Publishing Proteomics Data: How to Win Reviewers and Influence Editors

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University of Texas Health Science Center at San Antonio

St. Louis - ASMS 2015
San Antonio - ASMS 2016
Why do we publish?

Dissemination
Education
Promotion/tenure/funding
Masochism?
Who do we need to satisfy?

“Technology” check may be conducted before or in parallel with scientific review

Editor sends article to experts in the article’s specific area

Reviewer sends suggestions for revisions and for publication

Reviewer reads article for clarity, accuracy, appropriate methodology, and theoretical base

Author plans, designs, implements and writes up his research. (perhaps for a 2nd or 3rd time.)

Author sends manuscript to editor

Editor decides who should review article

Editor sends response to author: publish (rare), publish with revisions, redesign/add research, or reject paper.

https://psychanded.files.wordpress.com/2009/10/peer-review2.png
Reviewers need to be able understand (and believe) what you did and what you found.

Readers need to be able to understand what was done, have enough information to conduct similar experiments and mine the data.
For submission, be prepared to:
Provide a list of “preferred reviewers.”
Pick people who are knowledgeable in the area of the study.
Don’t count on your friends being easy on you. They don’t want you to publish a less than stellar paper.
Don’t pick the “top names” in the field. They most likely won’t have time for the review and it will delay the evaluation process.
Provide a list of people you truly feel will be biased against your work.

Abstract
Introduction
Methods
Results and Discussion
Conclusions
Supporting information

STOP
READ THE INSTRUCTIONS FOR AUTHORS
Elements of a manuscript

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Abstract
Introduction
Methods
Results and Discussion
Conclusions
Supporting information
Elements of a manuscript

Succinctly describe your project and results. This may be all that the editor reads before inviting reviewers.
Elements of a manuscript

Here’s your chance to make the case for your study. Keep on topic. This isn’t a review article.
Elements of a manuscript

Now the details . . .
How much is enough?

Abstract
Introduction
Methods
Results and Discussion
Conclusions
Supporting information
Protein analysis by mass spectrometry. Proteins were separated by 1-D SDS-PAGE and the gel lanes were divided into slices and then digested with trypsin. The digests were analyzed by HPLC-tandem mass spectrometry followed by data processing as previously described (21).
2.11. Protein identification by mass spectrometry

Proteins were separated by 1-D SDS-PAGE. Gel slices containing proteins in each gel lane were digested in situ with trypsin (Promega). The digests were analyzed by capillary HPLC electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) on a Thermo Fisher Orbitrap Velos mass spectrometer. The MS data were searched against the rodent subset of the NCBInr protein database (NCBInr_20130102; 316,972 sequences) by Mascot (Matrix Science). The Mascot results were subjected to a subset search in Scaffold (Proteome Software).
Methods section - samples

Easy to provide

Source
Animals
  Species
  Strain/genetic background
Cells
  Cell line name
    designation
  Source
  Genetic alteration(s)
Labeling strategy (SILAC, iTRAQ/TMT, SILAM)
Number of biological and/or technical replicates
Methods section - sample preparation
Easy to provide

Protein isolation
Proteolytic digestion (if used)
Internal standards added (if used)
Chemical modification (if used), including labeling scheme
Off-line chromatography/cleanup
PTM-specific enrichment/isolation
Methods section - MS analysis

Easy to provide

HPLC

Instrument vendor/model

Column, mobile phases, flow rate, gradient, auxiliary detection details

Strategy for sample injection order

Mass spectrometry

Instrument vendor/model

Instrument parameters/scan strategy

HPLC-ESI-tandem-MS

Data-dependent analysis: MS1 mass resolution, MS1 scan range, charge-state screening parameters, mass window for precursor ion isolation, fragmentation mode, relative collision energy (or other parameter, as appropriate), mass analyzer for tandem-MS, MS2 mass resolution (where appropriate), number of product ion spectra per scan cycle, dynamic exclusion

Data-independent analysis: MS1 mass resolution, MS1 scan range(s), relative collision energy, mass analyzer for tandem-MS, MS2 mass resolution (where appropriate)
Methods section - data processing

Easy to provide

Software/method for peak list generation

Database searching

Software name(s), vendor(s) or literature citation, version

Databases

Name/source
Date/version
Taxonomy
Number of sequences

Search parameters

Precursor and product ion mass tolerances
Enzyme specificity
Charge states considered
Fixed and variable modifications
Other settings (e.g., $^{13}$C number in Mascot)
Quantitation method (where applicable)
Decoy search option

Do not say “using the default parameters”
Methods section - data processing

Getting harder

Criteria for acceptance of peptide assignments and protein identifications

- Mascot ions scores/expect values
- SEQUEST $X_{corr}$ cutoffs
- X! Tandem scores

Post-processing with additional software

- PeptideProphet/ProteinProphet
- Scaffold
- Proteome Discoverer
- In-house software (need to document)

