DAY 1

Freeze dry samples - need 2.5 mg dried tiss.

8-10 samples at one time

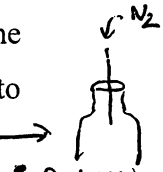
FA Extraction protocol (from Ursula Strandberg) – SY updated 27-Nov-2012

Flammables - Chl.: MeOH 2:1

Re-use reagent-specific pipettes (in hood)

imp't.

1. Rinse all tubes (burned overnight at 440 Celsius) and Teflon lined caps with chloroform: methanol 2:1 (vol: vol). You will need 3 pre-labeled centrifuge tubes and caps for each extraction.
2. Place a ~10 mg (less for fatty samples, e.g. fish) sample in centrifuge tube labeled *sample#*-A. Add 2 mL chloroform. ($CHCl_3$) (For storage flush with nitrogen and store in freezer).
3. Add 1 mL of methanol — micropipette - / tips to R of hood
4. Add 1 mL of 2:1 chloroform: methanol and then ½ mL of water (milliQ). Recap the tube and sonicate (Rm 319) the mixture for ~10 mins, then vortex for 30 seconds, and then centrifuge (add ice, Rm 320 (code: 2446)) the test tube for 3 min. at 3000 rpm. (Rm 319) ↓~6
5. Remove the organic layer (bottom layer) using a pasteur pipette. Depress the pipette bulb as the tip travels through the upper layer (push out bubbles), so that the pipette only draws from the lower layer. As the pipette is removed from the lower layer, squeeze the bulb slightly so that no upper layer is entrained into the pipette. Remove the entire lower layer. Transfer the removed organic layer into the pre-cleaned tubes labeled *sample#*-B. Begin N_2 evap. of B →



- adjust reg. so that ball dia. at 20 psi
- open cylinder
- ~~open flow~~
- open needles at top (½ turn)
- open flow slowly → look for small flutter on sample surface
- H2O bath set to 2 La ~30°

6. Add 2.7 mL of chloroform to sample A. Sonicate, vortex, and centrifuge the samples (same times/speeds) and pipette the lower layer when separated, using new pipettes each time. ~~Repeat, (so that the organic layer has been removed 3 times)~~ pooling all organics into sample vial B.
7. To store, flush organics with nitrogen and store in freezer.
8. Evaporate sample vial B under a gentle stream of nitrogen, and continue to transmethylation procedure.

*polypropylene pipette tips are ok to use for transferring of chloroform and methanol. Refer to page 18 of https://fscimage.fishersci.com/images/D16542~.pdf

Bring:
glass pipettes
gloves

Standard solutions

Prepare 2% $KHCO_3$ (Potassium bicarbonate):
To prepare 1 L 2% $KHCO_3$, weigh 20 g of $KHCO_3$, add it to a 1 L cylinder and add 1 L of water (milliQ).

Prepare hexane: diethyl ether:
To prepare 1 L hexane: diethyl ether, add 500ml hexane and 500 ml diethyl ether to a 1 L glass bottle.

Weight/volume percentage: weight of solute in grams/volume of solution in ml

Prepare 1% H_2SO_4-MeOH
Add 1 ml of H_2SO_4 to 99 ml of MeOH. Always add acid to the solution (not the other way around).

Notes

Remember to buy diethyl ether without BHT already in it. (1 L bottles are good so that they are used up in a timely manner, and not left to sit for long periods of time)

Glassware prep:

- Soak tubes in water with soap (micro-90 or nitro-x)
- Use brush to scrub tubes
- Rinse with DI water (at least 5 times to remove all traces of soap)
- Dry tubes and caps in oven
- Burn tubes in muffle furnace at 440 C
- Rinse tubes with cap using 2:1 chloroform: methanol (in a fume hood)
- Invert and allow for evaporation until dry and ready to use

For preparing reagent bottles

- Wash
- Rinse with Acetone finally