Fluxomics analysis by LC-MS and GC-MS

Analysis summary

The assay is intended to evaluate the flux of metabolites through various biochemical pathways by following the faith of stable-isotope labeled precursor compounds in a pulse-chase experiment.

Analyte extraction and sample preparation

Cell culture extraction (samples supplied on culture plates)

Extraction solvent – methanol : chloroform 1 : 1.

- Place the sample plates and extraction solvent on dry ice.
- Clean cell scraper with paper tissue soaked in methanol.
- One plate at a time, move each plate on regular ice, add 1.5 mL of extraction solvent, and scrape cells, then scrape cell suspension to the side of the tilted plate.
- Transfer cell suspension to a pre-labeled 2mL micro-centrifuge tube, place the tube on dry ice.
- Centrifuge for 10 min at 4°C, 15,000g.
- Transfer 600µL of supernatant to glass auto-sampler vials, store samples at -20^{o}C until LC-MS.
- Create pooled sample by combining 10µL aliquots of each individual extract.
- Transfer 100µL aliquot of extract to glass auto-sampler vial for GC-MS derivatization.
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Cell culture extraction (samples supplied as precipitated cells in micro-centrifuge tubes)

Extraction solvent - methanol : chloroform : water 8 : 1 : 1

- Add 300µL of extraction solvent to each cell sample, vortex to completely re-suspend the pellet.
- Sonicate at 40% output power, 20% duty cycle for 20 seconds, keep samples on ice throughout the procedure.
- Leave for 5 minutes at 4°C or on ice, vortex.
- Centrifuge for 5 min at 4°C, 14,000rpm.
- Transfer 100µL of supernatant to auto-sampler vial with glass insert for LC-MS analysis.
- Create pooled sample by combining 10µL aliquots of each individual extract.
- Transfer 100µL aliquot of extract to glass auto-sampler vial for GC-MS derivatization.

Sample derivatization for GC-MS analysis

- Dry sample extracts and standards in a vacuum centrifuge at 45^{o}C.
- While samples are drying, prepare a 20mg/mL solution of methoxyamine hydrochloride in pyridine in a glass vial; use glass syringe or pipette to dispense pyridine, vortex to dissolve.
- Add 50µL of the methoxyamine hydrochloride solution to dried samples, cap the vials and incubate at 37°C for 90min (preferably in a dry box).
- Uncap the vials, add 50µL of MTBSTFA + 1% TBDCMS to all vials using glass syringe or pipette, re-cap vials and incubate at 70°C (sand bath) for 60min; alternatively leave overnight at room temperature.
- Cool the vials to room temperature; if contents is cloudy, centrifuge for 2min; transfer contents to auto-sampler vials with glass inserts using a glass Pasteur pipette, cap the vials, promptly analyze on GC-MS.

LC-MS

- Chromatographic column - Luna® 3 µm NH2 100 Å, LC Column 150 x 1 mm, Ea (Phenomenex Inc.).
- LC gradient
    - Phase A: 5mM ammonium acetate in water, pH 9.9 (adjusted using LC-MS grade ammonium hydroxide).
    - Phase B: 100% acetonitrile.
    - timetable – listed in table 1 below.
- Auto-sampler temperature 4°C.
- Injection volume 10 µL (may vary between experiments).
- Mass-spectrometer parameters
    - Instrument - Agilent 6520 Q-TOF
    - Mode – ESI negative.

The specific LC-MS method details are provided in supplementary material (QTOF-002-HILIC-35min-1mm_LC_PARAMS.xml and QTOF-002-HILIC-35min-1mm_MS_PARAMS.xml files).

Table 1. LC gradient timetable

Time, min	%B	Flow, ml/min
0	80	0.075
15	0	0.075
20	0	0.075
20.1	80	0.075
25	80	0.075
30	80	0.09
34.9	80	0.09
34.99	80	0.075

GC-MS

Samples are analyzed on DB-5MS, 250µm ID x 30m column from Agilent or equivalent. The specific GC-MS method details are provided in supplementary material (Evans_70-300C_20Cpmin_scan.txt file).

References