Methanol/chloroform Extraction

This extraction should be performed on lyophilized tissue that has been preweighed to get dry mass.

Preparation

* Make sure all glass test tubes are labeled and correspond to the samples
* Turn on centrifuge
  + Setting 2000xg at 4C for 5 minutes
* Aliquot water, methanol and chloroform in small portions into beakers (if all is not used, put in waste beaker)
* Get ice bucket for vials to go into

Performing the extraction

* Prepare glass test tubes with nonpolar solvents
  + In each test tube add 1mL CHCl3 and 0.5mL H20
* Bead beat dried flies for 20 seconds at speed 4
  + Using bead beater, load samples ensuring all are evenly spaced
  + Be sure to evenly tighten the silver knobs
  + Close lid and adjust time and speed if needed, and press run
* Add polar solvents to lyophilized flies in bead beating tube
  + 1mL MeOH and 0.4mL H20
  + MeOH goes in first (do this very quickly after bead beating to stop enzyme activity)
* Bead beat for 20 seconds at speed 5
* Add polar solvent/fly mixture to nonpolar solvents in glass vial
* Vortex for 30 seconds
  + Always keep glass test tubes on ice when not in use
* In the ice bucket put on shaker for 10 minutes
* Centrifuge at 2000xg at 4C for 5 minutes
  + Settings should be adjusted in preparation step when turning on centrifuge
  + Be sure it is set to rcf
* Remove upper polar phase and place in second set of pre-weighed and labeled microcentrifuge tube
  + Use disposable Pasteur pipets
* Freeze at 80°C if not ready to centrivap
* Centrivap until dry
* Weigh to get dry metabolites
* Rehydrate with 620µL 0.1M sodium phosphate buffer (pH 7.3)
* Vortex for 30 seconds
* Centrifuge 10000rpm for 5 minutes at 4C
* Transfer 600µL to labeled NMR tube
* Save bottom, nonpolar layer in small glass (pre-weighed and labeled) vial and freeze at 80C
* Dry under stream of N2 gas until dry- weigh to get dry metabolites
* Rehydrate with 620µL deuterated chloroform
* Transfer 600µL to labeled NMR tube