Lipid extraction from plant leaves (Bligh and Dyer's method)

1. Macerate plant leaves (about 1 g fresh weight) in a motor on ice (or with liquid nitrogen)
2. Add 1 ml of chloroform:methanol (1:2) and homogenize well
3. Transfer the homogenate to a glass tube (15 mm) with a stopper
4. Add another 2 ml of chloroform:methanol (1:2) to the motor for wash
5. Transfer the wash to the same glass tube
6. Centrifuge at 3,000 rpm for 5 minutes using the swing rotor of a table-top centrifuge machine
7. Transfer the supernatant to another glass tube with a stopper
8. Add 3 ml of chloroform:methanol (1:2) and 0.8 ml of 1% KCl to the pellet left at #7
9. Vortex well
10. Centrifuge at 3,000 rpm for 5 minutes
11. Transfer the supernatant to the tube prepared in #7
12. Add 2 ml of chloroform and 1.2 ml of 1% KCl to the collected supernatant
13. Vortex well
14. Centrifuge at 3,000 rpm for 5 minutes
15. Transfer the lower layer ("lipid extract") to another glass tube (weigh it before use) with a Pasteur pipette
16. Dry under nitrogen stream
17. Weigh the tube with lipid and calculate the weight of total lipids
18. Dissolve in an adequate volume of chloroform:methanol (2:1) for further use

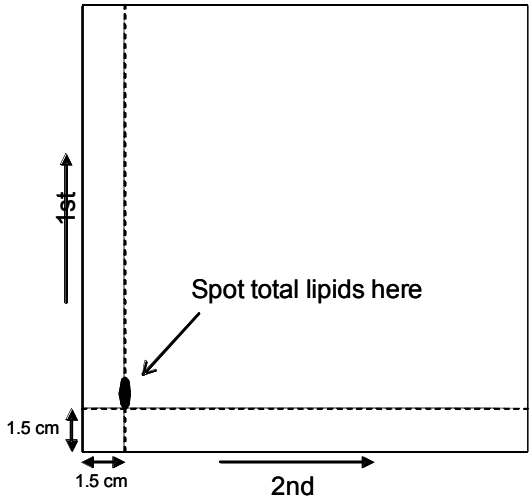
Separation of lipid classes by thin-layer chromatography (TLC)

(This is a protocol for the separation of glycerolipids in plant leaves)

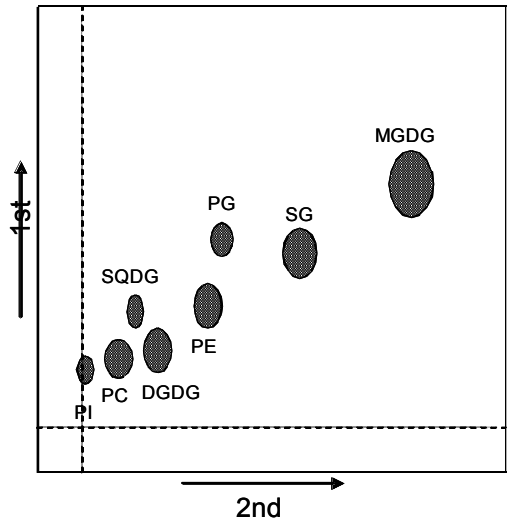
1. Perform 2-dimension TLC of about 500 μg total lipids using a Silica gel 60 TLC plate

   1st: chloroform:methanol:7N ammonia water= 120:80:8

   2nd: chloroform:methanol:acetic acid:H_2O=170:20:15:3



2. Visualize lipid classes under UV light after spraying 0.001% primline in 80% acetone to the TLC plate



MGDG, monogalactosyldiacylglycerol; SG, sterolglucoside; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol

Preparation of gas chromatography (GC) samples

1. Mark the lipid spots with a pencil under UV light
2. Scrape off the each spots from the plate and transfer to a glass tube with a screw cap individually
3. Add 100 μl of 1 mM of control fatty acid (pentadecanoic acid, 15:0) and 350 μl of methanol-HCl and close the cap tight
4. Incubate at 85C for an hour
5. Add 500 μl of hexane and vortex well
6. Transfer the upper layer to another tube (no need to be with a cap)
7. Add another 500 μl of hexane to the lower layer left over and vortex well
8. Transfer the upper layer to the tube prepared in #6
9. Dry under nitrogen stream
10. Add 60 μl of hexane and dissolve the lipid
11. Transfer to a GC vial and inject 3 μl of the sample for GC analysis