

Column Chromatography

Name of Person/People Completing template

Maurizio Ronci

1. General features

1.(a) Global Descriptors

Date stamp: 2009-07-31

Responsible person: Maurizio Ronci

1. (b) Sample

Brief description of sample: Salmonella typhimurium

Processing applied to the sample: Salmonella t. pellets were dissolved in 100 mM Tris pH 7.5 containing 6 M Urea. After reduction and alkylation of proteins, samples were digested with 50:1 (w/w) sequence grade trypsin at 37° C overnight. Samples were diluted with 0.1% formic acid to a final concentration of 0.375 ug/ul in presence of Enolase *Saccharomyces cerevisiae* digestion (50fmol/ul).

Sample injection: sample temperature 15 °C; injection volume = 2µl, full loop

2. Equipment

2.(a) Product details for Column

Manufacturer: Waters Corp.

Model: nanoACQUITY UPLC BEH300 C18, 75µm X 250 mm, 1.7µm

Separation Mode: Reverse Phase

2. (b) Physical Characteristics of Column

Dimensions: length 250 mm; inner diameter 75µm

Description of stationary phase: C18, particle size 1.7µm

Additional accessories: nanoACQUITY UPLC Trap Symmetry C18, 180µm X 20 mm, 5µm

2. (c) Chromatography System Used for the Separation

Manufacturer: Waters Corp.

Model: NanoAcquity UPLC

Controller software and version: MassLynx v.4.1

3. Mobile Phase

Name of Mobile phase: A; Description of the constituents: 99.9% water/0.1% formic acid

Name of Mobile phase: B; Description of the constituents: 99.9% acetonitrile/0.1% formic acid

4. Properties of the Column Run

Separation run

Time:151 min

Flow: 0.25 µl/min

Time(min): 0	% A:97	% B:3	Curve = -
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Time(min): 1.00	% A:97	% B:3	Curve = 6
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Time(min): 120.00	% A:60	% B:40	Curve = 6
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Time(min): 135.00	% A:10	% B:90	Curve = 6
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Time(min): 150.00	% A:10	% B:90	Curve = 6
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Time(min): 151.00	% A:97	% B:3	Curve = 6
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Temperature: 35 °C

5. Pre and Post Run Processes

Type: Trapping, pre run process

Substance: 100% A

Time: 5.00 min

Flow rate: 10 µL/min

Type: Equilibration, post run process

Substance: 97% A, 3%B

Time: 29.0 min
Flow rate: 0.25 μ L/min

6. Column Outputs

6.(a) Detection

Equipment used for detection: Waters Corp., Q-Tof Premiere
Type: mass spectrometer
Equipment settings: Expression analysis
Timescale over which data was collected: 10-150 min

6. (b) Fractions

Fraction description: Fractions not collected, eluent directed to MS

MIAPE: Mass Spectrometry

1. General features

1.(a) Global descriptors

Date stamp: 2009-07-31
Responsible person: Maurizio Ronci
Instrument manufacturer, model: Waters Corporation, Q-Tof Premiere

1. (b) Control and analysis software

Software name and version: MassLynx v4.1 and ProteinLynx Global Server, v2.3
Switching criteria: Expression (Electrospray-shotgun) (see Ref. 1)
Acquisition times: start 10 minutes, end 150 minutes
Acquisition ionization mode: Source: ESI; Polarity: positive; Analyzer mode: V mode
TOF MS: MS survey over the range: Start 50 Da, End 1990 Da; MS survey scanning conditions: scan time 1.5 seconds
Expression criteria: Ramp high energy from 15 to 40 volts; Low energy 4 volts
Lock mass: Reference scan: Scan time 1.5 seconds, frequency: 30 seconds; Sampling cone 35 volts; Collision Energy 4 volts.

