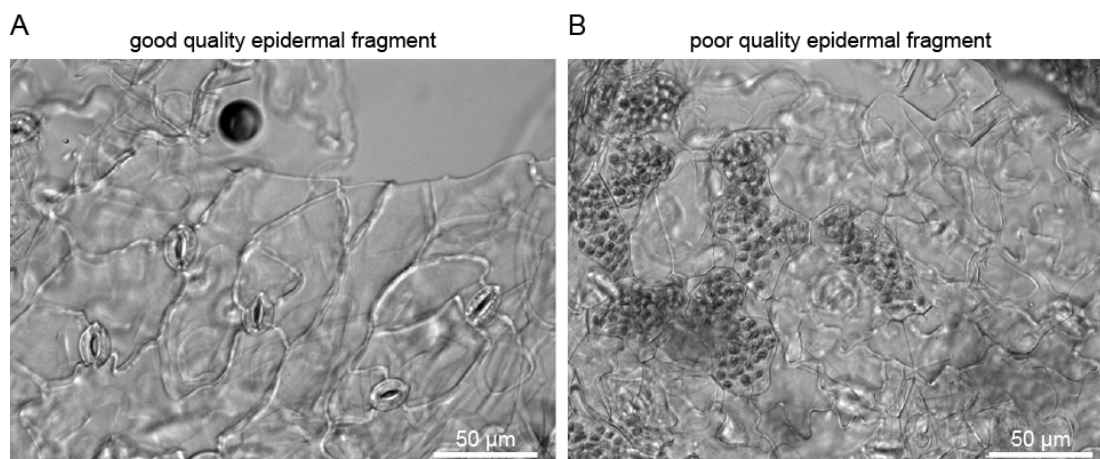


Figure 4. Isolated guard cell enriched fragments under a microscope. A. Sample isolated with the Braun blender; B. Sample isolated with a small 220 W blender.

To control the quality of guard cell enrichment, real-time quantitative PCR can be done with guard cell expressed genes like *HT1* and *GORK*, by comparing their expression level in guard cell enriched sample versus a whole leaf sample (Figure 5; for details about the qPCR conditions see Jalakas *et al.*, 2017).

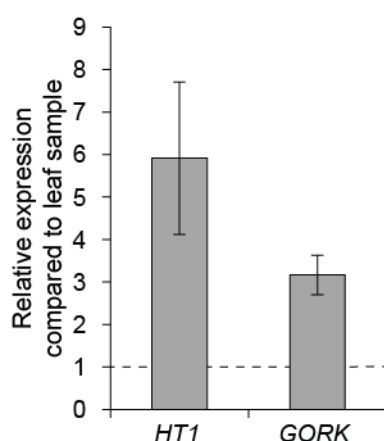


Figure 5. Gene expression in Col-0 guard cell enriched sample compared to leaf sample.

The mean of three biological replicates are shown; error bars depict \pm SEM.

Acknowledgments

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