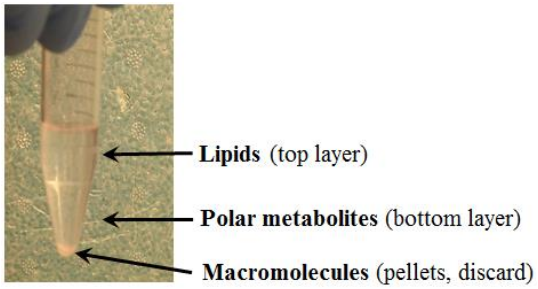
LOCASALE LAB PROTOCOLS

Lipids and Polar Metabolites Extraction from Cell Culture

1. Grow cells in 6-well plate (bigger plate or dish is not acceptable) to 50% - 80% confluence. NOTE: try your best to extract the same number of cells when comparing across a set of conditions (e.g. WT vs. KO). Let us know if this is not possible and we can think of alternative approaches.
2. Tilt the plate, and immediately aspirate medium at room temperature (about few seconds per well). TRY TO REMOVE THE MEDIUM AS MUCH AS POSSIBLE SINCE THE CONSISTENCY OF THIS STEP WILL AFFECT SENSITIVITY AND REPRODUCIBILITY.
3. Immediately place the plate on dry ice, and add 1 mL 80% methanol/water (both HPLC grade) (pre-cooled in -80^{o}C freezer for at least 1hr) to each well.
4. Transfer the plate to a -80^{o}C freezer and leave it there for 15min to further inactivate enzyme activities.
5. Remove the plate from -80^{o}C freezer and put it on dry ice, scrape cells into extraction solvent.
6. Transfer the cell extract of each well into individual 15ml plastic tube on regular ice.
7. Vortex for 1min, and then add 2.4 ml of ice cold MTBE (HPLC grade, pre-cooled on ice, **DO NOT** put MTBE into -80^{o}C freezer!) on ice, vortex for 1min.
8. Add 0.6 ml ice cold water (HPLC grade, pre-cooled on ice), vortex for 1 min. The final extraction solvent composition is MeOH/MTBE/H2O 1:3:1 (v/v/v, all HPLC grade).
9. Centrifuge (3500 g for 10min) at 4°C, two layers should form. The top layer contains lipids, while bottom layer contains polar metabolites.



10. Split top layer into two clean 2ml Eppendorf tubes (~ 1.2ml per split). Split the bottom layer (not the pellets!) into two clean Eppendorf tubes (~550-600 ul per split).
11. Speed vacuum dry all tubes (~ 1 hr for top layer, ~ 2hrs for bottom layer).
12. Store dry pellets in -80^{o}C freezer for further LC-MS analysis.

Prepared by Locasale Research Group (March 2017)