Draft Guidelines for Publication of Peptide and Protein Identification Data

Journal of Molecular and Cellular Proteomics Working Group on Publication Guidelines

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Publication Guidelines for Peptide and Protein Identification Data in MCP

Goals:

- try to insure that high quality, significant data are entering the proteomics literature
- develop minimal guidelines for publication of peptide and protein identification data in MCP
- Initial focus on how identifications were made and validated
- guidelines should not be burdensome nor should they dictate what tools to use
- Initiate discussion on requiring submission of data as a condition for acceptance of manuscript and logistics involved

Why are guidelines needed?

Dramatic increase in the number of large data set papers being published

- Lack of accepted and widely available computational tools for reviewers and readers to determine if results are valid
- Published studies often do not contain enough information for the reader to assess how the data was processed and what the criteria for identification were
- Lack of understanding and misuse of algorithms contribute to large false positive error rates
- Likely that we are publishing many incorrect interpretations

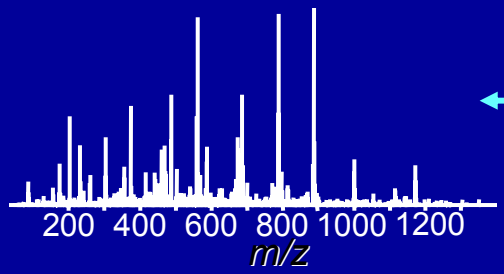
Why are guidelines needed?

- Finding a peptide match in a DB is easy, but knowing whether it is correct is not