2. Ion sources (a) Electrospray ionization (ESI)

Supply type: Fed nanoelectrospray: NanoLockSpray source equipped with a reference spray (Lock mass)
Interface manufacturer: nanoACQUITY UPLC, Waters Corporation
Sprayer type: PicoTip EMITTER SilicaTip, 10 \pm 1 μ m, New Objective, Inc.
Relevant voltage: capillary 2.8 kV, sampling cone 35, extraction cone 2.5, ion guide 2.5
Nebulizing gas: cone 26 l/hr, nanoflow 0.40 bar

3. Post-source componentry (b) TOF drift tube

Reflectron status: ON (ion optics in V-mode)

3. Post-source componentry (d) Collision energy

Collision energy: Expression criteria (Electrospray-shotgun): continuous gradient of collision energies from 15 to 40 V. The radiofrequency applied to the quadrupole mass analyzer was adjusted such that ions from m/z 300 to 2000 were efficiently transmitted, ensuring that any ions less than m/z 300 observed in the LC-MS data only arose from dissociations in the collision cell.

3. Post-source componentry (f) Detectors

Detector type: microchannel plate/time to digital converter (MCP/TDC)

4. Spectrum and peak list generation and annotation

4. (a) Spectrum description

Source files: 6926_220709_1a2_01, 6926_220709_1a2_02, 6926_220709_1a2_03, 6926_Zn_220709_1a2_01, 6926_Zn_220709_1a2_02, 6926_Zn_220709_1a2_03, 123_22072009_01, 123_22072009_02, 123_22072009_03, 123_Zn_22072009_01, 123_Zn_22072009_02, 123_Zn_22072009_03

MS level for each spectrum: MS and MS^E

Ion mode for each spectrum: positive

Precursor m/z and charge: see

SupplFile3_PeptideTable_Ciavardelli_01.pdf

SupplFile4_PeptideTable_Ciavardelli_02.pdf

SupplFile5_PeptideTable_Ciavardelli_03.pdf

SupplFile6_PeptideTable_Ciavardelli_04.pdf

SupplFile7_PeptideTable_Ciavardelli_05.pdf

SupplFile8_PeptideTable_Ciavardelli_06.pdf

SupplFile9_PeptideTable_Ciavardelli_07.pdf

SupplFile10_PeptideTable_Ciavardelli_08.pdf

SupplFile11_PeptideTable_Ciavardelli_09.pdf

SupplFile12_PeptideTable_Ciavardelli_10.pdf

SupplFile13_PeptideTable_Ciavardelli_11.pdf

SupplFile14_PeptideTable_Ciavardelli_12.pdf

4. (b) Peak list generation

This task was performed with the specific algorithm of the ProteinLynx Global Server (PLGS) v2.3 developed for Electrospray-Shotgun mass acquisition: peak apexes are located, the data calibrated and lock-mass corrected (using Apex3D), and then deconvoluted (using Peptide3D) (see Ref.2, 3).

Processing parameters applied:

Chromatographic peak width: Automatic

MS TOF resolution: Automatic

Lock mass for charge 2: 785.8426 Da/e

Lock mass Window (Tolerance): 0.25 Da

Low energy Threshold: 250 counts

Elevated energy Threshold: 100 counts

Retention time window: automatic

Intensity threshold value: 1500counts

Mass Spectrometry Informatics

1. General features (a) Global decriptors

Date stamp: 2009-07-31

Responsible person: Maurizio Ronci

Software name, version and manufacturer: ProteinLynx Global Server, v2.3, Waters Corporation

Location of the file generated: contact Maurizio Ronci, project:

Proj__12482609942810_12440940968040748

2. Input data and parameters

2.(a) input data

Description and type of MS data:

MA6926 LC-MS run1 = 6926_220709_1a2_01,

MA6926 LC-MS run2 = 6926_220709_1a2_02,

MA6926 LC-MS run3 = 6926_220709_1a2_03,

MA 6926 + Zn(II) LC-MS run1 = 6926_Zn_220709_1a2_01,
MA 6926 + Zn(II) LC-MS run2 = 6926_Zn_220709_1a2_02,
MA 6926 + Zn(II) LC-MS run3 = 6926_Zn_220709_1a2_03,
SA123 LC-MS run1 = 123_220709_1a2_01,
SA123 LC-MS run2 = 123_220709_1a2_02,
SA123 LC-MS run3 = 123_220709_1a2_03,
SA123 + Zn(II) LC-MS run1 = 123_Zn_220709_1a2_01,
SA123 + Zn(II) LC-MS run2 = 123_Zn_220709_1a2_02,
SA123 + Zn(II) LC-MS run3 = 123_Zn_220709_1a2_03

