Bioinformatics for Proteomics

Mass spectrometry based proteomic studies typically aim at measuring and comparing changes in biological samples. This however strongly relies on our ability to interpret the massive amounts of data produced by modern mass spectrometers. Therefore, methods and tools were developed tackling four major topics:

1. Identification
2. Quantification
3. Mapping of the results with biological knowledge
4. Interact with public repositories

The present tutorial aims at providing the basis for any user to go through this workflow: (A) identify peptides, proteins and their modifications, (B) quantify the identified, (C) interact with external resources to bring biological knowledge and (D) share data using online repositories and reprocess public data. Notably, we will only use user friendly and open source software applicable to any kind of mass spectrometer. Despite our efforts however, such software or the resources they rely on are not always available yet. For example, proteomics resources are heavily focused on a few model organisms – investigating other species can become challenging very fast. If you encounter issues not tackled by the present tutorial, feel free to contact the authors.

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There are several tools that partly or fully support such workflows. A detailed list mainly focused on free software is given in the appendix below. Among these, we recommend the use of the following tools:

1. To convert raw files we recommend **MSConvert** as part of the **Proteowizard[1](#_ENREF_1" \o "Kessner, 2008 #240)** package (<http://proteowizard.sourceforge.net>). We recommend **OpenMS[2](#_ENREF_2" \o "Sturm, 2008 #2)** for files manipulation and **TOPPAS**[**3**](#_ENREF_3) for workflow design (<http://openms.de>).
2. We recommend **UniProt[4](#_ENREF_4" \o "Apweiler, 2004 #2)** ([www.uniprot.org](file:///C:\Users\hba041\Teaching\WT%20Course%20EBI%202012\www.uniprot.org)) databases and for their manipulation **dbtoolkit[5](#_ENREF_5" \o "Martens, 2005 #19)** (<http://dbtoolkit.googlecode.com>).
3. To match peptides to spectra, we will here use two distinct, freely available search engines: **OMSSA**[**6**](#_ENREF_6) and **X!Tandem[7](#_ENREF_7" \o "Craig, 2004 #46)**, both of which are made easily accessible *via* a free tool called **SearchGUI[8](#_ENREF_8" \o "Vaudel, 2011 #18)** ([http://searchgui.googlecode.com](http://dbtoolkit.googlecode.com)).
4. To analyze the search results, and to do the peptide and protein inference, we recommend the use of **PeptideShaker** (<http://peptide-shaker.googlecode.com>).
5. For the validation of the identifications we also recommend the use of **PeptideShaker** (<http://peptide-shaker.googlecode.com>).
6. Many external resources are available on the internet. Among these we will use:   
   **UniProt**[4](#_ENREF_4" \o "Apweiler, 2004 #2) (<http://www.uniprot.org>), **Reactome**[9](#_ENREF_9" \o "Haw, 2011 #139) (<http://www.reactome.org>),   
   **PICR**[10](#_ENREF_10) (<http://www.ebi.ac.uk/Tools/picr>) and **Dasty**[11](#_ENREF_11" \o "Jones, 2005 #81) (<http://www.ebi.ac.uk/dasty>).   
   Note that additional resources are listed in **PeptideShaker**, and will also be used to conduct the gene ontology analysis of the data.
7. In order to make your data publicly available, you can upload them in public repositories. We recommend **ProteomeXchange** (<http://proteomexchange.org>) and **PRIDE**[**12**](#_ENREF_12) (<http://www.ebi.ac.uk/pride>).

You will find a folder named software containing all the software needed for this tutorial as well as folders corresponding to the different chapters. Although it is recommended to follow the tutorial in its entirety, the chapters can be followed independently. For every chapter, the resources folder contained in the chapter folder provides all the files needed. If you experience any difficulty with a program, feel free to contact the developers. We will also welcome any feedback on these tutorials.

***All chapters are also available online:*** [***http://compomics.com/bioinformatics\_for\_proteomics\_tutorial***](http://compomics.com/peptide_and_protein_identification_tutorial)

Appendix: Proteomics Software

The concepts and methods introduced in the present tutorial can be applied to other software and packages. The table below provides a (non-exhaustive) list of software dedicated to proteomics, with brief descriptions and corresponding references that will help you to get started.