MS/MS Database Search

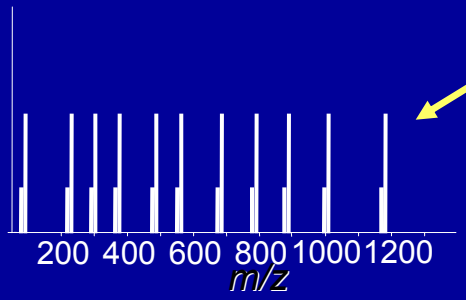
Acquired MS/MS spectrum

Sequence Database



correlate

Theoretical spectrum



ISLLDAQSAPLR
VVEELCPTPEGK
DLLLQWCWENGK
ECDVVSNTIIAEK
GDAVFVIDALNR
VPTPNVSVVDLTNR
SYLFCMENSAEK
PEQSDLRSWTAK

similarity score

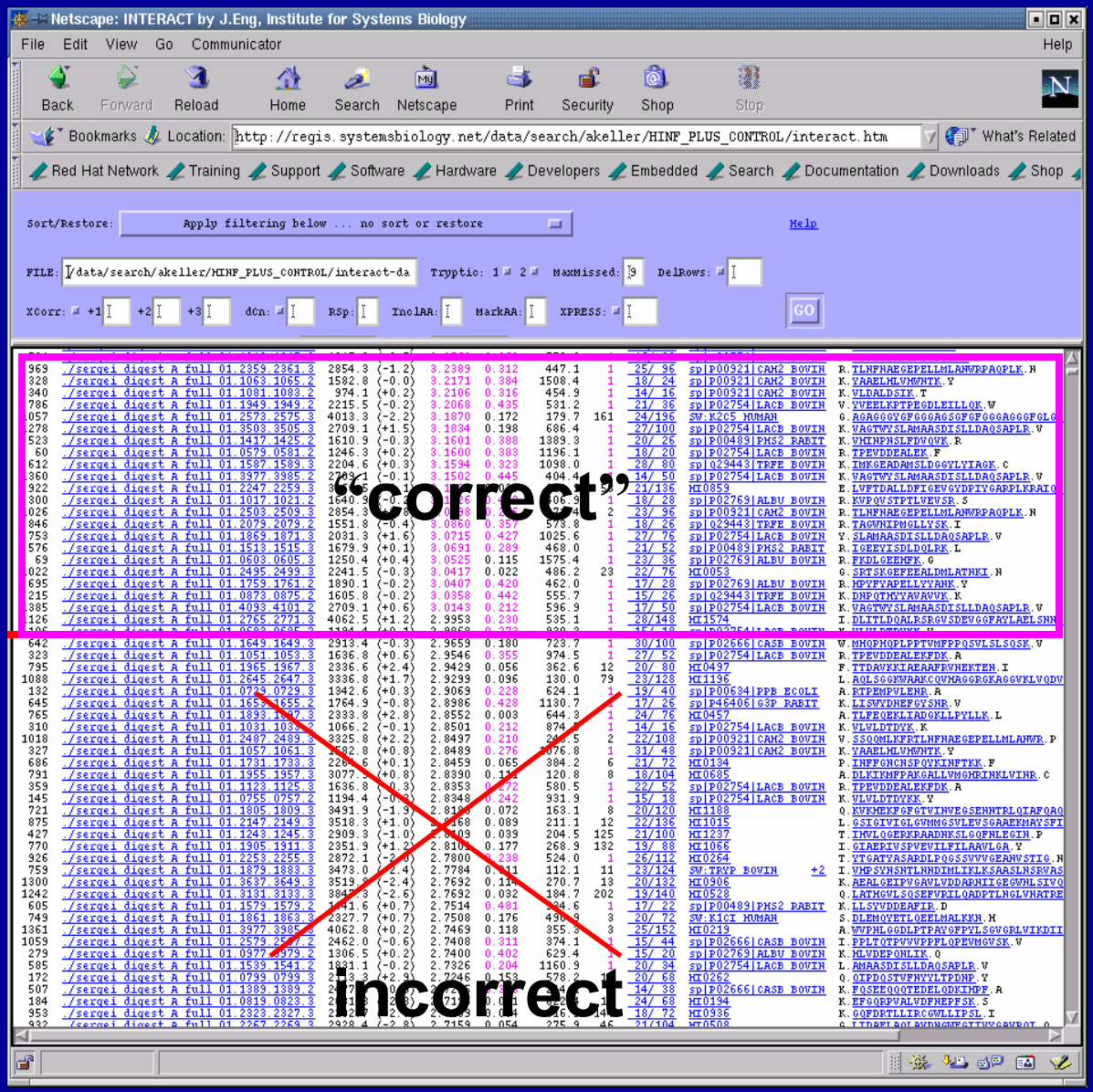
best matching database peptide

Algorithms: SEQUEST, Mascot, Sonar, SpectrumMill, …

best matching peptide in database may be correct or incorrect

Slide courtesy of Alexey Nesvizhskii, ISB

Threshold Model



threshold

SEQUEST:
Xcorr > 2.0
ΔC_n > 0.1

MASCOT:
Score > 30

Slide courtesy of Alexey Nesvizhskii, ISB

Why are guidelines needed?

- Finding a peptide match in a DB is easy, but knowing whether it is correct is not
  - It is almost always possible to match a MS/MS spectrum to a peptide in the database
  - Incorrect matches often (but not always) result from use of low quality peptide MS/MS data to search the database
  - Even high quality data can produce invalid identifications
    - actual peptide sequence is not in the database searched (under the search conditions used)

Why are guidelines needed?

- Unknown and variable false positive error rates are associated with each algorithm
  - Commercial algorithms uses thresholds and scoring methods to move most probable hit to top of list
  - Recommended settings are empirically derived and are not universally applicable
  - Use of conservative scoring and filtering thresholds reduces number of misassigned peptides and proteins, but does not eliminate false positives
  - Probability of a false positive assignment is much higher for “one-hit-wonders”
- statistical methods to validate peptide assignments to MS/MS spectra of peptides have shown promising results, but are not yet widely available or accepted

Publication Guidelines for Peptide and Protein Identification Data in MCP

Working group assembled January, 2004

- Ruedi Aebersold, ETH Zurich and Institute for Systems Biology
- Michael Baldwin, University of California, San Francisco
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- Steven Carr, Broad Institute of MIT and Harvard (Chair)
- Karl Clauser, Broad Institute of MIT and Harvard
- Alexey Nesvizhskii, Institute for Systems Biology
- Additional contributions from: Robert Chalkley, Kirk Hansen, Kati Medzihradszky, UCSF; Andrew Keller, ISB and Ron Beavis, Beavis Informatics, Ltd.

Guidelines published Mol. Cell. Proteomics June 2004; 3: 531.

Guideline 1

Describe search engine used and how peptide and protein assignments were made using that software

All papers must provide:

- The method and/or program used to create the “peak list” from raw data
  - note factors that affect the quality of the subsequent database search (e.g., smoothing, de-isotoping)
- Name and version of DB search program used and parameters used for its operation
  - include precursor-ion mass accuracy; fragment-ion mass accuracy; modifications allowed for; enzyme specified or not; any missed cleavages; etc.

Guideline 1, con't.

