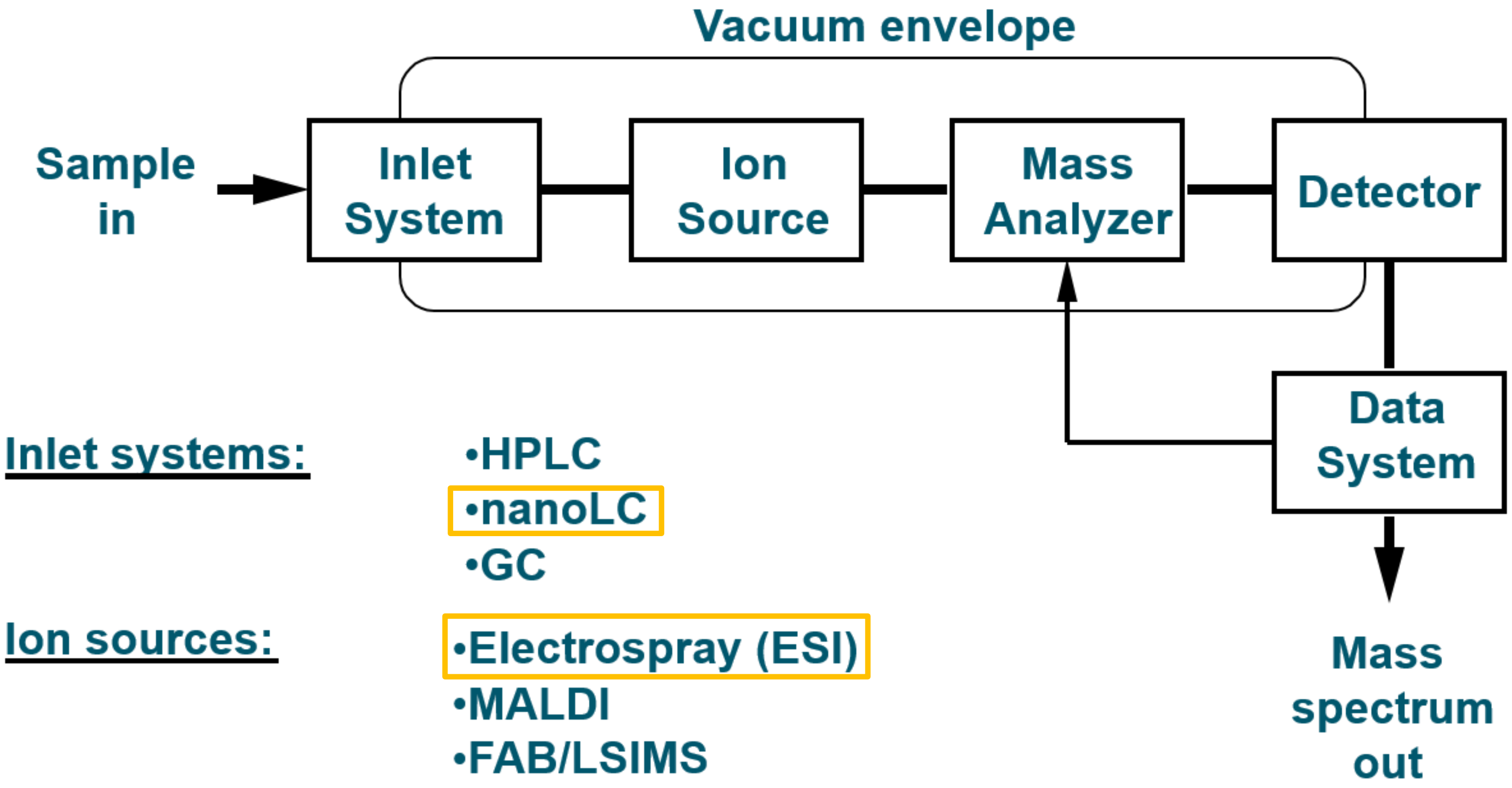
Orbitrap ELITE

World Top 10 University by 2030

연구지원본부, 생체고분자 질량분석실
서정곤, 김도은.
UCRF,
Biomolecular Mass Spectrometry Resource (BMSR)
Jeong Kon Seo, Do Eun Kim.

