Embryo Extractions

1. To cell sample in 50 µL of water, add 950 µL 2:2:0.75 cold MeOH:ACN:H2O MAW solution (should have final MAW ratio of 2:2:1).
2. Lyse cells by doing the following three times:
    a. 30s vortex
    b. 1m liquid nitrogen bath
    c. 2m thaw in sonicator; submerge slowly & carefully
    d. Bath sonicate at 25º C for 10m
3. Store samples at -20º C 1-2 hours or overnight
4. Isolate supernatant
    a. Centrifuge at 14k rpm and 4º C for 10m. Orient front of Eppendorf tube to the outside of the centrifuge so that the upper edge of the pellet is oriented away from the tube's cap joint (see step 4b).
    b. Pour supernatant to new Eppendorfs at 4ºC. To leave as little solvent as possible without disturbing the pellet, pour in the direction of the pellet's upper edge (which should be at the front, away from the cap joint).
5. Dry pool with speedvac; no heat; 2-15 hrs; don't overdry.
6. Resuspend metabolites
    a. Add 100 µL 1:1 water:acetonitrile to residue
    b. Do this twice: bath sonicate for 5m at 25º C, then vortex for 1m
    d. Store for 1 hr at 4º C
7. Prepare final sample
    a. Centrifuge at 14k rpm and 4º C for 10m
    b. Transfer 90µL supernatant to LC vials without disturbing insoluble pellet
    c. Store at -80 º C