- Name and version of sequence database used
  - Include number of protein entries at time of search
- Scores used to interpret MS/MS data
- Thresholds and values specific to judging certainty of identification and description of how applied
- Describe any statistical analysis that was applied to validate the results and of how it was applied
  - e.g. reverse database search

Guideline 2

Provide sequence coverage observed for each protein identified

- the total number of peptides belonging to each protein must be explicitly stated (not # of MS/MS spectra)
- different forms of the same peptide are to be counted as only a single peptide
  - Differing charge states of same peptide or common sample handling artifacts (e.g., ox) all count as 1
- encourage providing tables that list sequences of all identified peptides/protein

Guidelines 3 and 4

Increase the stringency of information required to use single peptide identifications for protein assignment

Protein assignments based on single peptide assignments must include:

- the sequence of the peptide used to make each such assignment, together with the amino acids N- and C-terminal to that peptide's sequence
- the precursor mass and charge (not just m/z) observed
- the scores for this peptide

Guidelines 3 and 4, con't.

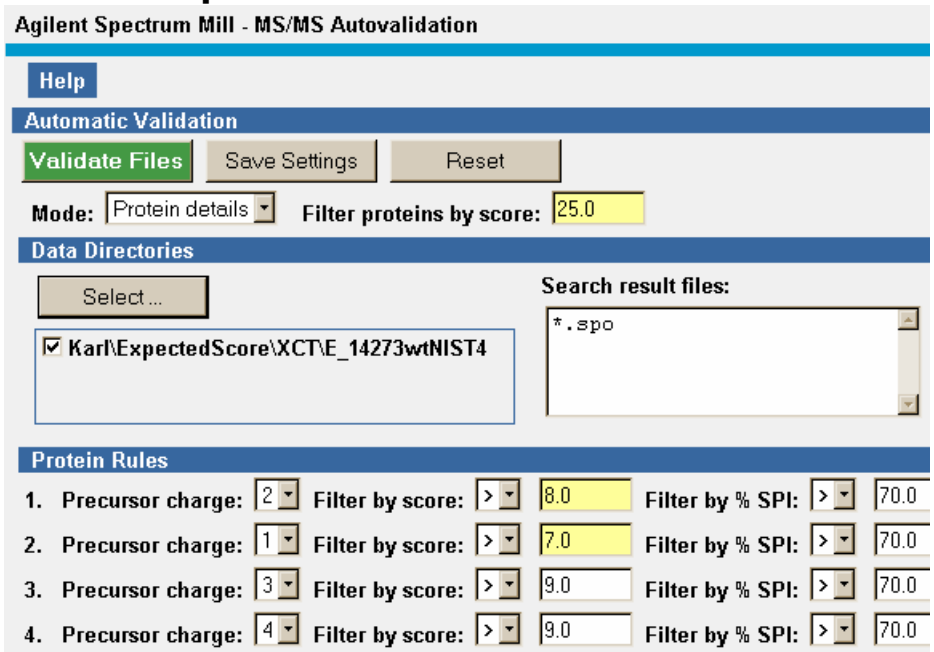
- Biological conclusions based on a single peptide id's or to a posttranslationally modified form of that protein, must be supported by inclusion of the MS/MS spectrum

- Single peptides from ICAT and similar experiments are covered by this guideline as well
  - For large ICAT datasets we have not yet required that spectra for all single-peptide id's be provided