ULSAN NATIONAL INSTITUTE OF
SCIENCE AND TECHNOLOGY
2009

Mass spectrometer



Vacuum envelope

Sample in → Inlet System — Ion Source — Mass Analyzer — Detector

Data System

Mass spectrum out

Inlet systems:

- HPLC
- nanoLC
- GC

Ion sources:

- Electrospray (ESI)
- MALDI
- FAB/LSIMS
- Electron ionization (EI)

Mass analyzers:

- Quadrupole
- Time-of-flight
- Ion trap
- Magnetic sector
- FTMS (Orbitrap)

Orbitrap ELITE specification

1. Mass range : m/z 50-2000

2. Resolution : Max. 240,000 Full Width at Half Maximum(FWHM)
at m/z 400, 4Hz scan rate

3. Mass accuracy : < 3 ppm with external calibration
< 1 ppm using internal calibration

4. MS/MS techniques : CID, HCD and ETD

5. Sensitivity : 100 fg of reserpine has 100:1 signal-to-noise ratio

6. MS^n : n = 1 to 10

- 월 평균가동률 : 328.5 % (가동률 기준 : 128hr/월 = 100%)
- 평균 대기시간 : 6주
- 7월13일 기준, 현재 대기일 : 8월 29일

Applications

Approach	Application	Prerequisite	Analysis Tool
Protein ID (Bottom up)	Shotgun (gel), OFFGel (solution)	High Speed High Sensitivity	SEQUEST MASCOT*
Protien ID (Top down)	De novo sequencing	High mass accuracy	PeakStudio, ProSight
PTM	Phosphorylation, Glycosylation, Ubiquitination, Methylation, Acetylation…..	Soft fragmentation	Scaffold PTM, MaxQuant
Quantification	Metabolic/chemical/ enzymatic labeling, Label free	High mass accuracy	SIEVE Progenesis

* In-house server is essential.