False discovery rate (FDR) determination

- Decoy database details
- Method for FDR calculation

De novo sequencing

- Approach (e.g., manual or computational)
- Validation
Methods section - quantitative analysis

Getting even harder

Software name, vendor or literature citation, version
Quantitation parameters
Normalization
Methods section - quantitative analysis

Getting even harder

Considerations for acceptance of peptide values

SILAC/metabolic labeling
  Variability of ratios across an EIC
  Fraction of the EIC window
  Agreement with predicted isotope pattern

Reporter ion-based methods
  Variability assessment for reporter ion ratios of within-sample replicates

Spectral counting
  Minimum number of assigned peptides/spectra
  Spectrum designation
    Total spectra, unique spectra, unique sequence

Intensity-based methods
  Method for peak integration/intensity determination

Variability assessment for peptides assigned to a protein
Methods section - quantitative analysis

Getting even harder

Considerations for acceptance of peptide values

SILAC/metabolic labeling

Variability of ratios across an EIC

Fraction of the EIC window

Agreement with predicted isotope pattern

Reporter ion-based methods

Variability assessment for reporter ion ratios of within-sample replicates

Spectral counting

Minimum number of assigned peptides/spectra

Spectrum designation

Total spectra, unique spectra, unique sequence

Intensity-based methods

Method for peak integration/intensity determination

Variability assessment for peptides assigned to a protein
Variability assessment of peptides assigned to a protein

Relative quantities of selected tRNA synthetases in *C. albicans* grown at different temperatures

<table>
<thead>
<tr>
<th>tRNA synthetase</th>
<th>Accession</th>
<th>30 min</th>
<th>60 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L/H</td>
<td>SD(geo)</td>
<td>#</td>
<td>L/H</td>
</tr>
<tr>
<td>Val</td>
<td>orf19.1295</td>
<td>3.13</td>
<td>2.60</td>
<td>89</td>
</tr>
<tr>
<td>Ile</td>
<td>orf19.2138</td>
<td>4.13</td>
<td>3.77</td>
<td>33</td>
</tr>
<tr>
<td>Leu</td>
<td>orf19.2560</td>
<td>5.09</td>
<td>2.08</td>
<td>5</td>
</tr>
<tr>
<td>Ala</td>
<td>orf19.5746</td>
<td>5.94</td>
<td>1.09</td>
<td>6</td>
</tr>
<tr>
<td>Asn</td>
<td>orf19.6702</td>
<td>7.18</td>
<td>1.76</td>
<td>24</td>
</tr>
<tr>
<td>Gin</td>
<td>orf19.7084</td>
<td>5.55</td>
<td>1.51</td>
<td>11</td>
</tr>
</tbody>
</table>

Values were obtained from Mascot Distiller processing of the MS data and search results as multi-file projects for each time-point. H/L, median ratio of peak areas of extracted ion chromatograms for peptides assigned above the homology threshold; SD(geo), geometric standard deviation of the assigned peptides; #, number of peptides used for relative quantification.

Weintraub, S.T. et al. ASMS 2014 ThP08 076
Methods section - statistics

May be really difficult

Software/program
Test(s) applied
Significance levels

“I can prove it or disprove it! What do you want me to do?”
Provide a clear, succinct report of your results and insightful interpretations.

Highlight key findings. Do not cover every detail presented in the results tables and figures.

Focus on truly meaningful observations. Do you really need to discuss the biological significance of every protein you identified?
Table 3
Mass spectrometry of fractions 22 and 23 revealed the presence of several molecular chaperones. For a complete list please see Table S1.

<table>
<thead>
<tr>
<th>Identified proteins (8/223)</th>
<th>Accession number</th>
<th>MW</th>
<th>fr.22</th>
<th>fr.23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducible heat shock protein 70 (HSP72) [Heteroccephalus glaber]</td>
<td>gi</td>
<td>13242237 (+26)</td>
<td>71 kDa</td>
<td>28</td>
</tr>
<tr>
<td>Ubiquitin-like modifier-activating enzyme 1 [Heteroccephalus glaber]</td>
<td>gi</td>
<td>351699501</td>
<td>119 kDa</td>
<td>20</td>
</tr>
<tr>
<td>78 kDa glucose-regulated protein [Heteroccephalus glaber]</td>
<td>gi</td>
<td>351702099</td>
<td>72 kDa</td>
<td>10</td>
</tr>
<tr>
<td>Inducible heat shock protein 70 [Mus musculus]</td>
<td>gi</td>
<td>118490060 (+7)</td>
<td>70 kDa</td>
<td>8</td>
</tr>
<tr>
<td>Hsp90a1 protein [Mus musculus]</td>
<td>gi</td>
<td>118142832 (+23)</td>
<td>66 kDa</td>
<td>8</td>
</tr>
<tr>
<td>Heat shock protein 90 beta [Equus caballus]</td>
<td>gi</td>
<td>12082134 (+17)</td>
<td>82 kDa</td>
<td>7</td>
</tr>
<tr>
<td>Protein disulfide-isomerase [Heteroccephalus glaber]</td>
<td>gi</td>
<td>351706419</td>
<td>57 kDa</td>
<td>7</td>
</tr>
<tr>
<td>Stress-70 protein, mitochondrial [Mus musculus]</td>
<td>gi</td>
<td>162461907 (+9)</td>
<td>73 kDa</td>
<td>7</td>
</tr>
</tbody>
</table>

Spectral counts (quantitative value) determined by Scaffold (v3) are shown in the table under the columns labeled fr.22 and fr.23.

