Lipid extraction from Arabidopsis rosette leaves

Sampling Table

Sample name (date):

Sample No.	Example	No. 1	No. 2	No. 3
Fresh weight (g)	1.0			
H_2O from material (ml) —— (W)	0.9			
2-Propanol (ml)	5	5	5	5
Homogenate (ml) Methanol	5	5	5	5
Chloroform (1)	5	5	5	5
H_2O =4.0-(W)	3.1			
Pellet washing (ml) Methanol	2	2	2	2
Chloroform (2)	1	1	1	1
H_20	0．8	0．8	0．8	0．8
Combined $CHCl_3$ layer = (1) + (2)	6	6	6	6
H_20	6	6	6	6
Empty vessel (g)				
Total lipid extracts (mg)				

Day 1

Place weighed rosette leaves (1 g) in a 50 ml screw-capped glass tube.
Flash 5 ml hot 2-propanol (preheated in 80˚C water bath) over the rosette leaves using a 10-ml silicon-rubber-capped glass pipette so that all leaves are immediately immersed in 2-propanol. The resultant samples (in a 50-ml tube) should be immediately put into the 80˚C water bath.

↓ 5 min, 80˚C

Chilled on ice.
Add methanol, chloroform, and water as calculated from the **Sampling Table**.

↓

Homogenize samples on ice to homogeneity, using a Polytron (max. speed),

↓ 3000 rpm x 15 min (r.t.)

Recover the supernatant (A) into a fresh 50-ml screw-capped glass tube by decantation (if the pellet is soft, remove by pipetting).

↓

Resuspend the pellet in methanol, chloroform and water, as listed in the **Sampling Table**, and mix well using a vortex mixer.

↓ 3000 rpm x 15 min (r.t.)

Recover the supernatant (B) and combine with the supernatant A.

↓

Add 6 ml chloroform and 6 ml H_2O to the combined supernatant and mix vigorously using a vortex mixer.

↓ 3000 rpm x 15 min (r.t.)

Recover the lower (chloroform) layer into a fresh 50-ml screw-capped glass tube with an equal volume of 0.9% KCl.

↓ 3000 rpm x 15 min (r.t)

Recover the lower (chloroform) layer*[1] into a 20 ml weighed pear-shaped flask, and evaporate the solvent on a rotary evaporator*[2].

*[1]Special attention should be paid not to recover any material floating in the intermediate phase.

*[2]If you see water droplets on the bottom of flask, add a few ml of ethanol and evaporate again.

↓

Dry up the residual solvent in a vacuum desiccator and determine the lipid dry

weight.

↓

Add chloroform to make a 10 mg/ml lipid solution while placing the flask on ice to prevent solvent evaporation.

↓

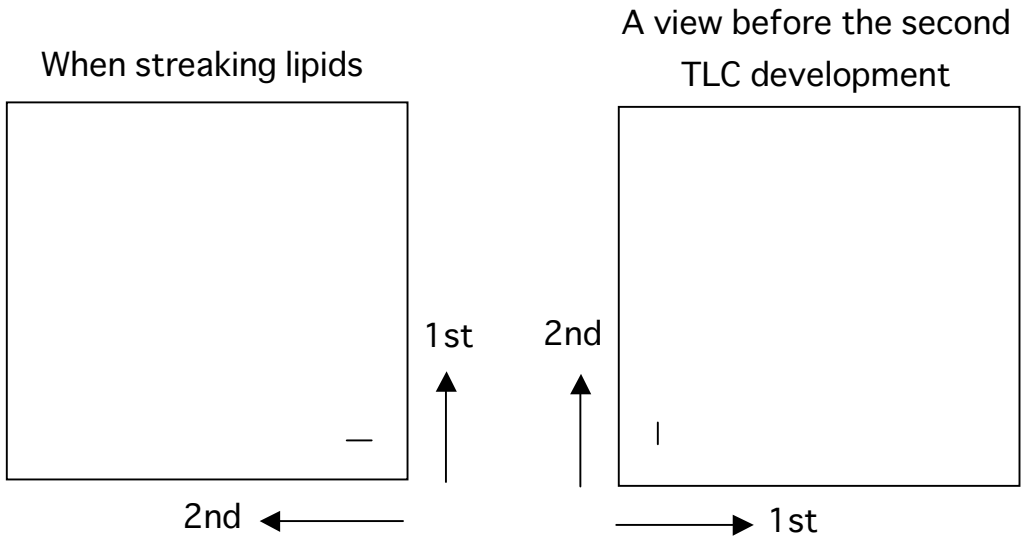
Transfer the lipid solution into a 1-ml screw-capped sample tube*[3].

*[3]Because the cap of the tube is so small, special attention should be paid to ensure that the cap is screwed tightly on the tube. Each cap should then be sealed with parafilm and stored at -30 ˚C. If you store the sample for more than one week, the antioxidant butylhydroxyltoluene (BHT) should be added at a final concentration of 10 μg/ml.

Day 2

Separation of lipids by two-dimensional silica gel thin-layer chromatography

Using a 100-µl glass micro syringe, streak 100 µl of the lipid extract (1 mg lipid equivalent) on a 1-cm line marked in the 2-cm-from-right and 2-cm-from-bottom position of a TLC plate (20 x 20 cm, Silica gel 60, Merck 1.05721.) as shown below.



Estimated weight of lipid classes in 1 mg total lipid extracts:

Pigments	0.59 mg
Glycolipids	0.2 mg
Neutral phospholipids	0.12 mg
Acidic lipids	0.06 mg

1st dimension：

Acetone/Benzene/Methanol/water = 8:3:2:1（by volume)

↓

Air-dry the plate (you may use warm air from a hair dryer).

↓

2nd dimension：

Chloroform/Methanol/Acetic acid/Water =170:25:25:4（by volume）

↓

Dry the plate using warm air from a hair dryer.

↓

Spray 0.01% Purimurin/80% acetone solution over the plate.
Dry the plate using warm air from a hair dryer.

↓

Mark lipid spots under long-wave UV light (360 nm), using a soft pencil (HB). The fluorescence image must be taken on a digital camera.

↓

Scrape off each spot on medicine paper, and carefully transfer all silica gel powder into a 15-ml screw-capped glass tube*[4].

*[4]Special attention should be paid not to use any tubes with cracks on the mouth or the screw mound.

↓

Add 10 µl each of a 15:0 standard/toluene solution, using a fresh glass capillary pipette.

↓

Add 10 µl of a BHT/toluene solution, using a fresh glass capillary pipette.
(If 14:0 is expected in the lipid samples, BHT must not be added, because 14:0-Me and BHT show the same retention time on a gas chromatograph.)

↓

Add 3 ml each of HCL/methanol solution. Screw on the tube and mix vigorously with a vortex mixer.

↓ 80 ˚C for 3 hrs on an aluminum heating block.

Day 3

Chill all tubes on ice.

↓

Extract FA methyl esters with 3 ml distilled hexane.

↓

Evaporate hexane, using a vacuum centrifugation evaporator. Dissolve dried lipid residues in ~5 drops of hexane from a Pasteur pipette and subject to gas chromatograph analyses.

(The hexane solution should be transferred to a 1-ml sample tube for storage or a glass tube for an automatic sample injector.)

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