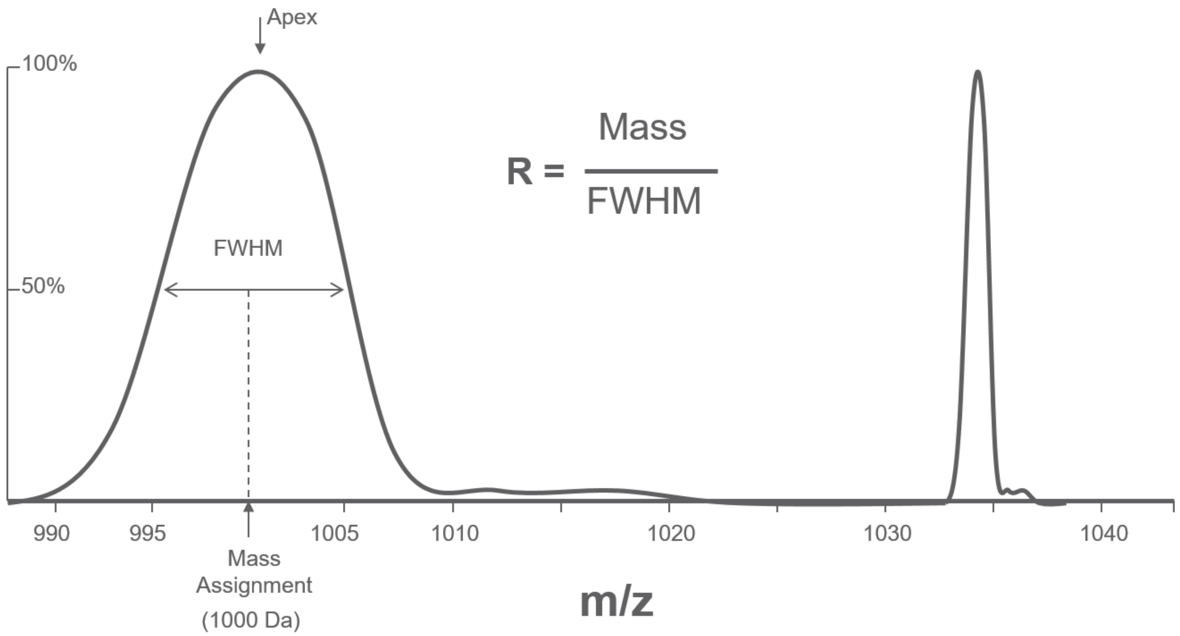
Accuracy

$$\textbf{Mass accuracy}\ (\text{ppm}) = \frac{\text{observed mass} - \text{theoretical mass}}{\text{theoretical mass}} \times 10^6$$

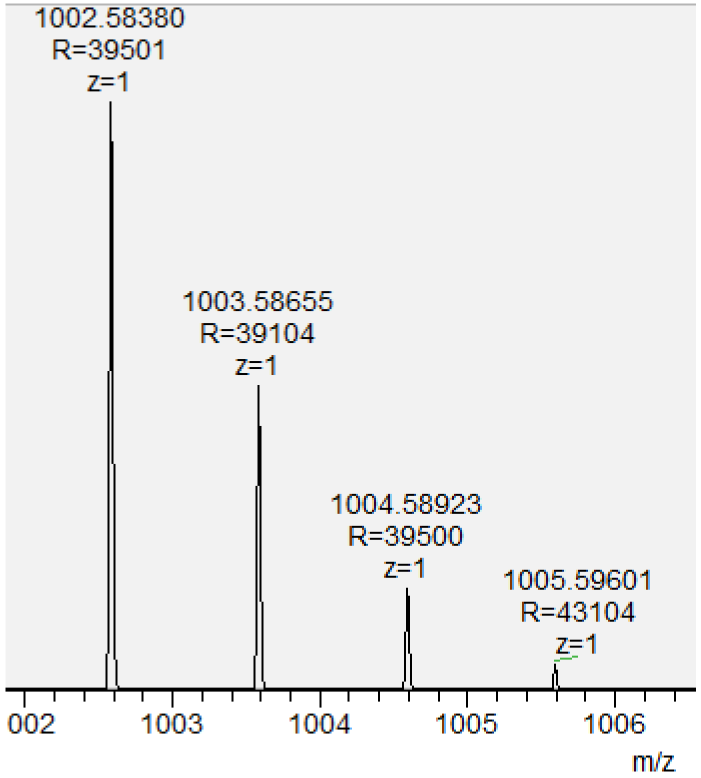
1234.1234 (m/z) 기준
1 ppm : 1234.1224-1234.1236 m/z
5 ppm : 1234.1199-1234.1260 m/z
10 ppm : 1234.1168-1234.1291 m/z
50 ppm : 1234.0921-1234.1538 m/z

567.5678 (m/z) 기준
1 ppm : 567.5675-567.5680 m/z
5 ppm : 567.5663-567.5692 m/z
10 ppm : 567.5649-567.5706 m/z
50 ppm : 567.5536-567.5819 m/z

