Lipid extraction from Arabidopsis mitochondria

Sampling Table

Sample name (date):

Sample No.	Example	No. 1	No. 2	No. 3
Mit suspension (ml)	1.0			
H_2O from material (ml) —— (W)	1.0			
2-Propanol (ml)	5	5	5	5
Extraction Methanol	5	5	5	5
Chloroform (1)	5	5	5	5
H_2O =4.0-(W)	3.0			
Phase separation $CHCl_3$	5	5	5	5
H_2O	5	5	5	5
Washing with 0.9% KCl	10	10	10	10
Empty vessel (g)				
Total lipid extracts (mg)				

Day 1

Place 1 ml mitochondrion suspension (1 ml) in a 30 ml screw-capped glass tube (kept frozen at -80°C before use). Just before starting, warm frozen samples in running tap water until the suspension is almost thawed. Then keep the samples on ice.

Flash 5 ml hot 2-propanol (preheated in a 80°C water bath) into chilled mitochondrial suspension, using a 10-ml silicon-rubber-capped glass pipette, and immediately put the resultant suspension into the 80°C water bath.
   ↓ 5 min, 80°C
Chill on ice.
Add methanol, chloroform, and water as calculated from the **Sampling Table**.
The solution must be homogenous.
   ↓

Add 5 ml chloroform and 5 ml H_2O, and mix vigorously using a vortex mixer.
   ↓ 3000 rpm x 15 min (r.t.)
Recover the lower (chloroform) layer into a fresh 30-ml screw-capped glass tube, and then wash 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 tightly screwed 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 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.

When streaking lipids

A view before the second TLC development

1st   2nd

2nd ←   → 1st
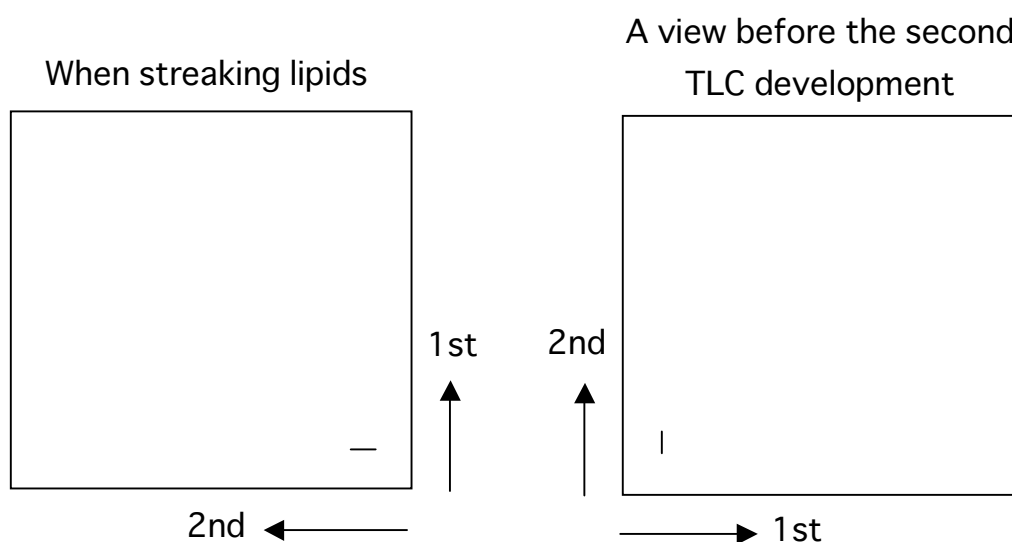
Estimated weight of lipid classes in 1 mg total lipid extracts:

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 was 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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