|  |  |  |
| --- | --- | --- |
| Type | Software | Description |
| Converter | ProteoWizard[1](#_ENREF_1" \o "Kessner, 2008 #240) | Converter accepting most mass spectrometer proprietary formats and converting them into open formats |
| mzML parser | jmzML[13](#_ENREF_13" \o "Cote, 2010 #150) | Mass spectrometry mzML file parser |
| General proteomics package | OpenMS[14](#_ENREF_14" \o "Bertsch, 2011 #15) | Package of tools for proteomics allowing the design  of workflows with a graphical interface |
| TPP[15](#_ENREF_15) | Package of tools for proteomics mainly command line driven |
| MaxQuant[16](#_ENREF_16" \o "Cox, 2008 #103) | Package for identification and quantification  of entire proteomes |
| PeptideShaker\* | Interpretation of proteomics identifications  from multiple search engines |
| Identification post-processor | MassSieve[17](#_ENREF_17" \o "Slotta, 2010 #128) | Identification processing software |
| De novo sequencing | PepNovo[18](#_ENREF_18" \o "Frank, 2005 #97) | De novo sequencing tool |
| PEAKS[19](#_ENREF_19) | De novo sequencing tool (commercial) |
| Tag sequencing | GutenTag[20](#_ENREF_20" \o "Tabb, 2003 #191) | Finds peptide patterns in spectra |
| DirecTag[21](#_ENREF_21" \o "Tabb, 2008 #190) | Finds peptide patterns in spectra |
| Database search engine | Sequest[22](#_ENREF_22" \o "Yates, 1995 #99) | Database search engine (commercial) |
| Mascot[23](#_ENREF_23) | Database search engine (commercial) |
| OMSSA[6](#_ENREF_6) | Database search engine |
| X!Tandem[7](#_ENREF_7" \o "Craig, 2004 #46) | Database search engine |
| Morpheus[24](#_ENREF_24) | Database search engine |
| Inspect[25](#_ENREF_25) | Database search engine |
| MyriMatch[26](#_ENREF_26) | Database search engine |
| MassWiz[27](#_ENREF_27" \o "Yadav, 2011 #101) | Database search engine |
| MS Amanda | Database search engine (Proteome Discoverer only) |
| Andromeda[28](#_ENREF_28) | Database search engine (MaxQuant only) |
| User friendly interfaces | SearchGUI[8](#_ENREF_8) | Graphical interface for search engines |
| PRIDE Inspector[29](#_ENREF_29) | Graphical interface for the inspection of PRIDE XML files |
| TOPPAS[30](#_ENREF_30) | Graphical interface for the design of OpenMS workflows |
| Spectral library searching | NIST MS search[31](#_ENREF_31) | Spectral libraries search engine |
| X!Hunter[32](#_ENREF_32" \o "Craig, 2006 #106) | Spectral libraries search engine |
| SpectraST[33](#_ENREF_33" \o "Lam, 2007 #108) | Spectral libraries search engine |
| Identification file parsers | MascotDatFile[34](#_ENREF_34" \o "Helsens, 2007 #59) | Java parser for Mascot .dat files |
| OMSSA parser[35](#_ENREF_35) | Java parser for OMSSA .omx files |
| X!Tandem parser[36](#_ENREF_36) | Java parser for X!Tandem XML files |
| Data structure | compomics-utilities[37](#_ENREF_37) | Java object structure for the handling and visualization  of identifications from different search engines |
| PSM rescoring | Percolator[38](#_ENREF_38) | Machine learning algorithm rescoring  PSMs and attaching them a p-value |
| PeptideProphet[39](#_ENREF_39" \o "Keller, 2002 #27) | Machine learning algorithm attaching  PSMs a PEP (integrated in TPP) |
| PepArML[40](#_ENREF_40" \o "Edwards, 2009 #179) | Machine learning algorithm merging results from  different search engines with web interface: https://edwardslab.bmcb.georgetown.edu/pymsio |
| Database manipulation | dbtoolkit[5](#_ENREF_5" \o "Martens, 2005 #19) | Tool allowing the manipulation of databases  and creation of custom ones |
| Peptide inference | iProphet[41](#_ENREF_41" \o "Shteynberg, 2011 #126) | Tool for statistical post-processing of PSMs  (integrated in TPP) |
| Protein inference | ProteinProphet[42](#_ENREF_42" \o "Nesvizhskii, 2003 #10) | Tool for protein inference (integrated in TPP) |
| IDPicker[43](#_ENREF_43" \o "Ma, 2009 #127) | Tool for protein inference |
| MassSieve[17](#_ENREF_17" \o "Slotta, 2010 #128) | Identification processing software |
| Protein annotation | UniProtKB[4](#_ENREF_4" \o "Apweiler, 2004 #2) | Protein knowledge database |
| Dasty[11](#_ENREF_11" \o "Jones, 2005 #81) | Cross reference tool for protein databases |
| GO enrichment | GOTree[44](#_ENREF_44" \o "Zhang, 2004 #134) | GO enrichment tool |
| Onotologizer[45](#_ENREF_45" \o "Bauer, 2008 #135) | GO enrichment tool |
| DAVID[46](#_ENREF_46) | Interface for enrichment of identification results |
| 3D structures | jmol[47](#_ENREF_47" \o "Hanson, 2010 #138) | Tool for the display of 3D structures |
| Pathways | Reactome[9](#_ENREF_9" \o "Haw, 2011 #139) | Pathway investigation interface allowing the mapping  of one’s results and pathway coverage estimation |
| Interactions | STRING[48](#_ENREF_48) | Protein interaction investigation interface |
| Repository | PRIDE[12](#_ENREF_12) | Protein identification repository |
| PeptideAtlas[49](#_ENREF_49" \o "Deutsch, 2008 #142) | Peptide identification repository |
| GPMDB[50](#_ENREF_50) | Peptide and protein identification repository |
| Quality control | SimpatiQCo[51](#_ENREF_51" \o "Pichler, 2012 #1) | Quality Control for proteomics |
| Local data management | MASPECTRAS[52](#_ENREF_52) | LIMS system |
| Proteios[53](#_ENREF_53" \o "Hakkinen, 2009 #160) | LIMS system |
| ms\_lims[54](#_ENREF_54" \o "Helsens, 2010 #161) | LIMS system |

*\* PeptideShaker is not yet published, available at* [*http://peptide-shaker.googlecode.com*](http://peptide-shaker.googlecode.com)*.*

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