Resolution

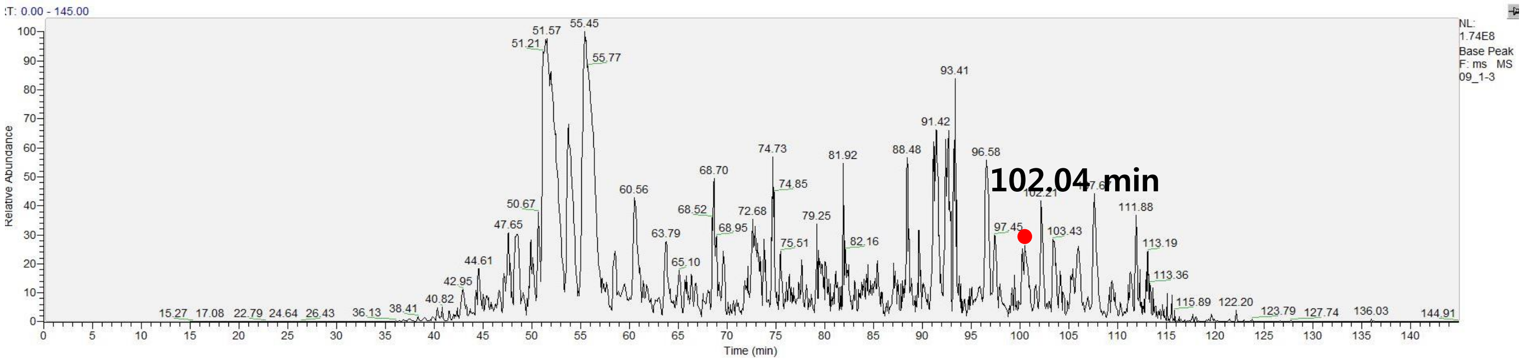


$$R = \frac{\text{Mass}}{\text{FWHM}}$$



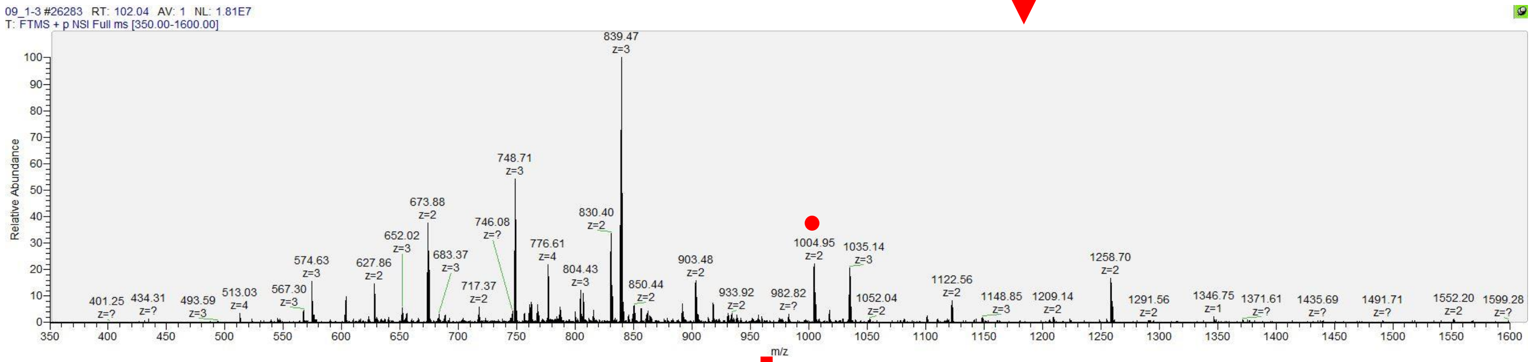
R=40,000 at 1000 m/z (Scan setting : R=60,000)

