

Sep 28, 2022

Version 2

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](#)

 [Journal of Experimental Botany](#)

DOI

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LBNL omics

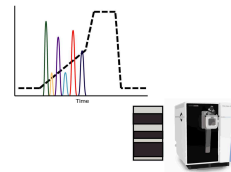
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Protocol status: Working

We use this protocol and it's working

Created: September 19, 2022

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Keywords: Proteomics, Liquid chromatography, Mass spectrometry, DDA, Orbitrap, standard flow liquid chromatography for shotgun proteomic, discovery proteomic data, proteomic data, shotgun proteomic, ms data acquisition, standard flow liquid chromatography, data acquisition method, liquid chromatography, bioenergy research, biotechnology

Disclaimer

This protocol is for research purposes only.

Abstract

This protocol details steps in discovery proteomic data-independent acquisition with a standard-flow UHPLC-Orbitrap 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.

Materials

MATERIALS

⊗ Acetonitrile LCMS quality **JT Baker Catalog #9829-02**

⊗ LCMS grade water **VWR International (Avantor) Catalog #BJLC365-2.5**

⊗ Isopropanol **VWR International (Avantor) 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 Orbitrap Exploris 480 (Thermo Fisher Scientific) coupled with an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, CA)

Protocol materials

⊗ Acetonitrile LCMS quality **JT Baker Catalog #9829-02**

⊗ LCMS grade water **VWR International (Avantor) Catalog #BJLC365-2.5**

⊗ Isopropanol **VWR International (Avantor) Catalog #BJ650447-4L**

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

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 start

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 μ L 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).

Equipment

Obitrap Exploris 480

NAME

Mass spectrometer

TYPE

Thermo Fisher

BRAND

BRE725532

SKU

<https://www.thermofisher.com/order/catalog/product/BRE725532#/BRE725532>^{LINK}

Equipment

1290 Infinity UHPLC

NAME

Ultra-high performance liquid chromatography system

TYPE

Agilent Technologies

BRAND

1290 Infinity UHPLC

SKU

<https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems>

LINK

- 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 ($\pm$ 2.1 mm ID , $\pm$ 100 mm length , $\pm$ 1.9 μm particle size , 120-Å pore size) (Agilent, Cat.#695675-902) coupled with an Agilent InfinityLab Poroshell 120 EC-C18 guard column ($\pm$ 2.1 mm ID , $\pm$ 5 mm length , $\pm$ 1.9 μm particle size , 160-Å pore size) (Agilent, Cat.#821725-940). The column is operated at 60°C .

- 4 Twenty micrograms $20\text{ }\mu\text{g}$ 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 15 minute total acquisition time with a 10 minute linear elution gradient of chromatographic separation is as follows:

45m

	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

	A	B	C	D
	5	20	80	13.5
	6	98	2	14.0
	7	98	2	15.0

Table 1. Chromatographic gradient table

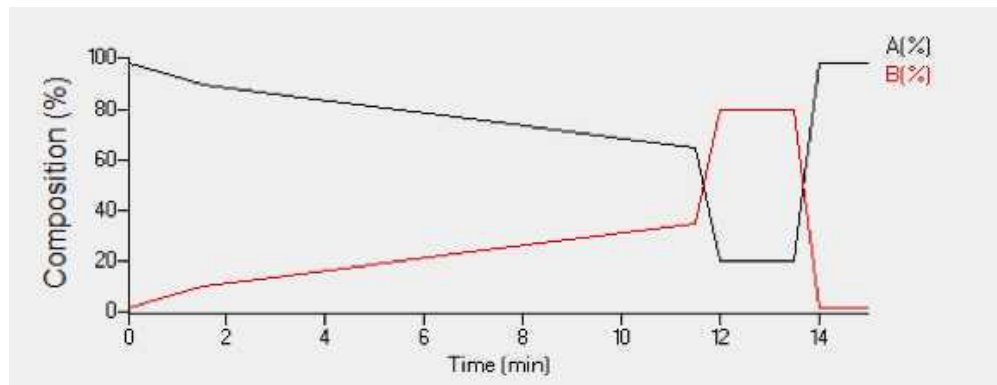


Figure 1. Chromatographic gradient diagram

Note

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 OptaMaxTM 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
Sheath gas	50

A	B
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:

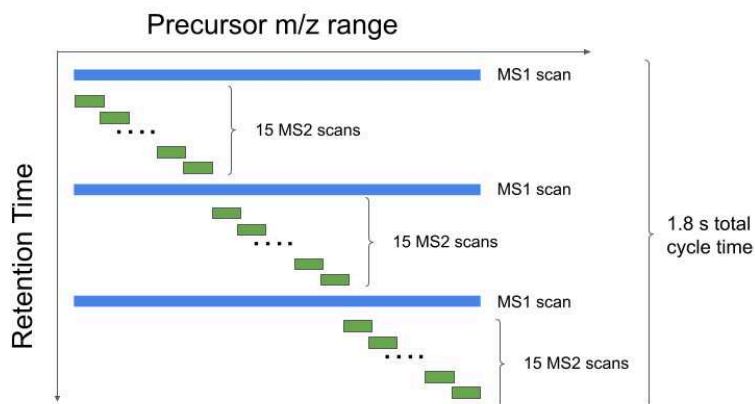


Figure 2. Sequencing scheme of the DIA acquisition method

A	B
Survey scan orbitrap 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 precursor isolation window	13.5 m/z
MS2 scan range	145 to 1450 m/z
MS2 scan orbitrap resolution	15K
MS2 scan AGC target	1000%

A	B
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

Software

Thermo Fisher Scientific

NAME

Thermo Fisher Scientific

DEVELOPER

<https://www.thermofisher.com/order/catalog/product/OPTON-30965#/OPTON-30965>

SOURCE
LINK

- 8 The acquired DIA raw data files were analyzed by an integrated software suite DIA-NN v 1.8.1.

Citation

Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M (2019)
. 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>

LINK

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

A	B
Enzyme	Trypsin
Maximum missed cleavages	1
Precursor and Fragment MS accuracies	Automatically determined
Precursor length range	7-30
Precursor charge range	2-4
Fixed modifications	Carbamidomethyl (Cys)
Variable modifications	Deamination (Asn, Gln); Oxidation (Met)
Precursor and protein identification 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

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.

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

Protocol



NAME

Label-free quantification (LFQ) proteomic data analysis from DIA-NN output files

CREATED BY

Christopher J Petzold

Preview

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.
- 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-Orbitrap 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.

Citations

Step 8

Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput.

<https://doi.org/10.1038/s41592-019-0638-x>