Will readers know what this is?

What were the criteria for acceptance of peptide assignments and protein identifications?

Was an FDR assessed? How was it used?

Bacteriophage 201\(\phi\)2-1 structure and morphology
1-D SDS PAGE of phage 201φ2-1 structural proteins

Full-length separation

Separation for ~1.5 cm

24 bands excised

Cut into 7 slices
1-D SDS PAGE of phage 201φ2-1 structural proteins

76 proteins identified
≥2 peptides/protein
Scaffold post-processing
95% confidence (peptides)
≥99.9% probability (protein)
Characterization of *Pseudomonas chlororaphis* myovirus 201Φ2-1 via genomic sequencing, mass spectrometry, and electron microscopy

Julie A. Thomas, Mandy R. Rolando, Christopher A. Carroll, Peter S. Shen, David M. Belnap, Susan T. Weintraub, Philip Serwer, Stephen C. Hardies

**Department of Biochemistry, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900, USA**

**Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, USA**

**Abstract**

*Pseudomonas chlororaphis* phage 201Φ2-1 is a relative of *Pseudomonas aeruginosa* myovirus φKZ. Phage 201Φ2-1 was examined by complete genomic sequencing (316,674 bp), by a comprehensive mass spectrometry survey of its virion proteins and by electron microscopy. Seventy-six proteins, of which at least 69
### Table 1
MS data and homologues for the 201ϕ2-1 proteins identified by HPLC-ESI-MS/MS

<table>
<thead>
<tr>
<th>gp</th>
<th>M (kDa)</th>
<th>Unique peptides</th>
<th>Total spectra</th>
<th>Spectra/Mw</th>
<th>% coverage</th>
<th>Function, homologues, paralogues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Proteins of established virion function according to Fokine et al. (2007)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>77.5</td>
<td>22</td>
<td>355</td>
<td>4.6</td>
<td>44</td>
<td>major sheath protein, KZ29 (63% over 693); EL6 (21% over 707)</td>
</tr>
<tr>
<td>200</td>
<td>82.4</td>
<td>22</td>
<td>696</td>
<td>8.4</td>
<td>38</td>
<td>major capsid protein, KZ120 (64% over 749); EL78 (20% over 325)</td>
</tr>
<tr>
<td>276</td>
<td>251.8</td>
<td>23</td>
<td>34</td>
<td>0.1</td>
<td>10</td>
<td>cell-puncturing device, KZ181 (33% over 2387); KZ144 (45% over 187); EL183 (22% over 270)</td>
</tr>
<tr>
<td><strong>B. RNA polymerase-related virion proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>139</td>
<td>49.6</td>
<td>8</td>
<td>34</td>
<td>0.7</td>
<td>24</td>
<td>RNA polymerase, beta subunit, KZ80 (56% over 449); EL44 (23% over 447)</td>
</tr>
<tr>
<td>273/274</td>
<td>173.4</td>
<td>24</td>
<td>87</td>
<td>0.5</td>
<td>19</td>
<td>RNA polymerase, beta subunit, KZ78 (33% over 1548); EL186 (23% over 1142); EL187 (26% over 356)</td>
</tr>
<tr>
<td>275</td>
<td>62.7</td>
<td>8</td>
<td>27</td>
<td>0.4</td>
<td>15</td>
<td>RNA polymerase, beta subunit KZ80 (68% over 490); EL184 (32% over 491)</td>
</tr>
</tbody>
</table>

---

1. All proteins had a protein identity probability of 100%, as determined by Scaffold (Proteome Software), with the exception of gp164 (99%) and gp229 (96%). Results displayed were obtained from a combined data set of the Gel-LCMS analysis, with the exception of gp276 which was only detected in analysis of an individual gel band (see text).

2. Homologues were determined using Psi-Blast and BlastP (% identities over the homologous region are provided). The best matching ϕKZ and EL homologue for each 201ϕ2-1 protein is listed. Paralogue families are as follows: paralogue family a refers to a domain found in 201ϕ2-1 gp16, 217, 218, 219, 220. A homologous domain exists in ϕKZ gp131, 132, 133, 134 and 135 and EL gp113, 114 and 115; paralogue family b refers to a domain found in 201ϕ2-1 gpl55, 156, 157, 246, 247. Homologous domains exist in ϕKZ gpl53, 94, 95, 162 and 163; paralogue family c refers to a domain found in 201ϕ2-1 gpl46 and 452. Homologous domains exist in ϕKZ gpl83 and EL gpl55.

