Extraction Protocol: Liver
Platforms: CSH-QTOF (Lipidomics)/ GCTOF (Primary Metabolites)/ Proteomics/ Oxylipins
Amount of Tissue Provided: ~100-150mg

1) Keep Specimen on Dry Ice
2) Transfer Tissue Contents into a new 1.5mL labeled eppendorf tube; keep on dry ice at all times
3) Add three (3) 3mm metal grinding balls to each sample; store in -80C for 10minutes
4) Homogenize the entire tissue to fine powder using genogrinder; make sure that the metal grinding balls are ice-cold prior to homogenization (step 3)
5) Upon completion of homogenization, keep samples on dry ice
6) Weight out **two (2) aliquots**: a ~**5mg** aliquot for *CSH_lipidomics* and a ~**4mg** aliquot for *Primary Metabolites by GCTOF*
    a. Record the exact weight weighed out for each sample
    b. Keep all samples on dry ice
7) KEEP remaining tissue specimen (>90mg) for analysis of Oxylipins (store in -80C)
8) Analysis of Primary Metabolites (GCTOFMS)
    a. Add 1mL of ice-cold "degassed" 3:3:2 ACN/IPA/H2O
    b. Vortex for 10seconds
    c. Shake on shaker for 20min at -4C
    d. Centrifuge the samples for 2min at 14,000 rcf
    e. Transfer two (2) 500µL aliquots to new 1.5mL eppendorf tubes; one for backup the other to be dried to dryness using the SpeedVac
    f. **IMPORTANT:** The precipitated protein will be used for analysis of the proteome, DO NOT DISCARD THESE; Place these in a separate labeled box and store in -20C
    g. Keep all samples on ice during extraction period
    h. Dry down one (1) 500µL aliquot to complete dryness
    i. Perform cleanup on dried aliquot using 500µL of 50/50 v/v ACN/H2O
    j. Transfer supernatant and dry to completeness
    k. Submit for Derivatization
9) ***Analysis of Complex Lipids (LCQTOF)***
    a. Add 225µL of ice-cold "degassed" MeOH containing "ISTD mixture" to homogenized 5mg aliquot
    b. Vortex for 10 seconds
    c. Add 750µL of ice-cold "degassed" MTBE containing 22:1 CE ISTD
    d. Vortex for 10 seconds
    e. Shake on Orbital Mixer for 6min at 4C
    f. Add 188µL of room temperature H2O
    g. Vortex for 20 seconds
    h. Centrifuge for 2min at 14,000 rcf
    i. Transfer two (2) aliquots of 350µL of top layer, one for backup stored in -20C, the other for analysis
    j. Keep bottom layer and store in -20C
    k. Dry down one (1) 350µL aliquot to dryness using the Speedvac
    l. Resuspend samples in 108.6µL of 50ng/mL CUDA
    m. Vortex and sonicate for 5minutes
    n. Centrifuge for 2min at 14,000 rcf
    o. Transfer 90µL to an amber vial with micro-insert (non-diluted)

p. Transfer 10µL to a new 1.5mL eppendorf tube, dilute 20X with 50ng/mL CUDA in 90:10 MeOH:Toluene (10µL + 190µL CUDA) and transfer 100µL to amber vial with micro-insert (diluted for TGs)
    i. The dilution is based off previous experiences with liver samples (as described by Ingrid)