2. (b) input parameters

Database queried: UniProtKB/Swiss-Prot Protein Knowledgebase, release 57.5 of 07-July-09
containing 471472 sequence entries

Taxonomical restrictions: *Salmonella typhimurium*, 1700 sequence entries

Description of tool and scoring scheme: Search engine PLGS, ESI-QUAD-TOF, Ion accounting algorithm (see Ref.4)

Specified cleavage agent: Trypsin

Allowed number of missed cleavage: 1

Permissible amino acid modifications: Fixed: cysteine carbamidomethylation; variable: oxidation of methionine

Peptide tolerance: Automatic

Fragment tolerance: Automatic

Threshold, minimum scores for peptides, proteins: Minimum number of fragment ion matches for a peptide: 3; minimum number of fragment ion matches for a protein: 7; minimum number of peptide matches for a protein: 2.

Other relevant parameters: maximum protein mass 250000 Da; false positive rate 4%; data from Enolase *Saccharomyces cerevisiae* (UniProt/Swiss-Prot AC: P00924) was appended to the database.

3. The output of the procedure

3.(a) for identified peptides

See file:

SupplFile3_PeptideTable_Ciavardelli_01.pdf
SupplFile4_PeptideTable_Ciavardelli_02.pdf
SupplFile5_PeptideTable_Ciavardelli_03.pdf
SupplFile6_PeptideTable_Ciavardelli_04.pdf
SupplFile7_PeptideTable_Ciavardelli_05.pdf
SupplFile8_PeptideTable_Ciavardelli_06.pdf
SupplFile9_PeptideTable_Ciavardelli_07.pdf
SupplFile10_PeptideTable_Ciavardelli_08.pdf
SupplFile11_PeptideTable_Ciavardelli_09.pdf
SupplFile12_PeptideTable_Ciavardelli_10.pdf
SupplFile13_PeptideTable_Ciavardelli_11.pdf
SupplFile14_PeptideTable_Ciavardelli_12.pdf

3. (b) quantitation

Quantitation approach: Expression Analysis (PLGS, Waters Corp.)

Quantitation measurement: The intensity of peptides matched on the basis of their unique EMRT (Exact Mass Retention Time) signatures are compared to determine their relative abundance. The relative expression of each identified protein is calculated from the averaged ratio of multiple matched EMRT signatures (Ref. 5).

Number of replicates: 3

Acceptance criteria: Replicate filter settings: 2 out of three replicate for each condition; probability of upregulation settings: p-value lower than 0.05 and upper than 0.95.

Results: SupplFile1_SupplementaryTables.pdf

References:

- 1 Vissers JP, Langridge JI, Aerts JM. Analysis and quantification of diagnostic serum markers and protein signatures for Gaucher disease. *Mol Cell Proteomics*. 2007;6:755-66.
- 2 Geromanos SJ, Vissers JP, Silva JC, Dorschel CA, Li GZ, Gorenstein MV, et al. The detection, correlation, and comparison of peptide precursor and product ions from data independent LC-MS with data dependant LC-MS/MS. *Proteomics*. 2009;9:1683-95.
- 3 Silva JC, Denny R, Dorschel CA, Gorenstein M, Kass IJ, Li GZ, et al. Quantitative proteomic analysis by accurate mass retention time pairs. *Anal Chem*. 2005;77:2187-200.
- 4 Li GZ, Vissers JP, Silva JC, Golick D, Gorenstein MV, Geromanos SJ. Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures. *Proteomics*. 2009;9:1696-719.
- 5 Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ. Absolute quantification of proteins by LCMSE: a virtue of parallel MS acquisition. *Mol Cell Proteomics*. 2006;5:144-56.