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LC-MS3 Proteomics Data Acquisition

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Ruiqi Jian[1], Joanne Chan[1], lihua jiang[1]

[1]Stanford University

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Ruiqi Jian
Stanford University

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Abstract

This protocol described the LC, Mass spectrometry and database search methods used to acquire quantitative proteomic data. For peptides separation, we used a high-low pH 2D LC methods set up for 12 fractions. A MS3 mass spectrometry method was used for TMT10plex data acquision. Detailed paramters for database search was described as well.

Materials

REAGENTS

- Water LC-MS grade.
- Acetic Acid LC/MS grade.
- Amonia Formate LC/MS grade.
- Acetonitrile LC/MS grade.

EQUIPMENTS AND SUPPLIES

- Thermo Orbitrap Fusion Lumos Tribrid Mass Spectrometer
- Waters ACQUITY UPLC M-Class 2D System
- Waters Xbridge C18 column (300µm x 5 cm, 5.0µm)
- Waters Acquity UPLC M-class Trap 2D Symmetry C18 column (5µm, 180µmx20mm)
- New Objective Self-Pack PicoFrit Columns, no coating, 15um tip (360µm OD/100µm ID)
- Sepax GP-C18 resin (1.8um, 120A)
- Softwares: Thermo Proteome Discoverer 2.1

Troubleshooting

1 Proteomic samples were analyzed on a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer coupled online with a Waters 2D liquid chromatography (Waters MClass 2DnLC).

Liquid Chromatography Sepration

2 Peptides were separated by reverse phase chromatography at high pH in the first dimension, followed by an orthogonal separation at low pH in the second dimension.

3 In the first dimension, the mobile phases were buffer A: 20mM ammonium formate in water at pH10 and buffer B: 100% acetonitrile. Peptides were separated on a Waters Xbridge C18 column (300µm x 5 cm, 5.0µm) using 12 discontinuous step gradient of buffer B: 10.8%, 13.1%, 14.9%, 16.7%, 17.7%, 18.9%, 19.9%, 20.4%, 22.2%, 25.8%, 28.9%, 45% at 2 µl/min flow rate.

4 In the second dimension, the mobile phases were buffer A: 0.1% formic acid in water and buffer B: 0.1% formic acid in acetonitrile. An Waters Acquity UPLC M-class Trap 2D Symmetry C18 column (5µm, 180µmx20mm) and an in-house packed analytical column (360µm OD/100µm ID/15µm tip ID x 28cm length), packed with Sepex GP-C18 resin (1.8um, 120A) were used. Peptides were first trap on trap column , then seperated by analytical column with a gradient from 5% to 40% buffer B at a flow rate of 600 nl/min in 180 minutes.

MS3 Data Accquisition

5 Analytical column was heated at 45 °C . Eluted peptides were ionized in positive mode via flex ion source. The source was operated at 1.8-2.2 kV with the ion transfer tube heated at 275 °C .

6 The mass spectrometer was run in a data dependent mode. Full MS scan was acquired in the Orbitrap mass analyzer from 400-1500 m/z with resolution of 120,000.Precursors were isolated with an isolation window of 0.7 m/z and fragmented using CID at 35% energy in ion trap in rapid mode. AGC target was 1e4 and the maximum injection time was 100ms.

7 The top 8 fragment ions were selected for MS3 analysis, isolated with an m/z window of 1.6, and fragmented with HCD at 65% energy. Resulting fragments were detected in the Orbitrap at 60,000 resolution, with a maximum injection time of 150ms or until the AGC target value of 1e5 was reached.

Data Processing and Analysis

8 The acquired raw data were processed with the Proteome Discoverer 2.1 (Thermo).

9 Mass tolerance of 10ppm was used for precursor ion and 0.6 Dalton for fragment ions for the database search. The search included cysteine carbamidomethylation as a static modification. Acetylation at protein N-terminus, methionine oxidation and TMT at peptide N-terminus and Lysine were used as dynamic modifications. Up to two missed cleavages were allowed for trypsin digestion. Only unique peptides with minimum 6 amino acid length were considered for protein identification. The peptide false discovery rate (FDR) was set as less than 1%. Spectra with more than 50% interference were excluded for quantitative analysis.