3. An N-terminal peptide lacking only the initiator methionine was identified using semi-tryptic analysis.

4. ϕKZ refers to ϕKZ.

5. N-terminus is expected to be processed as the ϕKZ homologue is processed. Although no semi-tryptic fragments were found to define the mature ends, there is also a lack of peptide coverage in the N-terminal region that would be consistent with processing.

6. A mature N-terminus containing the initiator methionine was confirmed by semi-tryptic analysis.

7. Gel analysis indicated that the protein is processed consistent with a lack of MS sequence coverage in the N-terminal region of these sequences, except for gp246N which lacks MS coverage of the C-terminal region. The exact positions of the processed ends are unknown. The normalized spectrum count in parentheses was calculated using the apparent molecular weight of the processed form (Fig. 1).

8. Semi-tryptic analysis indicated removal of 63 and 60 N-terminal residues from gp238 and gp271, respectively.
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<table>
<thead>
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<th>gP</th>
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<td>696</td>
<td>8.4</td>
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<td>276</td>
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<td>23</td>
<td>34</td>
<td>0.1</td>
<td>10</td>
<td>cell-puncturing device, KZ181 (33% over 2387); KZ144 (45% over 187); EL183 (22% over 270).</td>
</tr>
</tbody>
</table>

A. Proteins of established virion function according to Fokine et al. (2007)

B. RNA polymerase-related virion proteins

1All proteins had a protein identity probability of 100%, as determined by Scaffold (Proteome Software), with the exception of gp164 (99%) and gp229 (96%). Results displayed were obtained from a combined data set of the GeLCMS analysis, with the exception of gp276 which was only detected in analysis of an individual gel band (see text).

2N-terminus is expected to be processed as the ΦKZ homologue is processed. Although no semi-tryptic fragments were found to define the mature ends, there is also a lack of peptide coverage in the N-terminal region that would be consistent with processing.

3A mature N-terminus containing the initiator methionine was confirmed by semi-tryptic analysis.

4Gel analysis indicated that the protein is processed consistent with a lack of MS sequence coverage in the N-terminal region of these sequences, except for gp246N which lacks MS coverage of the C-terminal region. The exact positions of the processed ends are unknown. The normalized spectrum count in parentheses was calculated using the apparent molecular weight of the processed form (Fig. 1).

5Semi-tryptic analysis indicated removal of 63 and 60 N-terminal residues from gp238 and gp271, respectively.
Elements of a manuscript

Abstract
Introduction
Methods
Results and Discussion
Conclusions
Supporting information

Conclusions - not a repeat of the Results
Elements of a manuscript

Think about what you’re including as supporting data.
Make sure the figures and tables are informative.
Use meaningful titles for the figures and tables.
Don’t provide every spreadsheet you or the software generated as a part of the analysis.
Rearrange spreadsheet columns as needed so that the key information is easily found.
What needs to be documented?
There is no consensus about whether all identified proteins need to be listed or only those that exhibited significant differences in quantity among experimental groups or are of special interest to the study.

Identification by HPLC-ESI-tandem-MS

Protein level report - spreadsheet format, PLEASE

Columns
  Protein name
  Accession number
  Molecular weight
  Number of assigned spectra (total and unique)
  Percent sequence coverage
  Probability of protein inference (if determined during post-processing)

Clear/meaningful column heading names

Legend - at the top of long tables or on a separate worksheet
  Explanations for abbreviated or non-standard column headings
  Significance level cutoff of assigned peptides
Supporting information

MALDI-TOF-MS (peptide mass fingerprint, PMF)

Only suitable for low-complexity samples

Protein level report

Columns

- Protein name
- Accession number
- Molecular weight
- Number of spectra searched
- Number of spectra assigned
- Percent sequence coverage
- Probability of protein inference

Clear/meaningful column heading names

Legend - at the top of long tables or on a separate worksheet

Explanation for abbreviated or non-standard column headings
MALDI-TOF/TOF
Protein level report
Columns
  Protein name
  Accession number
  Molecular weight
  Number of spectra searched for PMF
  Number of spectra assigned for PMF
  Number of tandem-mass spectra searched
  Number of tandem-mass spectra assigned
  Percent sequence coverage
  Probability of protein inference
Clear/meaningful column heading names
Legend - at the top of long tables or on a separate worksheet
  Explanations for abbreviated or non-standard column headings
Significance level cutoff of assigned peptides
Supporting information

Peptide level (grouped by protein)
For documentation of PTMs, sequence variations
Not usually necessary for manuscripts focusing on identification or relative quantification

Columns
Protein name
Peptide sequence - clearly showing modification(s)
Start/stop residue numbers
Observed $m/z$
Charge state
Mass error
Score/expect value for sequence assignment
Probability for localization of modification site (where appropriate)

Clear/meaningful column heading names
Legend - at the top of long tables or on a separate worksheet
  Explanations for abbreviated or non-standard column headings
  Significance level cutoff of assigned peptides
Supporting information
Annotated tandem mass spectra

There is no consensus about when annotated tandem mass spectra need to be provided.

