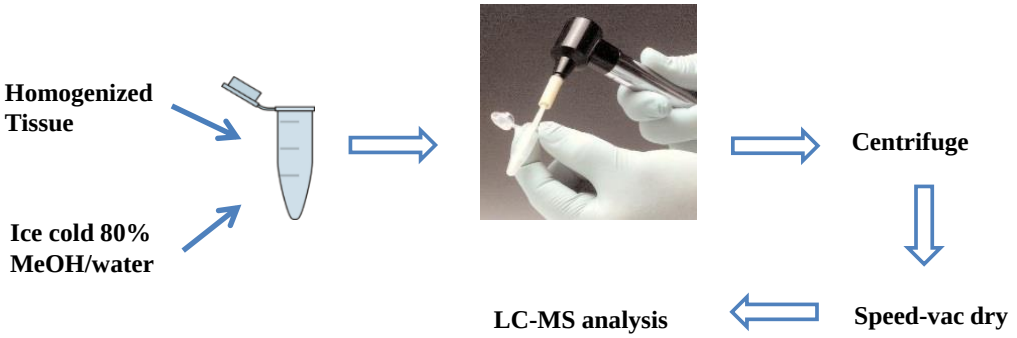
LOCASALE LAB PROTOCOLS

Tissue Sample Preparation



1. If tissue hasn't been homogenized yet, then dip Eppendorf tube containing the tissue into liquid N2 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. Put the wrap back to liquid N2 for additional 1 min. Take it out from liquid N2 and place it on a flat surface, open the wrap, use hammer or pestle to homogenize them.
4. Close the wrap, and put it back to liquid N2 for 1 min, and then transfer the ground tissue into a new tube sitting on dry ice.
5. Weigh about 3-10 mg tissue in a new Eppendorf tube on ice (dip samples into liquid N2 before weighing to keep them frozen). Write down the exact weight for normalization.
6. Add 200 µL ice cold 80% methanol, and stir until they are well mixed with solvent. (It could take a long time to mix, so try to ground them as much as possible in step 1). The mixer could be purchased from https://us.vwr.com/store/catalog/product.jsp?product_id=4830712 or other similar company. Note: Solvents should be HPLC grade.
7. Add another 300 µL ice cold 80% methanol, vortex.
8. Leave the mix on ice for 10min.
9. Centrifuge at 20 000 rcf for 10 min, 4^{o}C.
10. Transfer the supernatant into two tubes (roughly 200 µL per tube), save one tube as backup. Discard the old tube containing the pellet of cell debris. During transfer, try not to touch the bottom pellet, which will suppress mass spec signals. NOTE: the exact amount to be transferred should be normalized to the amount weighed in step 6 (ASK US IF YOU'RE UNSURE ABOUT WHAT WE MEAN HERE)
11. Speed vacuum dry the tubes at room temperature, which will take about 1 hr for 200 µL solvent. Note the dried pellet (containing the metabolites) should be a white to light yellow color and smaller than the volume of 5 µL liquid.
12. Store dry pellet in -80 ^{o}C freezer for further LC-MS analysis.

Prepared by Locasale Research Group (September 2016)