We Use Separate Thresholds for 1-hit Wonders

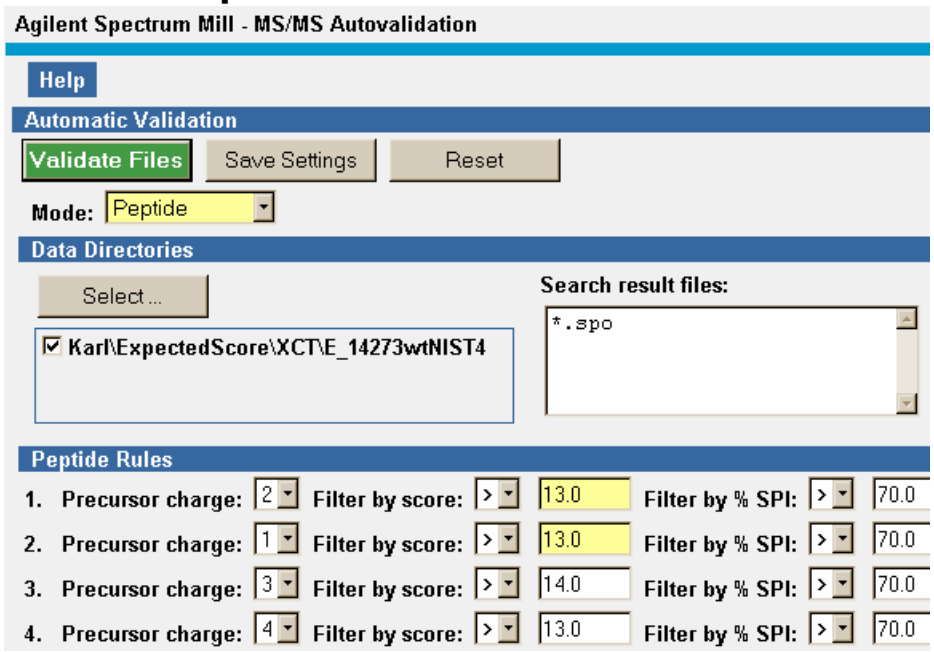
Step 1 - Protein Mode

- 2 or more peptides/protein
- Each spectrum: moderate or better score

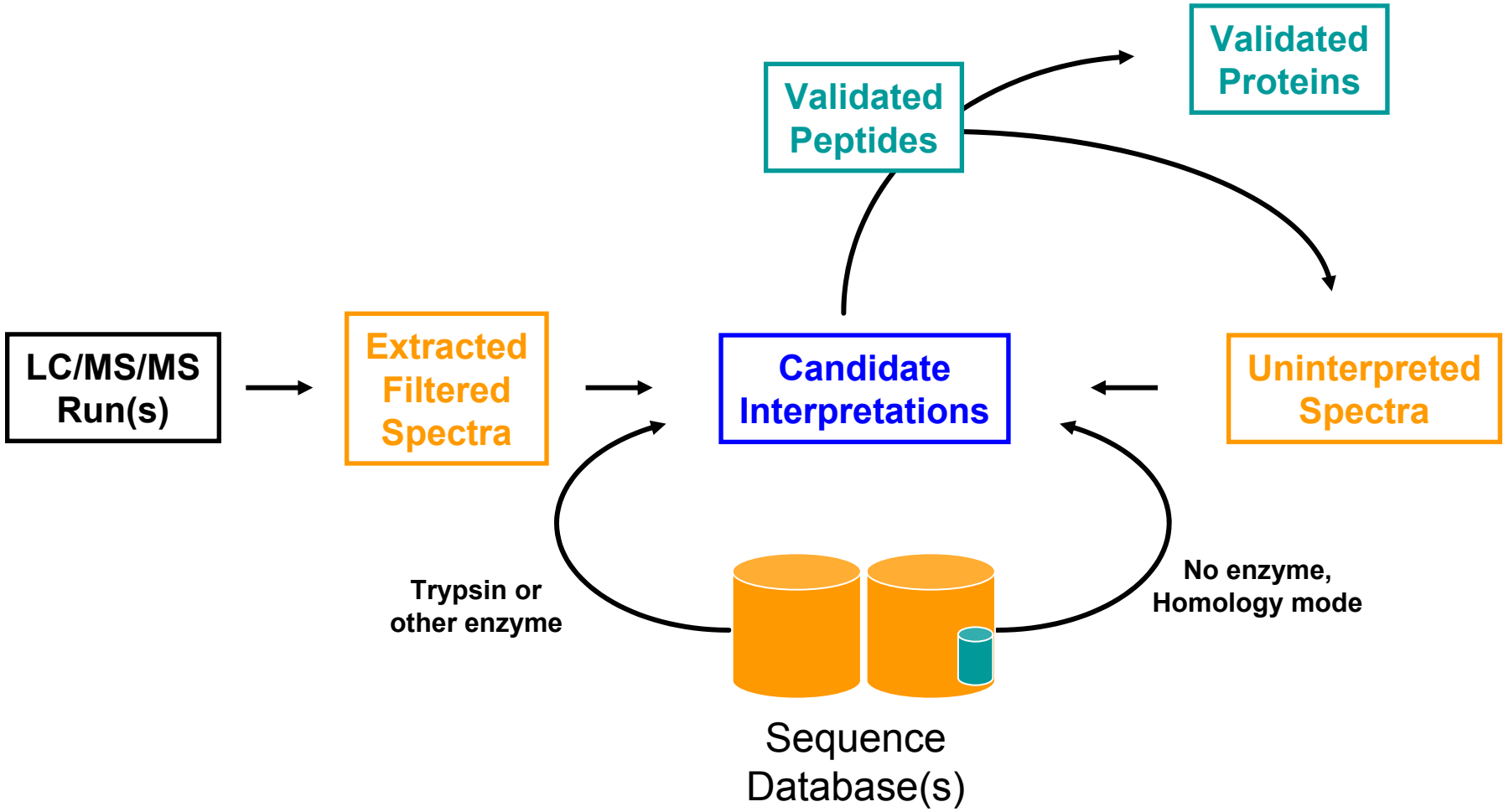


Step 2 - Peptide Mode

- 1 peptide/protein