Data Acquisition



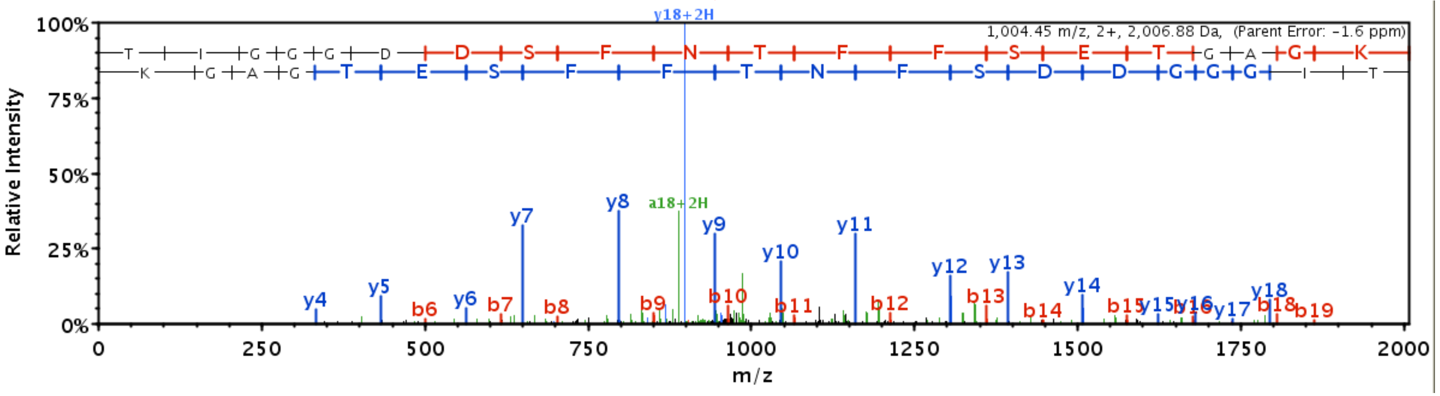
Chromatogram

32882 Spectra



MS spectrum

3512 peptides

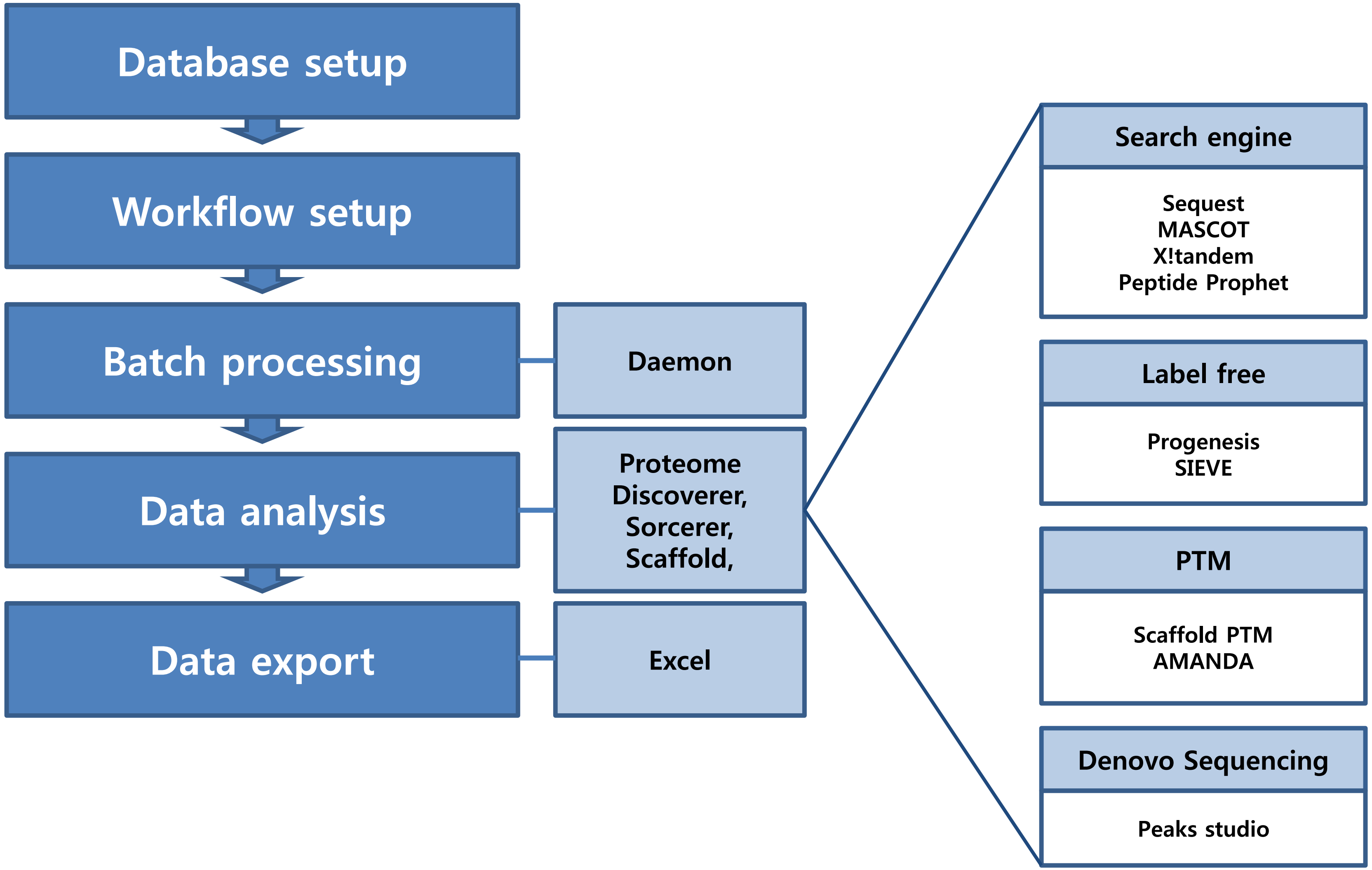


MS/MS spectrum

1284 proteins

FIRST IN CHANGE

WF of Data Analysis : Protein ID



Database setup

Workflow setup

Batch processing

Data analysis

Data export