Post-translational modifications
Do we need to see all spectra for a large phosphoproteomics study if acceptable parameters have been used for database searching and reasonable cutoffs have been applied to site localization probabilities? Unusual modifications or surprising findings that are the focus of the manuscript should be documented in the body of the manuscript.

Proteins identified by a single, high-confidence peptide assignment
Will examining the annotated spectra influence confidence about the assignment? Will you really look at them?

Annotated tandem mass spectra - annotate the following for each
- Peptide sequence
- Observed m/z
- Mass error
- Charge state
- Database search score
- Probability/expect score
- Site localization probability (where appropriate)
Supporting information
Annotated tandem mass spectra

Protein family 139
transketolase [Trichoderma atroviride IMI 206040]
Score, 106; matches, 2; match(sig), 2; sequences, 2; seq(sig), 2; eMPAI, 0.06

Print a PDF of the Mascot Peptide View
Peptide View

MS/MS Fragmentation of **LEGILPELVGGSA DL TGSN\textsuperscript{1 TP}**
Found in gi|358391264 in \textit{NCBImr}, transketolase [Trichodesma dioica]

Match to Query 6007: 2211.175448 from(1106.595000,2+) Title: 5776: Scan 10850 (rt=34.9916) [\texttt{lqqbio\textquotesingle MSL 1\textbackslash Wc\textbackslash h1}]

### All matches to this query

<table>
<thead>
<tr>
<th>Score</th>
<th>Mr(calc)</th>
<th>Delta</th>
<th>Sequence</th>
</tr>
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<tr>
<td>86.6</td>
<td>2211.1747</td>
<td>0.0008</td>
<td>ILEGILPELVGGSA DL TGS NLTR</td>
</tr>
<tr>
<td>86.6</td>
<td>2211.1747</td>
<td>0.0008</td>
<td>ILEGILPELVGGSA DL TGS NLTR</td>
</tr>
<tr>
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<td>2211.1648</td>
<td>0.0107</td>
<td>GLITVTYDVNPQIDIHTR</td>
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<tr>
<td>8.8</td>
<td>2211.1510</td>
<td>0.0244</td>
<td>VSSLPPWICHSFNAI STR</td>
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</tbody>
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### Monoisotopic mass of neutral peptide Mr(calc): 2211.1747
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 87  Expect: 3.2e-007
Matches: 58/212 fragment ions using 123 most intense peaks  (help)
Supporting information
Annotated tandem mass spectra

Protein family 376
uroporphyrinogen decarboxylase [Cryptococcus neoformans var. grubii H99]
Score, 30; matches, 1; match(sig), 1; sequences, 1; seq(sig), 1; eMPAI, 0.07
Peptide View

MS/MS Fragmentation of **VHSVLSQLSHPGVPITLFAK**
Found in gly405118227 in NCBInr, uroporphyrinogen decarboxylase 1 (Homo sapiens) (UniProt Q07791)

Match to Query 5835: 2129.199492 from(710.740440,3+)
Title: 5615: Scan 10667 (rt=34.4181) [\lcqbio\MSL 1\Wc

<table>
<thead>
<tr>
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<th>Mr(calc)</th>
<th>Delta</th>
<th>Sequence</th>
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<tbody>
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<td>0.0229</td>
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</table>

Monoisotopic mass of neutral peptide Mr(calc): 2129.1997
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 30  Expect: 0.023
Matches : 26/204 fragment ions using 33 most intense peaks  (help)
Supporting information

Quantitative analysis

Protein level report
- Documentation can be added to the ID report or in a separate table

Columns
- Protein name
- Accession number
- Molecular weight
- Number of assigned spectra (total and unique)
- Percent sequence coverage
- Probability of protein inference (if determined post-processing)
- Number of peptides used for quantitative analysis
- Variability of results for peptides assigned to a protein

Clear/meaningful column heading names

Legend - at the top of long tables or on a separate worksheet
- Explanations for abbreviated or non-standard column headings
- Significance level cutoff of assigned peptides
Supporting information

Quantitative analysis

Protein level report

Documentation can be added to the ID report or in a separate table

Columns

Protein name
Accession number
Molecular weight
Number of assigned spectra (total and unique)
Percent sequence coverage
Probability of protein inference (if determined post-processing)
Number of peptides used for quantitative analysis

Variability of results for peptides assigned to a protein

Clear/meaningful column heading names

Legend - at the top of long tables or on a separate worksheet

Explanations for abbreviated or non-standard column headings
Significance level cutoff of assigned peptides
Supporting information

You can generate the supplemental data tables needed for publication directly from Mascot by exporting a CSV file from the Mascot Results Report page.
1-D SDS PAGE - proteins of *Cryptococcus neoformans*

Mascot search results report

MASCOT Search Results

User: stweintraub
E-mail: stweintraub@uthscsa.edu
Search title: Wormley Chaturvedi M1415-058 156130FLW32_F3.raw (NCBInr, fungi; contam) trypsin-1 Ox(M) Carb(C) D(NQ) decoy (Wormley Chaturvedi M1415-058 265 D, slice F3, Sul, D8)
Databases: 11 contaminants 20120713 (247 sequences; 128,130 residues)
2: NCBInr 20140522 (39,649,990 sequences; 14,178,194,136 residues)
Taxonomy: 1: (none)
2: Fungi (2,653,696 sequences)
Timestamp: 15 May 2015 at 19:07:55 GMT

Re-search
Not what you expected? Try the select summary.

