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

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2 Works for me dx.doi.org/10.17504/protocols.io.bgbqjsmw

LBNL-omics

Yan Chen

ABSTRACT

This protocol details steps in discovery proteomic data-dependent acquisition with a standard-flow UHPLC-QTOF 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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36944

MATERIALS

NAME	CATALOG #	VENDOR
Acetonitrile LCMS quality	9829-02	JT Baker
LCMS grade water	BJLC365-2.5	VWR International
Isopropanol	BJ650447-4L	VWR International

STEPS MATERIALS

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NAME	CATALOG #	VENDOR
Agilent Tune Mix: G1969-85000	G1969-85000	

MATERIALS TEXT

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)

EQUIPMENT

NAME	CATALOG #	VENDOR
6550 iFunnel Q-TOF	6550 QTOF	Agilent Technologies
1290 Infinity UHPLC	1290 Infinity UHPLC	Agilent Technologies

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 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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1290 Infinity UHPLC
Ultra-high performance liquid chromatography system

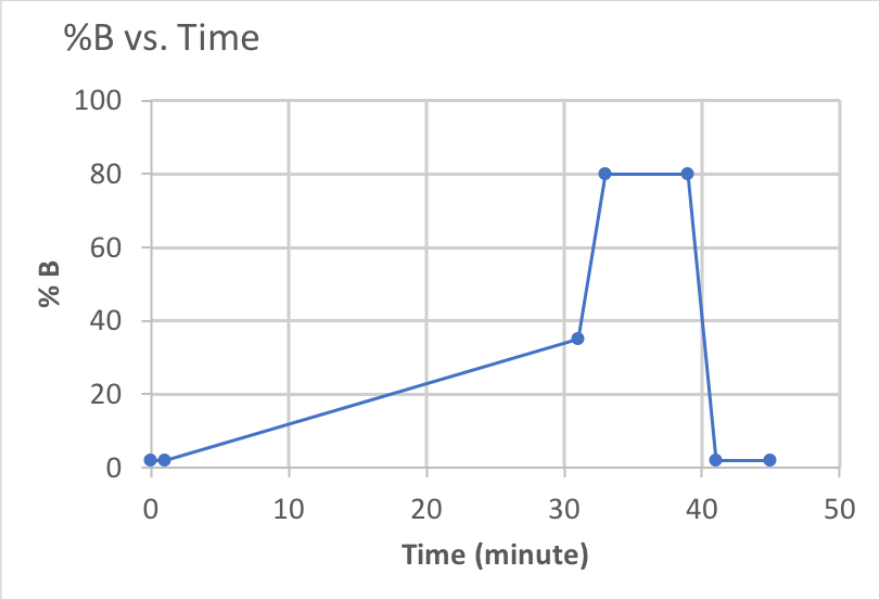
Agilent Technologies 1290 Infinity UHPLC

3 Samples were loaded into a temperature controlled autosampler operating at **4 °C** . The separation on the UHPLC is achieved by using an Agilent AdvanceBio Peptide Map column (**2.1 mm ID** , **250 mm length** , **2.7 µm particle size** , 120-Å pore size (Agilent, Cat.#651750-902)) coupled to a guard column (**2.1 mm ID** , **50 mm length** , **2.7 µm particle size** , and 160-Å pore size (Sigma-Aldrich, Cat.#53536-U)). 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 separaration 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:

Step	%A	%B	Time (minute)
1	98	2	0.00
2	98	2	1.00
3	65	35	31.00
4	20	80	33.00
5	20	80	39.00
6	98	2	41.00
7	98	2	45.00

Chromatographic gradient table



Chromatographic gradient graph

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

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

Gas temperature	250 °C
Drying gas flow	14 liters/min
Nebulizer pressure	35 psi
Sheath gas temparature	250 °C
Sheath gas flow	11 liters/min
Capillary voltage	3500 V
Fragmentor voltage	180 V
OCT 1 RF Vpp	750 V

6 The mass spectrometer is operated in data dependent acquisition (DDA) auto MS/MS mode with the following parameters:

Charge states	2 to 5
Precursor scan MS range	300 to 1,400 m/z
Maximum precursor ions allowed for MS2 event	20
Fragment ion scan MS range	100 to 1,700 m/z
Ion intensity threshold triggering MS2 analysis	1500 counts
Quadrupole resolution	Medium
Precursor ion accumulation	45,000 counts
Maximum MS2 accumulation time	333 ms
Precursor ion exclusion window	0.3 minutes

DDA parameters

7 The MS raw data were acquired using Agilent MassHunter version B.06.01

MassHunter B.06.01
by Agilent Technologies, Inc

8 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).

