January 2023

Cell Fixation Protocol

Notes before starting:

Depending on the plant tissue and/or species, the sampling method can be different. In the majority of cases, we recommend cutting samples into small pieces in order to facilitate the fixation with formaldehyde. A cold support such as a glass plate on ice can be used to cut the samples:

Arabidopsis seedlings (two weeks old) can be crosslinked intact. There is no need to cut them before crosslinking.

Arabidopsis adult rosette leaves can be sampled from 25 days old seedlings (18 leaves stage) and cut to small pieces of approximately 5 mm.

PLEASE NOTE THAT WE REQUEST 2 ADDITIONAL SAMPLES FOR EACH SAMPLE TYPE TO PROCEED WITH THE SHEARING OPTIMIZATION.

Reagents:

- 10X DPBS (Life technologies)
- Crosslinking bags (ask Diagenode to provide some bags)
- Formaldehyde 37% (Sigma, F8775-25ML)
- Glycine 1.25 M (VWR, 1.04201.0250)

Protocol :

1. Prepare 1x Crosslinking Buffer containing 1 % formaldehyde and store it on ice.

1x Crosslinking buffer	
10x PBS	3 ml
Formaldehyde	1 % (ex: 811 µl of Formaldehyde solution 37 %)
dH2O	Up to 30 ml

2. Harvest the plant material following the remarks in the Notes before Starting. Wash the plant material with dH2O after harvesting. Dry it using towel papers and keep it on ice until the crosslinking step in order to protect tissues from dryness. Plant material should not be in direct contact with ice.

3. Weigh the necessary quantity of plant material. Put the weighted sample in a crosslinking bag, make a node and pull simultaneously both ends of the thread. A second node can be made in order to close correctly the bag. Keep the crosslinking bag with the sample inside on ice until starting the crosslinking.
4. Introduce the closed crosslinking bag to the 50 ml falcon tube containing the crosslinking solution supplemented with formaldehyde. Make sure that the bag is submerged in buffer and not floating on top of it. A clean stainless steel paper clip can be fixed in the top of the crosslinking bag. This will somehow “lock” the crosslinking bag in the bottom of the tube and prevent it from floating on top of the solution. All samples should be introduced simultaneously to the 50 ml falcon tubes containing the crosslinking buffer supplemented with formaldehyde. Prolonged incubation of samples weighted first in crosslinking solution before applying the vacuum may induce variability.
5. Put the 50 ml falcon tubes containing samples in the desiccator (pre-filled with ice). During the crosslinking under vacuum, the tubes have to be opened. Otherwise, vacuum cannot be applied to the plant material. Close the desiccator lid and crosslink the plant tissue under vacuum (~ - 950 Millibars) during 15 min.
6. Turn off the vacuum pump and release the vacuum slowly. Replace 2.5 ml of the crosslinking buffer by 2.5 ml of Glycine. Mix the solution and apply vacuum for additional 5 min. Release the vacuum slowly.
7. Discard the Crosslinking Buffer. Add 40 ml of cold deionized water, close the tube and mix. Discard the water with dangerous products as it may contain formaldehyde. Repeat the washing step twice (3 washes in total).
8. Take the crosslinking bag out of the 50 ml falcon tube. Open the bag or cut it with scissors, remove plant tissue and dry it thoroughly using paper towels. Insert the plant tissue into new 50 ml tubes, close the tubes and snap-freeze them in liquid nitrogen. Store the samples at - 80 °C.