Daemon

Proteome Discoverer, Sorcerer, Scaffold,

Excel

Search engine

Sequest
MASCOT
X!tandem
Peptide Prophet

Label free

Progenesis
SIEVE

PTM

Scaffold PTM
AMANDA

Denovo Sequencing

Peaks studio

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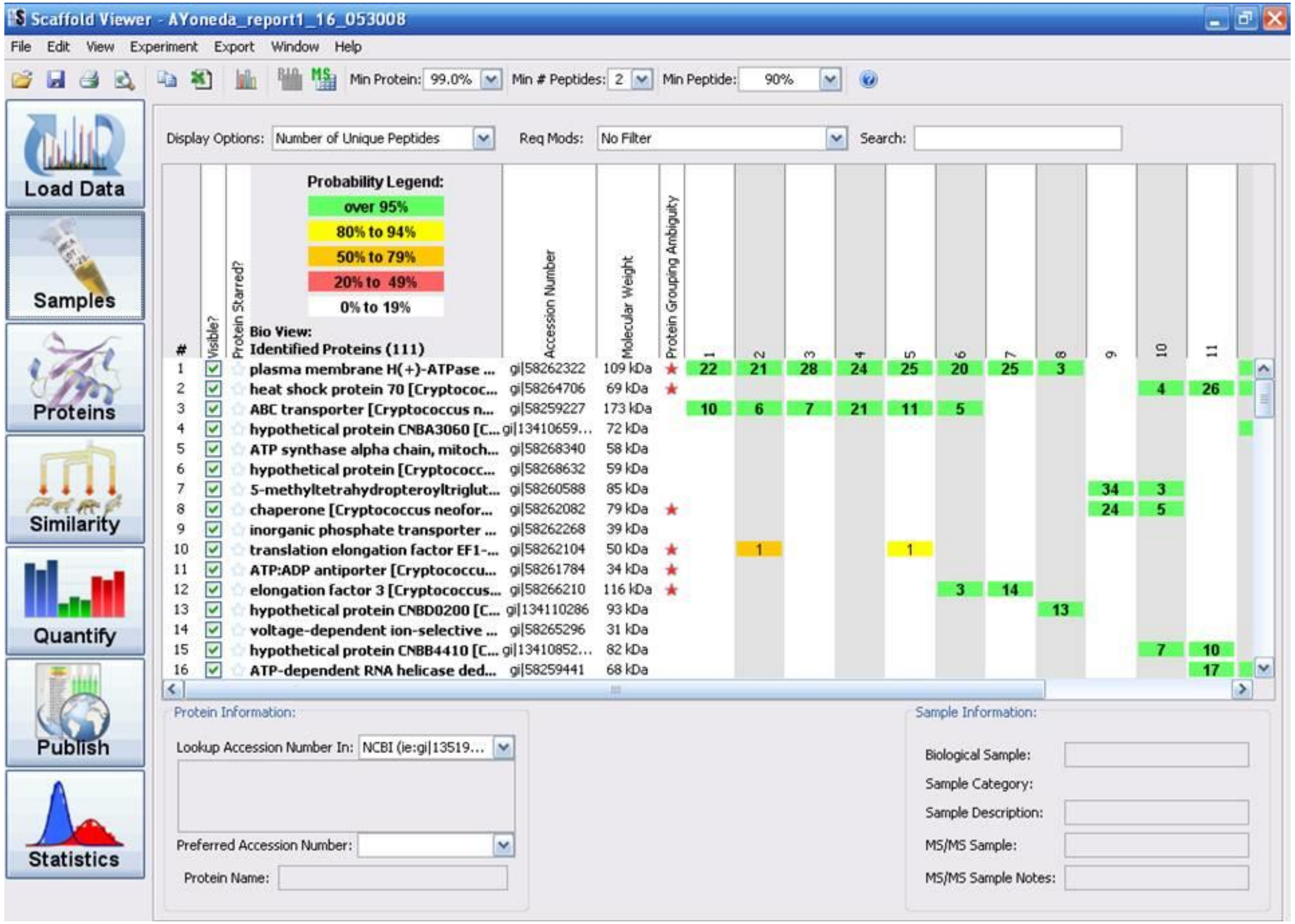
Data Analysis: Protein ID