Search parameters
Score distribution
Modification statistics
Legend

Protein Family Summary

Filter
Significance threshold p< Ions score or expect cut-off
Show Percolator scores
Preferred taxonomy

Decoy search summary (reversed protein sequences)
Proteins (389) Report Builder Unassigned (5102)

Protein families 1–10 (out of 389)
10 per page 1 2 3 4 5 6 39 Next Expand all Collapse all

1 2: zgi58261082 1222 14-9-3 protein [Cryptococcus neoformans var. neoformans JEC21]
Mascot search results report

MASCOT Search Results

User : stweintraub
E-mail : weintraub@uthscsa.edu
Search title : Wormley Chaturvedi M1415-058 156130FLUW22_F3.raw (NCBI:rf_fungi; contam) trypsin-1 Ox(M) Carb(C) D(NQ) decoy (Wormley Chaturvedi M1415-058 265 D, slice F3, Sul, D8)
Databases : 11 contaminants 20120713 (247 sequences; 128,130 residues)
2: NCBI nr 20140522 (39,649,990 sequences; 14,178,194,136 residues)
Taxonomy : 1: (none)
2: Fungi (2,653,696 sequences)
Timestamp : 15 May 2015 at 19:07:55 GMT

Search parameters

Type of search : MS/MS Ion Search
Enzyme : Trypsin
Fixed modifications : Carbamidomethyl (C)
Variable modifications : Deamidated (NQ), Oxidation (M)
Mass values : Monoisotopic
Protein mass : Unrestricted
Peptide mass tolerance : ± 20 ppm (# ¹³C = 1)
Fragment mass tolerance : ± 0.8 Da
Max missed cleavages : 1
Instrument type : ESI-TRAP
Number of queries : 8,057
**MASCOT Search Results**

**User:** stweintraub  
**E-mail:** stweintraub@uthscsa.edu  
**Search title:** Wormley Chaturvedi M1415:058 156130FLW32_F3.raw (NCBI(, fungi); contaminant) trypsin-1 Ox(M) Carb(C) D(NQ) decoy (Wormley Chaturvedi M1415:058 265 D, slice F3, Sul, D8)  
**Databases:**  
1: contaminants 20120713 (247 sequences; 128,130 residues)  
2: NCBI nr 20140522 (39,649,990 sequences; 14,178,194,136 residues)  
**Taxonomy:**  
1: (none)  
2: Fungi (2,653,696 sequences)  
**Timestamp:** 15 May 2015 at 19:07:55 GMT

**Score distribution**

**Peptide score distribution.** Ions score is $10 \log(P)$, where $P$ is the probability that the observed match is a random event. There are 1,263 peptide matches above identity threshold and 1,808 matches above homology threshold for 8,057 queries. Histogram score range is (0, 120). On average, individual ions scores $> 48$ (beyond green shading) indicate identity or extensive homology ($p<0.05$).

**Protein score distribution.** Score distribution for family members in the first 50 proteins. Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein families. **[Deprecated]**
Mascot search results report

MASCOT Search Results

User: stweintraub
E-mail: weintraub@uthscsa.edu
Search title: Wormley Chaturvedi M1415-058 156130FLW32_F3.raw (NCBIr.fungi; contam) trypsin-1 Ox(M) Carb(C) D(NQ) decay (Wormley Chaturvedi M1415-058 265 D, slice F3, S14, D8)
Databases: 11 contaminants 20120713 (247 sequences; 128,130 residues)
Taxonomy: 1: (none)
2: Fungi (2,653,696 sequences)
Timestamp: 15 May 2015 at 19:07:55 GMT

Not what you expected? Try the select summary.

