Preparing cells for a metabolomics experiment

General considerations for stable isotope (e.g. C-13, N-15, H-2 (D)) labeling:

The minimum labeling duration depends on the metabolic pathway of interest. For instance, metabolites in the glycolysis pathway only take minutes to reach isotopic steady-state for many cell types, while other metabolic pathways (e.g. lipids) might take days.

- plate about 200k (or more) cells per well in 6well plates (3 wells per condition minimum) and incubate o/n (for adherent cells, add additional well(s) for cell counts)
- after 24h rinse with 1x PBS and add fresh medium (with heavy tracer or not): 1.5-2 ml is sufficient to cover the cells for 24h, but keep in mind that the cells might deplete nutrients within that amount of time

Metabolite extraction with 80% MeOH

- rinse cells with cold 150 mM NH4AcO, pH 7.3
- add 1 ml 80% MeOH (-80C) and scrape off cells (if you have adherent cells)
- transfer suspension into Eppendorf tube and add 1-10 nmol norvaline (Internal standard: I usually prepare a 100 mM solution in H2O and dilute this 1:10 in MeOH (store at -20C), for an experiment I prepare a fresh 1:10 dilution in H2O)
- vortex each sample at least three times on ice
- spin down suspension at top speed for 5 min, transfer supernatant into glass vial, and re- suspend pellet in 200 µl 80% MeOH (2^{nd} extraction)
- spin down suspension at top speed for 5 min and combine solutions
- you can re-suspend the pellet in your favorite protein extraction buffer and measure the protein content as additional normalization information (or normalize to cell number if cell shape/size does not change)
- dry down metabolites in EZ-2Elite evaporator at 30C using program 3 (aqueous)
- keep samples at -80C at CNSI

Preparation of medium samples (metabolic footprint)

- remember that you will need 3 extra samples with 'fresh' medium: that is unused, spare medium you put on your cells
- add 20 µl medium (or blood serum) and 1-10 nmol norvaline to 500 µl 80% MeOH, vortext and spin for 5' at top speed
- transfer supernatant into glass tube and dry as described above
- keep samples at -80C at CNSI

Metabolite extraction from tissue*

- cut off 10-20 mg tissue on ice and homogenize in 1 ml 80% MeOH (-80C) (you might have to rinse the tissue to remove blood if possible)
- spin down at top speed (@4C) 1-2x for 5 min
- resuspend cell / tissue pellet in protein lysis buffer and measure protein concentration (see above)
- transfer fracture of supernatant (usually the equivalent of 1-5 µg protein) into glass tube, add 10 nmol norvaline

  dry samples using EZ-2Elite evaporator at 30C using program 3 (aqueous)
- keep dried samples at -80C at CNSI

Tissue extraction of mouse cecum for metabolomics*

- remove and snap-freeze about 20-50 mg cecal
- re-suspend cecum in 500 µl / sample water and disperse using a homogenizer
- spin samples at ~16 rcf for 5 min
- remove supernatant and measure protein content using BCA assay
- re-suspend the equivalent of 5 mg (protein) / sample into 500 µl 80% MeOH and 10 nmol DL-Norvaline
- Vortex sample for ~10 sec, spin at ~16 rcf for 5 min
- load supernatant into borosilicate glass vial
- evaporate samples using the EZ-2Elite evaporator at 30C using program 3 (aqueous)
- store samples at -80C at CNSI

Equipment and reagents needed for this protocol

- Ammonium acetate	A1542-500G	Fisher	for molecular biology, ≥98%
- glass vials:	03-410-151	Fisher	1.8 mL Volume; Clear Glass, 12x32 mm, 9 mm thread
- caps:	03-379-123	Thermo Scientific	Rubber/Silicone Septa
- MeOH:	A456-1	Fisher	Fisher Methanol (Optima* LC/MS)
- H2O:	W5-1	Fisher	Water, Glass Bottle; 1L
- Norvaline:	N7502-25G	Sigma	DL-Norvaline
Alternatively:	American Chromatography Supplies		
- glass vials:	VT009M-1232	ACS	1.8 mL Volume; Clear Glass, 12x32 mm, 9 mm thread
- caps:	C395E-09SB	ACS	Bonded PTFE/Silicone Septa
- caps:	C394-09SB	ACS	Bonded PTFE/Rubber Septa

C-13- and N-15-labeled metabolites (from Cambridge Isotope Laboratories if not otherwise stated)

- U13C Glucose:	CLM-1396-1	1 g

*Thanks to Marcus Seldin and Margarete Mehrabian