- Each spectrum: excellent score

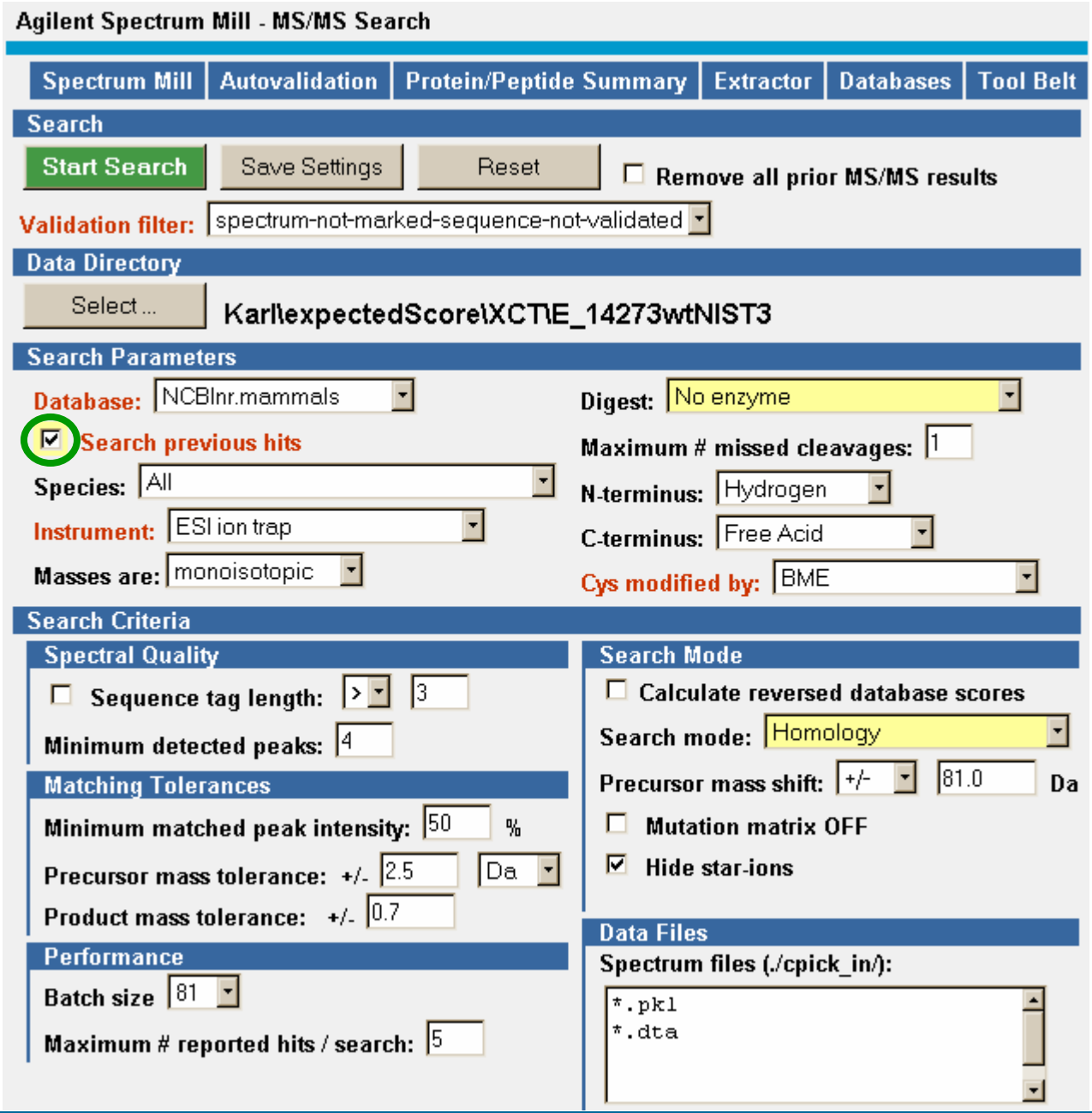


Disallow 1 Hit Wonders that are partial/non-tryptic



No enzyme and homology mode searching of remaining spectra

Using only the subset of proteins confidently identified from a previous trypsin search of the full database

