Sample preparation for shotgun lipidomics (Shevchenko lab)

For tissue: 5-20 mg wet weight should be more than enough, flash freeze tissue in liquid nitrogen and store at -80°C
For mammalian cells: starting from 0.5 x 10^6 cells. In case your protocol includes the washing step with PBS, please make sure that the PBS is freshly prepared and LC-MS grade water (Merck, 1.15333.2500) is used.

It is absolutely necessary to include blanks for protein determination and the lipidomic analysis. At least 10% of your samples should be blanks which go through the whole procedure together with the biological samples. For lipidomics use approximately the average volume of all biological samples for the blanks.

- Reconstitute samples in 100% isopropanol (IPA, LC-MS grade Honeywell Cat. 34965-2.5L, ~300-500 µl), use either 1.5 or 2 ml safelock eppis (Eppendorf, 0030120.086, 0030120.094)
    - Samples can be stored for a long time at -80°C without any detectable lipid degradation
- Keep samples on ice and add 1.0 mm zirconia beads (5-10; Carl Roth, N038.1)
    - Wash zirconia beads with H_20 (Merck, 1.15333.2500), methanol (MeOH; Merck, 1.06035.2500) and methyl-tert-butyl ether (MTBE; Honeywell, Cat. 650560-1L) before usage (prepare enough for multiple sample batches)
- Homogenize on a tissue lyser (Qiagen) for 2 x 5 min at 30 Hz at 4°C
    - Increase time if samples are not homogenized well
    - Some samples especially muscle or scared tissue are difficult to homogenize, if possible then use a proper tissue homogenizer
- Take an appropriate aliquot for protein determination, transfer it to a new 1.5 ml eppi and evaporate samples in a desiccator to complete dryness
    - For liver: 25 µl
    - For cells: ≤ 600000 cells 20 µl, ≥ 3 x 10^6 cells 10 µl
- Reconstitute samples in 1:1 ammonium bicarbonate buffer (150 mM in H_2O, ABC; Fluka, 40867-50G-F)/RIPA (ChemCruz, sc-24948A)
    - For liver: 200 µl
    - For cells: ≤ 600000 cells 100 µl, ≥ 3 x 10^6 cells 200 µl
- Incubate at room temperature shaking for at least 15 min
- Centrifuge for 15 min at highest speed at 4°C
- Take 10 µl for bicinchoninic acid assay (Pierce, 23227) in 96 well plate format (Thermo Scientific 167008)
    - Dilute 2 mg/ml BSA 1:1 with ABC buffer (in total 7 dilutions)

    - Use 150 µl of 50:1 A/B for the assay; incubate for 30 min at 37°C, detect absorbance at 562 nm
- Calculate volume corresponding to 50 µg of total protein
    - Pipette aliquot in a new 2 ml safelock eppi and store at -80°C; this will be used for lipid extraction

Disclaimer

This protocol was extensively test on liver samples and therefore the volumes are chosen so that these samples fall in the middle of the BSA calibration curve. For other tissues or cells, the used volumes (especially for reconstituting the samples for protein determination) have to be adjusted accordingly and tested again.

According to our experience for a proper statistical analysis is it is absolutely necessary to have at least 6 biological replicates per condition. Please consider this while planning your experiments and while performing data analysis. It is still possible to perform statistical analysis with less samples, however, the results have to be interpreted with more caution.

If necessary I can supply you with all necessary chemicals and materials. Additionally, you can use our desiccator.

For further questions just come back to me (knittelf@mpi-cbg.de).