Search parameters
Score distribution
Modification statistics

Modification statistics

<table>
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<tr>
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<th>Site</th>
<th>Above thr.</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Carbamidomethyl</td>
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<td>207</td>
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<tr>
<td>Deamidated</td>
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<td>130</td>
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<tr>
<td>Deamidated</td>
<td>Q</td>
<td>66</td>
</tr>
</tbody>
</table>

Protein families 1-10 (out of 389)

1292 14-9-3 protein [Cryptococcus neoformans var. neoformans JEC21]
Mascot search results report

MASCOT Search Results

User: stweintraub
E-mail: stweintraub@uthscsa.edu
Search title: Wormley Chaturvedi M1415-058 156130FLW22_F3.raw (NCBIr-fungl; contam) trypsin-1 Ox(M) Carb(C) D(NQ) decoy (Wormley Chaturvedi M1415-058 265 D, slice F3, Sul, D8)
Databases: 1: contaminants 20120713 (247 sequences; 128,130 residues)
2: NCBI nr 20140522 (39,649,990 sequences; 14,178,194,136 residues)
Taxonomy: 1: (none)
2: Fungi (2,653,696 sequences)
Timestamp: 15 May 2015 at 19:07:55 GMT

Protein Family Summary

Decoy search summary (reversed protein sequences)

Peptide matches in target in Decoy FDR
- above identity threshold 1263 26 2.06%
- above identity or homology threshold 1808 76 4.20%

Decoy results are available in the decoy report.
Mascot search results report

Select "Report Builder"
Mascot search results report
Mascot search results report - ions score cutoff 0, no FDR adjustment

Protein family 37
hexokinase 2 [Cryptococcus gattii WM276]

- Score: 256
- Matches: 23
- Match(sig): 13
- Sequences: 13
- Seq(sig): 7
- eMPAI: 0.87
Mascot search results report - ions score cutoff 0, no FDR adjustment

Protein sequence coverage: 35%

Matched peptides shown in **bold red**.

<table>
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<tr>
<th>Query Start - End</th>
<th>Observed Mr (exp)</th>
<th>Observed Mr (calc)</th>
<th>ppm</th>
<th>M</th>
<th>Score</th>
<th>Expect</th>
<th>Rank</th>
<th>Peptide</th>
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<tbody>
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<td>721.3964</td>
<td>2161.1673</td>
<td>2161.1671</td>
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<tr>
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<td>1123.5515</td>
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<td>1</td>
<td>U.R.CSAHNPVIVLLR.S</td>
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</table>

Unformatted sequence string: 488 residues (for pasting into other applications).

Sort peptides by: G Residue Number C Increasing Mass C Decreasing Mass
### Mascot search results report

Here is the Mascot search results from the email attachment.

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<tr>
<th>Accession</th>
<th>Score</th>
<th>Expect</th>
<th>Database</th>
<th>Description</th>
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<tr>
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<td>Heat shock protein 70 [Cryptococcus gattii WM276]</td>
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### Mascot search results report - ions score cutoff 0.05, no FDR adjustment

**Protein family 37**

**hexokinase 2 [Cryptococcus gattii WM276]**

- **Score**: 256
- **Matches**: 13
- **Match(sig)**: 13
- **Sequences**: 7
- **Seq(sig)**: 7
- **eMPAI**: 0.87

**Protein sequence coverage: 25%**

**25% coverage**

**Unformatted sequence string**: 488 residues (for pasting into other applications).

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<th>Mr(calc)</th>
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<th>M</th>
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<td>766.9161</td>
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<td>66</td>
<td>0.0093</td>
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<td>4467</td>
<td>190 - 204</td>
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</tr>
</tbody>
</table>
Adjust FDR to 1% (or other desired value)
Significance threshold altered by Mascot to value needed to achieve 1% FDR.
Mascot search results report - FDR adjustment

Ions score cutoff is still 0
All assignments will be displayed
Mascot search results report - FDR adjustment

Ions score cutoff 0.05
Only significant assignments will be displayed
Influence of Mascot significance and FDR settings

<p>| Hexokinase 2 [Cryptococcus gattii] gi|259120714 |
|------------------------------------------------|</p>
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<th>Significance Threshold</th>
<th>Ions score cutoff</th>
<th>FDR (%)</th>
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Mascot results spreadsheet generation

Expand column selection dialog

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Mascot results spreadsheet generation

**Protein hits (377 proteins)**

**Columns: Standard (12 out of 16)**

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- **Database**: Database Name
- **Accession**: Accession Number
- **Score**: Score
- **Mass**: Mass
- **Matches**: Matches
- **Match(sig)**: Matched Significance
- **Sequences**: Sequences
- **Seq(sig)**: Sequence Significance
- **emPAI**: emPAI
- **Description**: Description

**Select “<custom>”**

**Enable/disable columns for report**

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Mascot results spreadsheet generation

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Export as CSV
Move search parameter list to a separate worksheet.

Adjust column width as needed. Re-format/abbreviate column headings for readability. Use appropriate numbers of decimal points.

Give worksheets informative names.

