



Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure: Protein Identification following Data Dependent Acquisition (DDA) on LC/MS/MS

DToxS SOP Index: CO-1.0

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Quality Control (QC) steps are indicated with **green highlight**.

Metadata recording is highlighted with **yellow highlight and superscript indices**.

This SOP details computational procedures used following Protein Spectra Acquisition by Data Dependent Acquisition (DDA) on LC/MS/MS (DToxS SOP A-8.0)

1) Database Search:

- a. We use Proteome Discoverer (v. 1.4.12, Thermo Scientific, <http://www.thermoscientific.com/en/product/proteome-discoverer-software.html>) for generating the peak list, engaging a database search, and result filtering.
- b. Set the precursor mass range at 350-10,000 Da, and the S/N at 1.5 for peak filtering.
- c. Search the MS/MS spectra against the Swiss-Prot human database (Total 20,204 entries, we downloaded on 10/15/2015 from <http://www.uniprot.org/>) using both the Mascot search engine (V2.3) and the Sequest search engine. The Swiss-Prot database is updated every three months. The results are generated as .msf files. Important program options are set as follows:
 - i. Use Trypsin as the enzyme with 2 missed cleavages
 - ii. Set precursor mass tolerance to 10 ppm and the fragment mass tolerance to 0.1 Da
 - iii. Select protein N-terminus acetylation and methionine oxidation as variable modifications
 - iv. Select cysteine carbamidomethylation as a fixed modification.

- d. We use Scaffold software (V 4.4, Proteome Software, <http://www.proteomesoftware.com/products/free-viewer>) to display, filter, and merge the protein identification results from Proteome Discoverer.
- e. Accept protein and peptide identifications with false discovery rate less than 2%.
- f. Group proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone.
- g. Quantify relative protein abundance using normalized spectrum count.
- h. The raw data (.raw), Scaffold file (.sfd) and Excel table (.xlm) of protein identification and relative quantitation based on spectra counting are sent to Mount Sinai via dropbox.

Metadata

Mascot and Sequest search parameters: 1

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|----------------------------------|--|
| Protein Database: | Swiss-prot |
| Enzyme: | Trypsin |
| Maximum Missed Cleavage: | 2 |
| Taxonomy: | Homo sapiens |
| Precursor Mass Tolerance: | 10 ppm |
| Fragment Mass Tolerance: | 0.1Da |
| Dynamic Modifications: | Acetyl (Protein N-term) Oxidation (M) |
| Static Modification: | Carbamidomethyl (C) |