**reSpect – Tutorial version 0.1**

For this tutorial we will be using the Yeast Orbitrap data that we have analyzed in the PeptideProphet and iProphet tutorials.

The data were already converted to mzML using msconvert. The search results were searched using comet and X!Tandem, processed individually by PeptideProphet and combined using iProphet. This tutorial starts by running reSpect on the iProphet combined pepXML results.

1. Ensure that you are connected and have the latest Trans-Proteomic Pipeline (TPP)

* This tutorial is written with details for TPP on a Microsoft Windows operating system. If you have a different kind of operating system, this tutorial should still work, but the details of the installation and some file path locations will be somewhat different
* Ensure that you are Internet connected for this tutorial because you will download files
* This tutorial requires that you have TPP 5.2.0 or later installed on your system.
* If you have a pre-5.2.0 version of TPP already installed on your system, uninstall the older version first by clicking [Start] [Trans-Proteomics Pipeline] [Uninstall TPP]
* If TPP 5.2.0 or greater is not installed on your system, install that first by downloading and following the instructions at <http://tools.proteomecenter.org/wiki/index.php?title=TPP:5.2_Installation>. Note that you will need to restart your computer or manually start the Apache web server service

1. Launch the TPP web browser Graphical User Interface

* [Start] [Trans-Proteomic Pipeline] [TPP Web Interface] or open a web browser to: <http://localhost:10401/tpp>
* Login with the username ‘guest’ with password ‘guest’ (or alternate account if you have one)

1. In the web browser GUI, download the tutorial data:

* Click the [Files] tab at the top
* In the bottom right, create new directory: tutorials
* Click [TPP Tools] [fetch datasets]
* Click on [Show version information and available features]
* If there is a newer version, click on [Update to the latest version of fetchDataset]. Version 0.8.1 or greater is required
* Click [TPP Tools] [fetch datasets] again
* Click (Add Files)
* Checkmark the “tutorials” directory and click (Select)
* Paste the following URL into the [Dataset Identifier or URL] box:

http://www.tppms.org/tools/respect/RESPECT.zip

* Click (Fetch Dataset)
* Monitor the job by clicking [Refresh] until download and unzip is complete

1. Run reSpect on the example dataset

* Click [TPP Tools]:[Analysis Pipeline:Reanalyze Spectra]
* Click on (Add Files), and navigate to ***tutorials/reSpect/combined***
* This directory contains the iProphet pepXML results: ***interact.ipro.pep.xml***
* Checkmark interact.ipro.pep.xml and click (Select)
* Click on (Run reSpect*)*
* Click on [Refresh] to monitor progress. (About 1 minute on a modern computer)
* Ensure that the job did not end with an error
* The results of this command will be created in the ***reSpect/data*** directory containing the Original mzML data. The reSpect results append an “\_rs” suffix to the Original file’s basename and the will have the names:
  + OR20080317\_S\_SILAC-LH\_1-1\_01\_rs.mzML
  + OR20080320\_S\_SILAC-LH\_1-1\_11\_rs.mzML

