Discovery proteomic (DDA) LC-MS/MS data acquisition and analysis V.2

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Version 2

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1 *Works for me*

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LBNL-omics

Yan Chen

ABSTRACT

This protocol details steps in discovery proteomic data-dependent acquisition with a standard-flow UHPLC-Obitrap system and a subsequent Mascot database search. It was adapted from González Fernández-Niño, S. M., et al. "Standard flow liquid chromatography for shotgun proteomics in bioenergy research."*Frontiers in bioengineering and biotechnology,* 3 (2015): 44.

EXTERNAL LINK

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WHAT'S NEW

Updated to include Orbitrap Exploris 480 instrumentation.

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49737

MATERIALS TEXT

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

Analytical column: InfinityLab Poroshell 120 EC-C18 (**2.1 mm ID** , **100 mm length** , **1.9 µm particle size** , 120-Å pore size) (Agilent, Cat.#695675-902)

Guard column: InfinityLab Poroshell 120 EC-C18 guard column (**2.1 mm ID** , **5 mm length** , **1.9 µm particle size** , 160-Å pore size)(Agilent, Cat.#821725-940)

LC-MS system: Thermo Obitrap Exploris 480 (Thermo Fisher Scientific) 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 Spectromtery

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 a Thermo Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific, San Jose, CA) coupled with an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, CA).

Obitrap Exploris 480
Mass spectrometer
Thermo Fisher BRE725532

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1290 Infinity UHPLC
Ultra-high performance liquid chromatography system

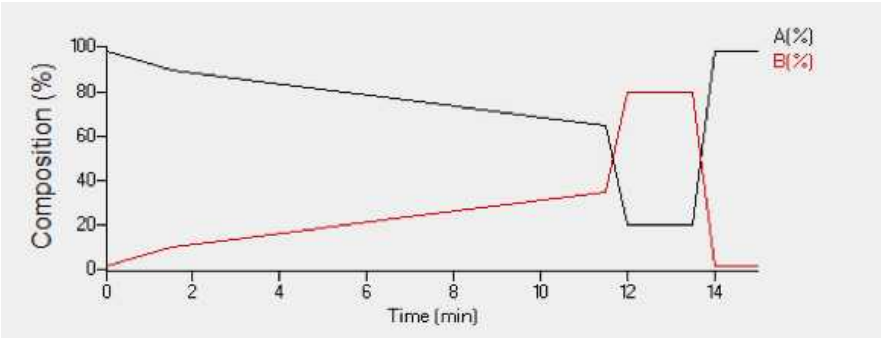
Agilent Technologies	1290 Infinity UHPLC

3 Samples were loaded into a temperature controlled autosampler operating at **4 °C** . The separation on the UHPLC is achieved by using an Agilent InfinityLab Poroshell 120 EC-C18 (**2.1 mm ID** , **100 mm length** , **1.9 µm particle size** , 120-Å pore size) (Agilent, Cat.#695675-902) coupled with an Agilent InfinityLab Poroshell 120 EC-C18 guard column (**2.1 mm ID** , **5 mm length** , **1.9 µm particle size** , 160-Å pore size)(Agilent, Cat.#821725-940). The column is operated at **60 °C** .

4 45m
Twenty micrograms **20 µg** 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 15 minute total acquisition time with a 10 minute linear elution gradient of chromatographic separation is as follows:

A	B	C	D
Step	%A	%B	Time (minute)
1	98	2	0.0
2	98	10	1.5
3	65	35	11.5
4	20	80	12.0
5	20	80	13.5
6	98	2	14.0
7	98	2	15.0

Table 1. Chromatographic gradient table



Chromatographic gradient diagram

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

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5 The eluted peptides were ionized via OptaMax^{TM} NG Electrospray Ion Source operating in **positive ion mode** with the following source parameters:

A	B
Vaporizer temp	250 °C
Ion transfer tube temp	300 °C
Positive ion voltage	3500 V
Shealth gas	50
Aux gas	20

Table 2. Source conditions

6 The mass spectrometer is operated in data dependent mode with cycles of a survey scan followed by 10 ddMS2 events. The survey scan and ddMS2 scan parameters are as follows:

A	B
Survey scan obitrap resolution	60K
Survey scan MS range	300 to 1,200 m/z
Survey scan AGC target	300%
Survey scan maximum ion injection time	60 ms
Ion intensity threshold triggering MS2 analysis	5.0e3
Isolation window	1.6 m/z
ddMS2 scan obitrap resolution	15K
ddMS2 scan AGC target	100%
ddMS2 scan maximum ion injection time	50s

DDA parameters

7 The MS raw data were acquired using Thermo Scientific Xcalibur version 4.3.73

Thermo Fisher Scientific 4.3.73

by Thermo Fisher Scientific

8 The acquired raw data were converted to .mgf files using RawConverter tool and searched against the protein database with Mascot search engine version 2.3.02 (Matrix Science).

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RawConverter 1.2.01
source by Yates Lab

Mascot Server 2.3.02
by 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.

9 The following Mascot search parameters are applied:

A	B
Enzyme	Trypsin
Maximum missed cleavages	1
Precursor ion tolerance	20 ppm
Fragment ion tolerance	0.1 Da
Fixed modifications	Carbamidomethyl (Cys)
Variable modifications	Deamination (Asn, Gln); Oxidation (Met)

Mascot search parameters

10 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 5.0.0
by Proteome software

LCMS QC and performance monitoring

11 The Exploris mass spectrometer is subjected to mass calibration check prior to analyzing samples to verify mass accuracy, intensity, and resolution of ions using Pierce™ FlexMix™ Calibration Solution purchased from Thermo Fisher Scientific.

Pierce™ FlexMix™ Calibration Solution **Thermo Fisher Catalog #A39239**

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12 A weekly mass calibration is performed to maintain <3 ppm mass accuracy without correction from internal calibrant.

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

14 UHPLC-Obitrap system performance is monitored at the beginning, middle, and end of large sample sets by running full LC-MS/MS data collection of 20 ug *E.coli* cell lysate protein tryptic digest. The protein identification, mass accuracy, peak shape, and resolution of peptides are evaluated.

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