Method for extracting lipids:

Modified Folch (Sweeting et al. 2006 Rapid Comm. Mass. Spec.)

1. Homogenize tissue in a 2:1 chloroform/methanol mixture, 20x tissue volume.
2. Sonicate homogenate for 10 min. at 35 kHz.
3. Centrifuge at 12,000 *g* for 5 min.
4. Remove supernatant.
5. Wash tissue with ultra-pure water, 20x tissue volume.
6. Sonicate and centrifuge again (removes remaining chloroform/methanol).
7. Oven dry remaining tissue at 50°C to constant weight.

Lipid content determined by weight difference and expressed as proportion of the weight of the original tissue sample.

Simplified method (Beaudoin et al. 2001, Sotiropoulos et al. 2004)

1. Soak tissue in 1:1 chloroform/methanol solution for three 10 min. intervals.
2. Rinse sample with distilled water and air-dry (Sotiropoulos addition)

Modified Folch (Kling et al. 1992, Ecology)

1. Dilute tissue (20x volume) with 2:1 chloroform/methanol.
2. Heat for 15 min. in a water bath at 60°C.
3. Filter homogenate through a Whatman GF/C glass fiber filter into centrifuge tubes.
4. Mix filtrate with 0.2x its volume of 50 mmol/L NaCl.
5. Allow mixture to separate into two phases.
6. Remove the upper phase.
7. Rinse inside tube wall with 3:48:47 chloroform/methanol/water. Remove rinse.
8. Evaporate lower phase at <60°C to dryness.