1. Run Comet on the reSpect generated data file
   * Create directory for the Comet search of the reSpect results, and create a comet.params file for searching reSpect results.
   * Open [Files]
   * Navigate to directory: c:/TPP/data/tutorials/reSpect/
   * In [SUB-DIRECTORIES]:[Create new directory] type the new directory name “comet\_rs” and click (Create new directory)
   * Navigate to directory: c:/TPP/data/tutorials/reSpect/comet
   * Checkmark comet.params and click (Copy)
   * Navigate to directory: c:/TPP/data/tutorials/reSpect/comet\_rs and click (Paste)
   * Click (Edit) and make the following changes to the params file C:/TPP/data/tutorials/reSpect/comet\_rs/comet.params
     + peptide\_mass\_tolerance = 3.1
     + peptide\_mass\_units = 0
     + isotope\_error = 0
   * Click (Save Changes)
   * Open [TPP Tools]:[Comet Search] and navigate to c:/TPP/data/tutorials/reSpect/data/
   * In “Please Choose Files”, select files OR20080317\_S\_SILAC-LH\_1-1\_01\_rs.mzML file and OR20080320\_S\_SILAC-LH\_1-1\_11\_rs.mzML (note the **\_rs**) and click (Select)
   * In “Choose Comet Parameters File” click (Add Files), navigate into “comet\_rs” subfolder and then checkmark the comet.params file and click [Select]
   * Next Choose a sequence database. Go up to reSpect folder, then into dbase folder. Checkmark uniprot\_Scerevisiae\_sep052014\_CONTAMINANTS\_RAND.fasta and click [Select]
   * Click on (Run Comet Search)
   * Click on [Refresh] to monitor progress. (About 40 to 60 minutes on a modern computer using 8 threads, longer on less powerful computers.)
   * Ensure that the job did not end with an error
2. Run the Prophets on the Comet results
   * Open [TPP Tools]:[Analyze Peptides]
   * Remove any selected input files if present by checkmarking them and clicking [Remove]
   * Navigate to the reSpect/comet\_rs folder
   * Checkmark files OR20080317\_S\_SILAC-LH\_1-1\_01\_rs.pep.xml file and OR20080320\_S\_SILAC-LH\_1-1\_11\_rs.pep.xml and click [Select]
   * Checkmark “Only use Expect Score as the discriminant …”
   * Checkmark “Use decoy hits to pin down the negative distribution.”
   * Next to “Decoy protein names begin with:” type **RAND0**
   * Checkmark “Use Non-parametric model (can only be used with decoy option)”
   * Under iProphet options, select “RUN iProphet”
   * Click on (Run XInteract)
   * Click on [Refresh] to monitor progress. (About 1 minute on a modern computer)
   * Ensure that the job did not end with an error
3. Explore the results
   * Click on the [PepXML] link next to interact.pep.xml (first output file)
   * Click on a probability and examine the models. Do they seem reasonable?
   * What is the probability threshold, number correct and incorrect at 1% FDR?
   * Close the model viewer and PepXML Viewer tabs
   * Click on the [PepXML] link next to interact.ipro.pep.xml (second output file)
   * Click on a probability and examine the models. Do they seem reasonable?
   * What is the iProphet probability threshold, number correct and incorrect at 1% FDR?
4. Compare the peptides identified at 1% FDR in the Original analysis to the peptides identified at 1% FDR in the reSpect analysis.
   * Open [Files]
   * Navigate to directory: c:/TPP/data/tutorials/reSpect/combined
   * Open the pepXML link to view the file interact.ipro.pep.xml in a new tab
   * Navigate to directory: c:/TPP/data/tutorials/reSpect/comet\_rs
   * Open the pepXML link to view the file interact.ipro.pep.xml in another new tab
   * In each analysis, identify the minimum probability required to reach a model estimated 1% FDR in each analysis.
   * Filter each analysis on the minimum probability for 1% FDR (identified in the previous step) or 50% probability, whichever is higher.
   * In the [PepXMLViewer]:[Other Actions] tab click (Export Spreadsheet). Do this for both analyses.
   * In a new tab in the web browser navigate to link: <http://bioinfogp.cnb.csic.es/tools/venny/>
   * Using Windows Explorer in Windows, open the Original analysis spreadsheet you’ve generated in Excel.
     + C:\TPP\data\tutorials\reSpect\combined\interact.ipro.pep.xls
   * Copy the peptides listed in Column E (exclude the header) to “List 1” on the Venny webpage.
     + Rename “List 1” to “Original.”
   * Close the file opened in Excel.
   * Using Windows Explorer in Windows, open the reSpect analysis spreadsheet you’ve generated in Excel.
     + C:\TPP\data\tutorials\reSpect\comet\_rs\interact.ipro.pep.xls
   * Copy the peptides listed in Column E (exclude the header) to “List 2” on the Venny webpage.
     + Rename “List 2” to “reSpect.”
   * How many unique peptides were identified in the Original search?
   * How many unique peptides were identified in the reSpect search?
   * Of the unique peptides identified in the reSpect search, how many were not seen in the Original search?
   * Explore a spectrum for a peptide missed in the Original analysis but found in the reSpect analysis: TATYDGEEGILAAK.
   * In the [PepXMLViewer]:[Filtering Options] for the reSpect analysis, in “Required peptide text (regex allowed):” enter TATYDGEEGILAAK, and click (Update Page)
   * Which spectrum matched this peptide?
   * In the [PepXMLViewer]:[Filtering Options] for the Original analysis, in “Required spectrum text (regex allowed):” enter the spectrum name of this spectrum, making sure to remove the “\_rs” suffix from the reSpect names for the same spectrum.