Database column can be deleted if only one database was used.
Differential expression analysis using SILAC (Stable Isotope Labeling with Amino Acids in Cell Culture)

- Unlabeled K and R
- $^{13}$C-labeled K and R
- Mix
- Excise gel slices
- Digest (in-gel) with trypsin
- Analyze by HPLC-ESI-MS/MS (Orbitrap)
- Assess intensity ratio between ions of labeled and unlabeled peptides to determine relative quantities of proteins

- equal quantity of protein
- or
- equal number of cells
Assessment of differences in protein expression of human cells infected with Marburg viruses using SILAC

Lethality: Marburg virus-Angola (MARV-Ang) > Marburg virus-Musoke (MARV-Mus)

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vertical slices to check incorporation

BioRad Criterion XT MOPS 12% SDS-PAGE reducing gel, “blue silver” stain
Mascot Distiller results

Quantitation table (proteins)

Total ion current chromatogram for slice A1
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<tr>
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<td>glyceraldehyde-3-phosphate dehydrogenase [Phoca largha]</td>
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<tr>
<td>2.3</td>
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Mascot Distiller results

Fatty acid synthase (protein 1.1)
136 of 190 peptides met criteria for quant

Peptide matches

Total ion current chromatogram for slice A1
Peptide matches: fatty acid synthase (protein 1.1)

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Peptide matches: fatty acid synthase (protein 1.1)

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<td>0.04262</td>
<td>0.6748</td>
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[Peptide match table]
Mascot Distiller results

Fatty acid synthase (protein 1.1)
136 of 190 peptides met criteria for quant

Peptide 9

red, experimental spectrum
black, theoretical isotope pattern

Extracted ion chromatograms

[M+2H]^2+
Mascot Distiller results

Cargo selection protein TP47 (protein 299)
3 of 11 peptides met criteria for quant
L/H = 1.64
SD = 1.05

Peptide 6

Extracted ion chromatograms

Light

Heavy (K6R6)

[M+2H]^2+
Cargo selection protein TP47 (protein 299)
3 of 11 peptides met criteria for quant
L/H = 1.64
SD = 1.05

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Mascot Distiller results

Fibronectin (protein 260.1)
5 of 16 peptides met criteria for quant
L/H = 3.22
SD = 3.17

Peptide 4

Extracted ion chromatograms

Light
Heavy (K6R6)

[M+2H]2+
**Fibronectin**
(protein 260.1)

5 of 16 peptides met criteria for quant

$L/H = 3.22$
$SD = 3.17$

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<td>15</td>
<td>4.13</td>
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<td>16</td>
<td>3.22</td>
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Differences in protein expression of human cells infected with Marburg viruses

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<tr>
<th>L/H</th>
<th>Median</th>
<th>SD(geo)</th>
<th>#</th>
<th>Description</th>
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<tbody>
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<td>NAD(P)H dehydrogenase [quinone] 1 isoform a [Homo sapiens]</td>
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<td>0.59</td>
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<td>unnamed protein product [Homo sapiens]</td>
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<td>0.59</td>
<td>1.16</td>
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<td>ornithine aminotransferase, OAT</td>
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<td>1.11</td>
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<td>transketolase [Homo sapiens]</td>
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<td>chaperonin 10 [Homo sapiens]</td>
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<td>60 kDa heat shock protein, mitochondrial [Homo sapiens]</td>
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<td>drebrin E2 [Homo sapiens]</td>
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<td>human rab GDI [Homo sapiens]</td>
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<td>1.61</td>
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<td>microtubule-associated protein RP/EB family member 1 [Homo sapiens]</td>
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<td>11</td>
<td>cargo selection protein TIP47 [Homo sapiens]</td>
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<tr>
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<td>myosin-9 [Homo sapiens]</td>
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<td>2.73</td>
<td>1.09</td>
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<td>nestin, isoform CRA_c [Homo sapiens]</td>
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Pathway analysis of differential expression of proteins in human cells infected with Marburg viruses (SILAC data)

Bars indicate the total number of proteins (y-axis left) involved in the indicated pathway; green, upregulated in MARV-Ang; red, upregulated in MARV-Mus. The line graph indicates the assigned –log(p-value) ratios (y-axis right) assessed via IPA for each respective pathway.

Lethality
MARV-Ang > MARV-Mus
Differences in protein expression of human cells infected with Marburg viruses

<table>
<thead>
<tr>
<th>L/H</th>
<th>SD(geo)</th>
<th>#</th>
<th>Description</th>
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<tbody>
<tr>
<td>0.09</td>
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<td>VP30 [Lake Victoria marburgvirus - Angola2005]</td>
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<td>93.21</td>
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<td>97.45</td>
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<td>9.57</td>
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<td>253.20</td>
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Differences in protein expression of human cells infected with lethal viruses using spectral counting and SILAC

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<th>Ebola</th>
<th>Angola</th>
<th>Musoke</th>
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<td>fatty acid synthase</td>
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<td>26</td>
<td>21</td>
<td>23</td>
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<tr>
<td>beta-actin</td>
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<td>32</td>
<td>42</td>
<td>42</td>
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<tr>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
<td>13</td>
<td>14</td>
<td>19</td>
<td>20</td>
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</table>

Significant differences were found for each protein by ANOVA (Scaffold)

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<thead>
<tr>
<th>Marburg-Angola (H), Marburg-Musoke (L)</th>
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<td>Protein</td>
<td>A</td>
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<tr>
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<tr>
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<td>0.89 (1.41)</td>
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<tr>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1.06 (1.30)</td>
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