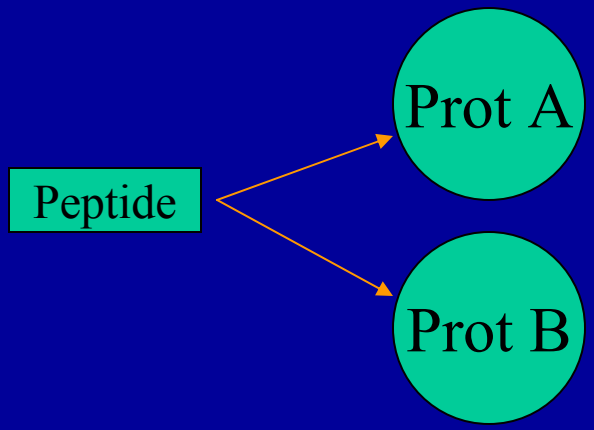


Guideline 6

How to count the number of unique proteins identified based on the peptides found

Protein Inference Problem

Degenerate peptides: correspond to more than a single entry in protein database



protein A or protein B ??

Or both?

In shotgun proteomics the connectivity between peptides and proteins is lost

Degenerate peptides are more prevalent with databases of higher eukaryotes due to the presence of:

- related protein family members
- alternative splice forms
- partial sequences

Slide courtesy of Alexey Nesvizhskii, ISB

Guideline 6

How to count the number of unique proteins identified based on the peptides found

Issue: same (or very similar) protein having different names and accession numbers in the database

– Authors must demonstrate that they are aware of the problem and have taken reasonable measures to eliminate redundancy

– When a single protein member of a multi-protein family has been singled out, explain how the other members of the group were ruled out, if at all

– If a protein from a different species than that studied is identified, then this must be mentioned and justified

Guideline 5

Peptide mass fingerprint data will continue to be accepted for peptide identification, but the standard of acceptability will be more stringent

- list the number of masses matched to the identified protein and the sequence coverage observed
- State the number of masses NOT matched
- Describe parameters and thresholds used to analyze the data (e.g., mass accuracy, res., how calibrated, etc.)
- Authors are encouraged to use and provide the results of scoring schemes which give measure of certainty of id, or perform some measure of false-positive rate

Guideline 7

MCP strongly encourages (but does not at present require) the submission of all MS/MS spectra mentioned in the paper as supplemental material.

- We will accept dta, pkl, mgf files

MCP is moving toward accepting and serving raw or minimally processed MS data, but we are not there yet

- Technical aspects of storing large repositories of raw mass spectrometric data has yet to be worked out
- Authors are encouraged to provide access to raw MS data using group websites etc.
  - **Not a viable, long-term solution. Public repositories are essential.**

Capacity Constraints on Repositories

File type	LCQ-Deca (centroid)	LTQ (centroid)	LTQ-FT (centroid)	QStar (profile)	Qtof (profile)
original/raw (MB)	15	65	200	75	500
Winzip compresses to (%)	71	83	83	50	50

Current/Future Utility Constraints on Readers/Reviewers

- Lowest common denominator currently is the original instrument vendor format.
    - Files contain all the interesting info in unprocessed form
        - parent peak intensities for quantitation
        - acquisition parameters

However…

- If repository stores original instrument vendor format, user needs instrument vendor's data system to read files

Current/Future Utility Constraints on Readers/Reviewers

- If repository stores XML format, then user needs compatible tools
  - –ISB provides converters from most instruments to mzXML and open source non-graphical mzXML reader
  - –mzData - similar XML format from HUPO, but no converters or readers available yet
- Will search engines support XML files?
- Will Instrument vendors formats continue to be compatible with XML converters?
  - Meetings like this need to have representatives from MS manufacturers present who are in decision-making capacity
- Will open source community provide viable graphical utilities for XML formats?
- Will they work on decreasing dataset size?

Next Steps

Meeting devoted to publication guidelines for proteomics data and data repository issues

Goals:

- to come up with an agreed upon set of standards for proteomics data publication/presentation
- to develop clear and actionable plans for data sharing with testable mechanisms to be put into place in 2005
  - journal editors
  - Tool developers
  - Instrument vendors
  - Power users

Coordinate with PSI-HUPO and other serious groups

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Over 15 million articles from over 4,500 PubMed journals, including 819,165 free full text articles from 779 HighWire-hosted journals