Discovery proteomic (DDA) LC-MS/MS data acquisition and analysis

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ABSTRACT


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Materials

**Materials**

- **Acetonitrile LCMS quality JT**
  - Baker Catalog #9829-02
- **LCMS grade water VWR**
  - International Catalog #BJLC365-2.5
- **Isopropanol VWR**
  - International Catalog #BJ650447-4L

**Step Materials**

- **2.1 mm ID**

Analytical column: Agilent AdvanceBio Peptide Map column (2.1 mm ID, 250 mm length, 2.7 µm particle size, 120-Å pore size) (Agilent, Cat.#651750-902)

Guard column: Ascentis guard column (2.1 mm ID, 50 mm length, 2.7 µm particle size, 160-Å pore size)(Sigma-Aldrich, Cat.#53536-U)

LC-MS system: Agilent 6550 QTOF mass spectrometer system coupled with an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, CA)

Safety Warnings

Wear proper PPE (gloves, safety goggle, and lab coat), and prepare solvents in a chemical fume hood. Store organic solvents in a flammable storage cabinet when not in use.

Before Starting

Prepare the following solvents:

- Needle wash solvents: Add 100 mL isopropanol into 900 mL water.
- Solvent A: Add 0.1 % volume formic acid into LC-MS grade water.
- Solvent B: Add 0.1 % volume formic acid into LC-MS grade acetonitrile.

Proteomics: HPLC and Mass Spectrometry

1. Thaw peptide samples on ice, and transfer 30 µl of each sample to LC autosampler vials (Agilent, Cat.#5182-0567,#5182-0564) or 96-well plate (Bio-Rad, Cat.#HSP9655).

2. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis is performed with an Agilent 6550 QTOF mass spectrometer system coupled with an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, CA).

6550 iFunnel Q-TOF Quadruple-time-of-flight (TOF) tandem Mass Spectrometer

Agilent Technologies 6550 QTOF

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Samples were loaded into a temperature controlled autosampler operating at \(4 \, ^\circ \text{C}\). The separation on the UHPLC is achieved by using an Agilent AdvanceBio Peptide Map column (\(2.1 \, \text{mm ID}, 250 \, \text{mm length}, 2.7 \, \mu\text{m particle size}, 120-\AA\) pore size (Agilent, Cat.#651750-902)) coupled to a guard column (\(2.1 \, \text{mm ID}, 50 \, \text{mm length}, 2.7 \, \mu\text{m particle size}, 160-\AA\) pore size (Sigma-Aldrich, Cat.#53536-U)). The column is operated at \(60 \, ^\circ \text{C}\).

Twenty micrograms \(20 \, \mu\text{g}\) of peptides are loaded onto the column from each sample and separated using a gradient separation with 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) operating at a flow rate of 0.4 ml/min. A 30 minutes linear elution gradient of chromatographic separation is as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>% A</th>
<th>% B</th>
<th>Time (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>35</td>
<td>31.00</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>80</td>
<td>33.00</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>80</td>
<td>39.00</td>
</tr>
<tr>
<td>6</td>
<td>98</td>
<td>2</td>
<td>41.00</td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>2</td>
<td>45.00</td>
</tr>
</tbody>
</table>

Chromatographic gradient table
The gradient length depends on the application of interest and the depth of proteome coverage a study is pursuing.

The eluted peptides were ionized via an Agilent Jet Stream ESI source operating in positive ion mode with the following source parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Drying gas flow</td>
<td>14 liters/min</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>35 psi</td>
</tr>
<tr>
<td>Sheath gas temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Sheath gas flow</td>
<td>11 liters/min</td>
</tr>
<tr>
<td>Capillary voltage</td>
<td>3500 V</td>
</tr>
<tr>
<td>Fragmentor voltage</td>
<td>180 V</td>
</tr>
<tr>
<td>OCT 1 RF Vpp</td>
<td>750 V</td>
</tr>
</tbody>
</table>

The mass spectrometer is operated in data dependent acquisition (DDA) auto MS/MS mode with the following parameters:
The MS raw data were acquired using Agilent MassHunter version B.06.01.

The acquired data were explored in Agilent Qualitative Analysis software version B.06.01. MS data were converted to .mgf files using inbuilt MGF file export and searched against the protein database with Mascot search engine version 2.3.02 (Matrix Science).

The latest protein database of interest was downloaded from the Universal Protein Resource database (https://www.uniprot.org/). Heterologous proteins of interest and common contaminant protein fasta sequences were subsequently added to the downloaded protein database, which then was used in the Mascot search.
The following Mascot search parameters are applied:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Trypsin</td>
</tr>
<tr>
<td>Maximum missed cleavages</td>
<td>1</td>
</tr>
<tr>
<td>Precursor ion tolerance</td>
<td>50 ppm</td>
</tr>
<tr>
<td>Fragment ion tolerance</td>
<td>0.1 Da</td>
</tr>
<tr>
<td>Fixed modifications</td>
<td>Carbamidomethyl (Cys)</td>
</tr>
<tr>
<td>Variable modifications</td>
<td>Deamination (Asn, Gln); Oxidation (Met)</td>
</tr>
</tbody>
</table>

Mascot search parameters

Mascot search results are refined by using Scaffold. Identified peptides are filtered by a 1% peptide-level false discovery rate. In addition, the false discovery rate at the protein level is calculated, and only the proteins with false discovery rate ≤1% are reported.

Scaffold 4.11.0 by Proteome Software, Inc

QTOF QC and performance monitoring

The QTOF mass spectrometer is subjected to TOF mass calibration (Check Tune) prior to analyzing samples to verify mass accuracy, intensity, and resolution of ions in 10 times diluted ESI low concentration tune mix purchased from Agilent.

Agilent Tune Mix: G1969-85000 Contributed by Agilent

A weekly TOF Quick Tune is performed to optimize ion transmission.

The mass spectrometer is subjected to a Standard Tune at least quarterly (and more frequently, if transmission tune fails, or performance issues arise).

UHPLC-QTOF system performance is monitored at the beginning, middle, and end of large sample sets by running full LC-MS/MS data collection of BSA tryptic digest standard (100 fmol). The HPLC retention times, mass accuracy, ion intensity, and resolution of BSA peptides are tracked via the PanoramaWeb server through an established AutoQC pipeline.
AutoQC loader by University of Washington


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