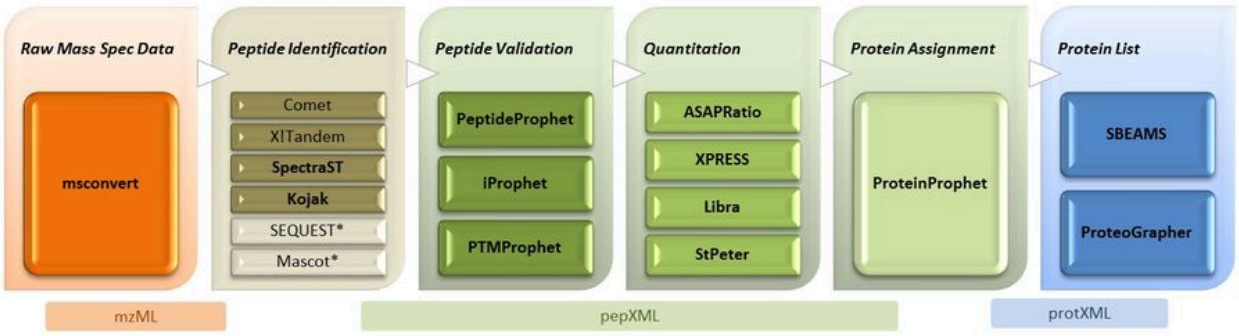
Analysis pipeline with xTandem



Follow these steps to convert, search, and analyze your x**Tandem** data:

1. RAW to mzML Conversion
Convert original .RAW files to the standard mzML input format used by the tools
2. Edit Tandem Default Parameters, if necessary
It is recommended that you copy the default input file to your local directory, and edit it as necessary
3. Peptide Database Search and Identification
Perform X-Tandem search.
4. Conversion to pepXML
Convert original search results to the pepXML input format used by TPP
5. Data Curation and (optional) Peptide Validation and Quantification
Use Xinteract and PepXMLViewer to filter, sort, group, and highlight data based on various criteria.
You can also validate peptide identifications using PeptideProphet, and further refine peptide-level analysis and combine results from multiple search engines using iProphet.
Calculate the relative abundances of peptides and proteins, and the corresponding confidence intervals, from isotopically-labeled data, using ASAPRatio and/or XPRESS. Or use Libra to do this for isobarically-labeled data (e.g. iTraq).
6. Protein Assignment and Validation
ProteinProphet provides a statistical model for validation of peptide identifications at the protein level.

Typical run for IDIRT

We followed the SILAC pipeline provided here.

TPP is normally installed in **c:/TPP.** The raw files were saved under **c:/TPP/data/** and all the results were saved in **c:/TPP/data/params** . You have to change the directories if you save the data in different folders.

Raw files can be find here:
https://rockefeller.box.com/s/8siuuxolaefafwcw3yzb2h6xsiv7w7er

Shell command could be wrapped in shell("..") in Rstudio
Tandem parameter file
Tpp_run.R

Or it could be run in command line as below:

1. Convert raw files to mzml:

"c:\Program Files (x86)\ProteoWizard 3.0.1908 64-bit\msconvert" c:/TPP/data/1a1.raw -v --mzML -o c:/TPP/data

Run the following command for all the raw files. For example for **1a1**

2. Running tandem:

   cd c:/TPP/data && c: && C:/TPP/bin/Tandem2XML c:/TPP/data/params/**1a1**.tandem c:/TPP/data/params/1a1.tandem.pep.xml
   cd c:/TPP/data && c: && C:/TPP/bin/tandem c:/TPP/data/params/**1a1**.tandem.params

3. Running peptideProphet, iProphet, ASAPRatio:

   cd c:/TPP/data/params && c: && C:/TPP/bin/xinteract -N**1a1**.interact.pep.xml -p0.0 -l2 -PPM -OA -i -A-IMKR-r0.05 **1a1**.tandem.pep.xml

4. Running proteinProphet:

   cd c:/TPP/data/params && c: && C:/TPP/bin/ProteinProphet c:/TPP/data/params/**1a1**.interact.ipro.pep.xml c:/TPP/data/params/**1a1**.interact.prot.xml IPROPHET ASAP_PROPHET