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Qualitative Analysis B.06.01

by Agilent Technologies, Inc

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:

Enzyme	Trypsin
Maximum missed cleavages	1
Precursor ion tolerance	50 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 4.11.0

by Proteome Software, Inc

QTOF QC and performance monitoring

11 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.

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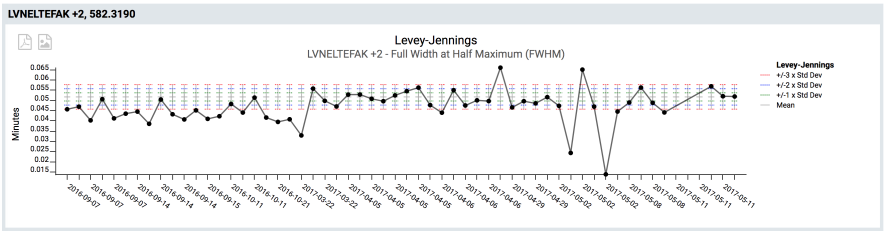
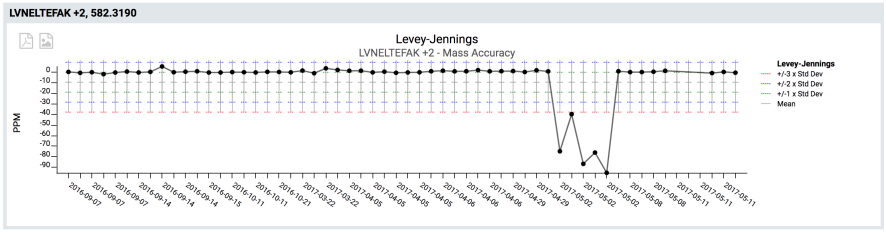
Agilent Tune Mix: G1969-85000
Catalog #: G1969-85000

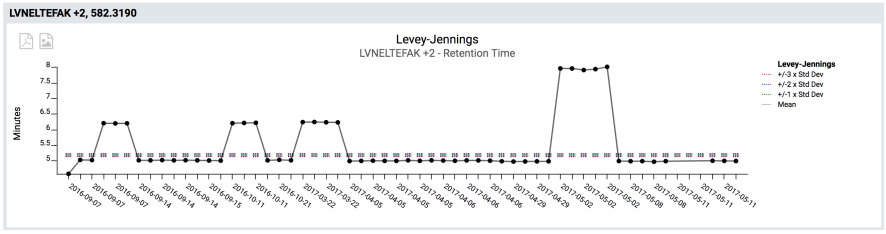
12 A weekly TOF Quick Tune is performed to optimize ion transmission.

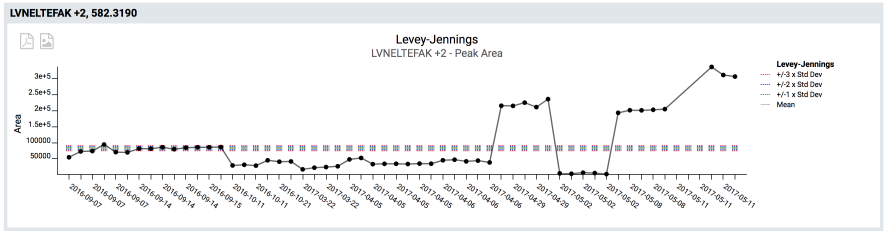
13 The mass spectrometer is subjected to a Standard Tune at least quarterly (and more frequently, if transmission tune fails, or performance issues arise).

14 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.









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AutoQC loader
by University of Washington

Bereman MS, Beri J, Sharma V, Nathe C, Eckels J, MacLean B, MacCoss MJ (2016). An Automated Pipeline to Monitor System Performance in Liquid Chromatography-Tandem Mass Spectrometry Proteomic Experiments.. Journal of proteome research.

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