Min Protein: 99.0%
Min # Peptides: 2
Min Peptide: 90%

Display Options: Number of Unique Peptides
Req Mods: No Filter
Search:

Probability Legend:

- 🟩 over 95%
- 🟨 80% to 94%
- 🟧 50% to 79%
- 🟥 20% to 49%
- ☐ 0% to 19%

#	Visible?	Protein Starred?	Bio View: Identified Proteins (111)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	1	2	3	4	5	6	7	8	9	10	11
1	☑	☆	plasma membrane H(+)-ATPase ...	gi|58262322	109 kDa	★	22	21	28	24	25	20	25	3			
2	☑	☆	heat shock protein 70 [Cryptococ...	gi|58264706	69 kDa	★										4	26
3	☑	☆	ABC transporter [Cryptococcus n...	gi|58259227	173 kDa		10	6	7	21	11	5					
4	☑	☆	hypothetical protein CNBA3060 [C...	gi|13410659...	72 kDa												
5	☑	☆	ATP synthase alpha chain, mitoch...	gi|58268340	58 kDa												
6	☑	☆	hypothetical protein [Cryptococc...	gi|58268632	59 kDa												
7	☑	☆	5-methyltetrahydropteroyltriglut...	gi|58260588	85 kDa										34	3	
8	☑	☆	chaperone [Cryptococcus neofor...	gi|58262082	79 kDa	★									24	5	
9	☑	☆	inorganic phosphate transporter ...	gi|58262268	39 kDa												
10	☑	☆	translation elongation factor EF1-...	gi|58262104	50 kDa	★		1			1						
11	☑	☆	ATP:ADP antiporter [Cryptococcu...	gi|58261784	34 kDa	★											
12	☑	☆	elongation factor 3 [Cryptococcus...	gi|58266210	116 kDa	★						3	14				
13	☑	☆	hypothetical protein CNBD0200 [C...	gi|134110286	93 kDa									13			
14	☑	☆	voltage-dependent ion-selective ...	gi|58265296	31 kDa												
15	☑	☆	hypothetical protein CNBB4410 [C...	gi|13410852...	82 kDa											7	10
16	☑	☆	ATP-dependent RNA helicase ded...	gi|58259441	68 kDa												17

Protein Information:

Lookup Accession Number In: NCBI (ie:gi|13519...

Preferred Accession Number:

Protein Name:

Sample Information:

Biological Sample:

Sample Category:

Sample Description:

MS/MS Sample:

MS/MS Sample Notes:

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QE Plus specification (will be installed in 2017)

1. Mass range : m/z 50-6,000

2. Max resolving power 140,000 at m/z 200

3. Mass accuracy : <1 ppm (Internal mass calibration)
<3 ppm (External mass calibration)

4. Sensitivity : SIM – 50 fg Buspirone on column S/N 100:1

5. Scan rate : Up to 12 Hz at resolution setting of 17,500 at m/z 200

THANK YOU

FIRST IN
CHANGE