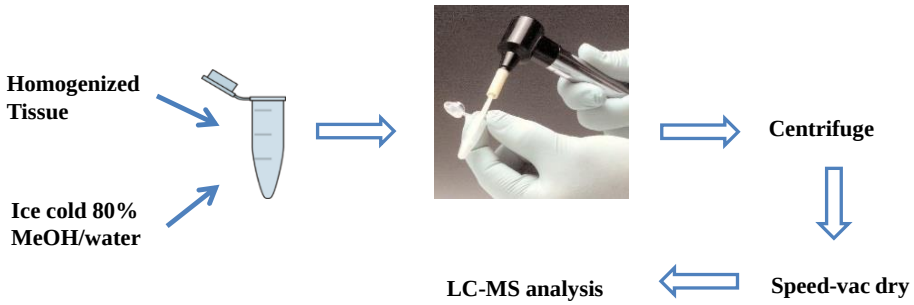
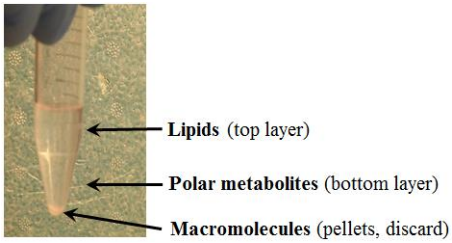
LOCASALE LAB PROTOCOLS

Lipids and Polar Metabolites Extraction from Tissues



1. If tissue hasn't been homogenized yet, then dip the Eppendorf tube containing the tissue into liquid N_2 for 2 min (keep the tube closed as tightly as possible to avoid pop up, wear goggle to protect eyes).
2. Transfer frozen tissue to a small piece of aluminum foil, and wrap it.
3. Place the wrapped tissue back into the liquid N_2 for an additional 1 minute. Take it out from liquid N_2 and place it on a flat surface, open the wrap, and use a hammer or pestle to homogenize the tissue.
4. Close the wrap, and place it back to liquid N_2 for 1 minute, and then transfer the ground tissue into a new tube sitting on dry ice.
5. Weigh about 5-10 mg tissue in a new 2ml Eppendorf tube on ice (dip samples into liquid N_2 before weighing to keep them frozen). Write down the exact weight for normalization.
6. Add 200 ul ice cold 80% methanol (HPLC grade), and stir until well mixed with solvent. (It can take a long time to mix, so try to ground it as much as possible in step 3). The mixer can be purchased from https://us.vwr.com/store/catalog/product.jsp?product_id=4830712 or a similar company. Note: solvents should be HPLC grade.
7. Add another 200 ul ice cold 80% methanol, vortex for 1 min.
8. Add 960 ul ice cold MTBE (**DO NOT** put MTBE into -80^{o}C freezer!), vortex for 1min.
9. Add 240 ul ice cold water, vortex for 2 min. The final extraction solvent composition is MeOH/MTBE/H2O 1:3:1 (v/v/v, all HPLC grade).
10. Centrifuge at 20 000 rcf 4^{o}C for 10 min, two layers formed. Top layer contains lipids, while bottom layer contains polar metabolites.



11. Split the top and or bottom layer (not pellets!) into two tubes (two tubes for top layer, two tubes for bottom layer, four tubes in total). One tube is used as a backup and shipped to us for future analysis or in case anything goes wrong with the analysis on our end (e.g. instrument glitch during the run, etc). NOTE: the exact amount to be transferred should be normalized to the amount weighed in step 5, ~ 2 mg per split (ASK US IF YOU'RE UNSURE ABOUT WHAT WE MEAN HERE) Speed vacuum dry all tubes.
12. Store dry pellets in -80^{o}C freezer for further LC-MS analysis.

Prepared by Locasale Research Group (March 2017)