SpectraST Tutorial

2018-09-20

1) Start the TPP Petunia web interface via the TPP tray or <http://localhost:10401/tpp>

- Login with ‘guest’ and the password

2) Verify the necessary spectral library files

- [Files]

- Go into the ‘class’ folder

- Go into the ‘dbase’ folder

- Go into the ‘speclibs’ folder

- You should see three “YeastNIST\_SILAC\_DECOY\*” files

3) Create a fresh working directory for a SpectraST search

- [Files]

- Go to /data/class/Yeast

- [Create new directory] spectrast

- Enter spectrast folder

4) Search the two mzML files with the provided library (light + heavy + decoys)

- Select SpectraST pipeline

- [TPP Tools] [SpectraST Search]

- Select the two mzML files in C:\TPP\data\class\Yeast\data

- Select the YeastNIST\_SILAC\_decoy library

- Select the sequence database file ‘uniprot\_Scerevisiae\_sep052014\_CONTAMINANTS\_RAND.fasta’ in /local/class/dbase

- In Output control, set output to: C:\TPP\data\class\Yeast\spectrast (as created in step 3)

- [Run SpectraST] (Will take ~1.5 minutes on a modern computer)

5) Run PeptideProphet

- [TPP Tools] [Analyze Peptides]

- Add the two new pep.xml files from step 4

- Select only the ‘Use accurate mass binning’ option (with default PPM)

- [Run Xinteract] (Will take ~1 minute on a modern computer)

6) Examine the results and models. How many total correct at 1% FDR? How does that compare to the Comet results?

7) Create an iProphet directory next to the spectrast directory and combine the Comet results and the spectrast results with iProphet. How many total correct at 1% peptide-level FDR

Finis!