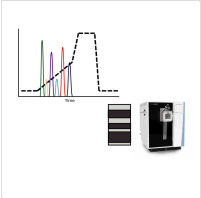

Version 2

Sep 28, 2022

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

Version 1 is forked from Discovery proteomic (DDA) LC-MS/MS data acquisition and analysis

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DISCLAIMER

This protocol is for research purposes only.

ABSTRACT

This protocol details steps in discovery proteomic data-independent acquisition with a standard-flow UHPLC-Obitrap system and a subsequent DIA-NN library-free database search. The data acquisition method 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.

DOI

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FORK NOTE

FORK FROM

Forked from Discovery proteomic (DDA) LC-MS/MS data acquisition and analysis, Yan Chen

KEYWORDS

Proteomics, Liquid chromatography, Mass spectrometry, DDA, Orbitrap

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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.

DISCLAIMER:

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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**

1290 Infinity UHPLC
Ultra-high performance liquid chromatography system
Agilent Technologies **1290 Infinity UHPLC**

Citation: Yan Chen, Jennifer Gin, Christopher J Petzold Discovery proteomic (DIA) LC-MS/MS data acquisition and analysis https://dx.doi.org/10.17504/protocols.io.e6nvwk1z7vmk/v2

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	90	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

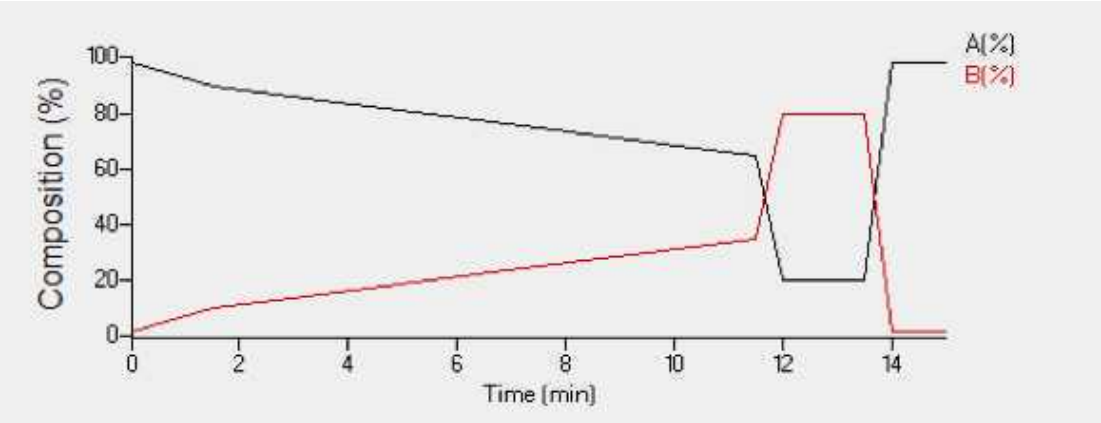

Figure 1. Chromatographic gradient diagram

The gradient length depends on the application of interest and the depth of proteome

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coverage a study is pursuing.

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 independent mode with a duty cycle of 3 survey scans and 45 MS2 scans . The survey scan and MS2 scan parameters are as follows:

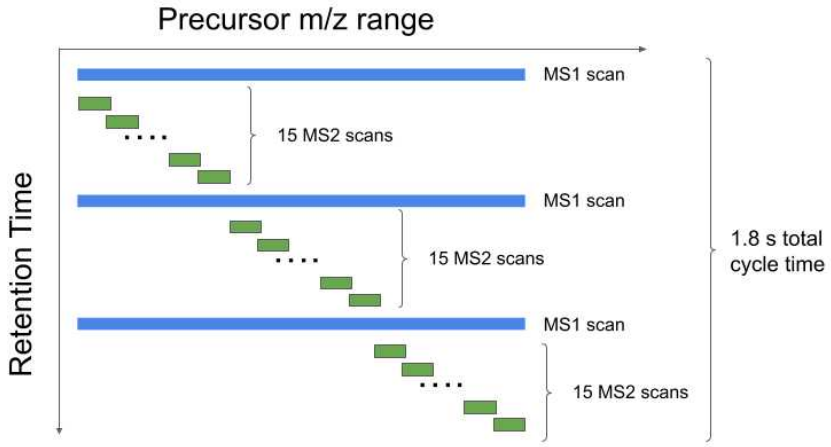

Figure 2. Sequencing scheme of the DIA acquisition method

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A	B
Survey scan obitrap resolution	60K
Survey scan MS range	380 to 985 m/z
Survey scan AGC target	300%
Survey scan maximum ion injection time	45 ms
DIA precusor isolation window	13.5 m/z
MS2 scan range	145 to 1450 m/z
MS2 scan obitrap resolution	15K
MS2 scan AGC target	1000%
MS2 scan maximum ion injection time	22 ms

Table 3. DIA survey scan and MS2 scan 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 DIA raw data files were analyzed by an integrated software suite DIA-NN v 1.8.1.

Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M (2020). DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput.. Nature methods. https://doi.org/10.1038/s41592-019-0638-x

9 DIA-NN configurations for Library-free search and peptide quantification:

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A	B
Enzyme	Trypsin
Maximum missed cleavages	1
Precusor and Fragment MS accuricies	Automatically determined
Precusor length range	7-30
Precusor charge range	2-4
Fixed modifications	Carbamidomethyl (Cys)
Variable modifications	Deamination (Asn, Gln); Oxidation (Met)
Precusor and protein identificaiton FDR	1%
Quantification strategy	Robust LC
Spectral library	Generated from fasta files of latest proteomes at Uniprot

Main configurations for DIA-NN search in library-free mode

Note: DIA-NN could also utilize experimentally generated spectral libraries to analyze LCMS raw data, such as DDA based spectral library, gas-phased fractionation (GPF)-DIA based spectral library, etc.

10 Protein quantity reported by DIA-NN was further processed and visualized using an jupyter notebook described in detail through an established protocol.

Label-free quantification (LFQ) proteomic data analysis from DIA-NN output files
by Christopher J Petzold,
Lawrence Berkeley National Laboratory

PREVIEW RUN

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**

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

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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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