Lipid extraction protocol using Chloroform Methanol solution.

This current protocol was adapted from a protocol sent to me by Dr. Maria Viozzi author of “Tissue-specific Isotopic Incorporation Turnover Rates and Trophic Discrimination Factors in the Freshwater Shrimp Macrobrachium borellii (Crustacea: Decapoda: Palaemonidae)” in which they lipid extracted the hepatopancreas of shrimp.

1. Prepare various flasks, vials, containers or test tubes. A clear container is best to work with as the color of the supernatant signifies completion of lipid extraction.
2. Measure 1.5mL of hepatopancreas in the 15mL falcon tube
3. Add chloroform:methanol in a ratio of 2:1 (ex. 300mL of CHCl3 + 150mL MeOH). Immerse the sample in the solution (for hepatopancreas I generally submerge in mL of C:M)
4. Stir the sample and solution using a glass rod or a spatula to ensure surface of sample is covered.
5. Allow the sample to rest for 30-45 minutes while lipids are being removed.
6. Repeat steps 3-5 for a standard number of times (I conduct 5 repetitions on the hepatopancreas as it is a very fatty tissue) if supernatant is still colored then conduct additional repetitions on individual samples. When C:M solution is clear then lipid extraction is complete.
   1. If you want to “test” your C:M solution clarity you can add DI water to compare. DI water will form a layer on top of the C:M so you can compare solution clarity to water. this is not e necessary step but is something you can do to check you work.
7. Finally dry the sample to grind and weigh. Sample can be left uncapped under a fume hood to dry overnight. If additional drying necessary, use a drying oven at 60°C