Isolation of mesophyll cells and vascular tissues from Arabidopsis rosset leaves

Components of enzyme solution:

P-sorbitol solution (reserved in ten-hold strength)

0.1% (w/v) Polyvinilpyrolidone (PVP)

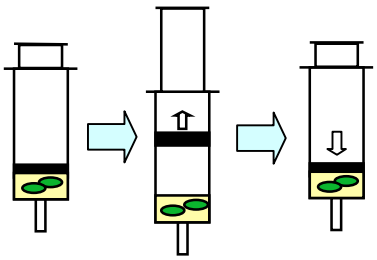
10 mM MES

1 mM $CaCl_2$

pH 5.6 (Tris)

0.3 M Sorbitol

0.6% (w/v) Sumizyme AP2



One hundred Arabidopsis leaves are put in a 50-100ml syringe with 20ml of enzyme solution, and the air inside of the syringe is forced out. The piston is pulled to create negative pressure inside of the syringe while sealing the tip of the syringe with a finger. After shaking the syringe while maintaining the pressure, the piston is pushed back to its original position. This step removes the air cellular vacancy. The additional pressure completes the infiltration of enzyme solution into the leaves.

↓

The leaves and enzyme solution are transfered to a flask.

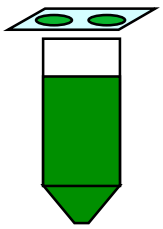
↓

The solution is incubated by shaking the flask at 80 rpm for 1 hour in a 31.5 °C water bath. After shaking, incubation is continued for another 30 minutes.

↓

The mesophyll cells and epidermal tissues are removed from the vascular tissues by gentle vortexing.

↓



The cell mixture is filtrated through 120-micrometer-pore nylon net to separate the mesophyll cells from the epidermal tissues, vascular tissues and the any remaining leaf fragments. The mesophyll cell suspension is collected in a 50ml tube.

↓

To collect the residue on the net in a beaker, the net is turned upside-down over the beaker, and the P-sorbitol solution is gently poured over the net.

↓

The epidermal tissues and vascular tissues are collected separately with the tip-cut micro pipetter tip.

Purification of mesophyll cells

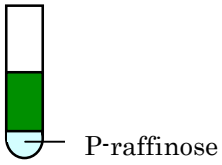
The mesophyll cell suspension is centrifuged at 1000rpm for 10 min. at 4°C.

↓

The supernatant is removed with an aspirator, the 2-3 ml of the P-sorbitol solution is added, and the suspension is vortexed gently.

↓

The cell suspension is put carefully with the tip-cut micro pipette tip on the P-raffinose solution in the test tube.

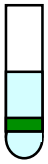


↓

The test tube is centrifuged at 1000rpm for 10 min. at 4°C.

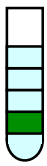
↓

The supernatant is removed with the aspirator, and the mesophyll cells on the P-raffinose phase are collected.



↓

The living cells are purified by P-sorbitol-solution-Percoll density gradient centrifugation. The solution which has a double strength of the P-sorbitol solution was mixed with Percol to prepare 50% Percoll, 15% Percoll and 10% Percoll. Create the layer with 50% percol, 15% Percol, cell suspension, 10% Percol and P-sor solution.



↓

The test tube is centrifuged at 1000rpm for 10 min. at 4°C.

↓

The cells appear at the two boundaries between 15% and 10% Percoll, and between 10% Percoll and the P-sorbitol solution layers are collected with the tip-cut micro pipette tip.