

Protein Identification — Database Search and FDR — Hands-on

Master the core steps of identifying proteins from LC–MS/MS data. This module walks through spectrum processing, database and FASTA preparation, search engine logic, target–decoy based FDR estimation, and validation workflows that yield high confidence peptide and protein identifications for downstream quantitative and functional analysis.

Protein Identification — Database Search and FDR

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Session 1

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From Raw Files to Search Inputs

LC–MS/MS raw data formats and conversion concepts

[vendor raw files \(overview\)](#) [mzML / mzXML / MGF](#)
[ideas](#) [profile vs centroid data](#)

Spectrum quality, charge states and precursor selection

[isotope patterns and monoisotopic peaks](#) [charge](#)
[state distribution](#) [simple spectrum QC ideas](#)

FASTA databases and search space definition

UniProt / reference proteomes (concepts)

contaminant and decoy entries (overview) **enzyme,**
missed cleavages, mass windows

Session 2

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Search Engines, Scoring & PSMs

Search engine families and workflows (conceptual)

Mascot / Sequest / Andromeda (overview) **MS-GF+,**
Comet and others (concepts) **search parameter files**

Peptide–spectrum matches and scoring logic

theoretical vs experimental spectra **score, e value,**
expectation value ideas **search tolerance and**
fragment mass error

Fixed and variable modifications in searches

carbamidomethyl Cys, oxidation Met **phospho**
(concept only here) **impact of too many variable**
mods

Session 3

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Protein Inference, FDR & Validation

Target–decoy strategy and FDR concepts

decoy generation ideas **PSM, peptide and protein**
level FDR **q values and score thresholds**

Protein inference and grouping principles

unique vs shared peptides **protein groups and**
leading proteins **parsimony ideas**

Validation and reporting of protein identification sets

1 percent FDR reporting conventions **minimum peptide and PSM filters** **export formats for downstream tools**

Session 4

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Mini Capstone: Search to Protein List

End to end walk through: from search setup to filtered identifications

Theory + Practical (guided)

Interpreting search result tables and QC plots (concepts)

score vs FDR curves **peptide and protein counts**
missed cleavages and modification patterns

Deliverables: parameter sheet, filtered protein list and QC summary note

search parameter template **exported peptide and protein tables** **one page QC and validation summary**