

Xpress and ASAPRatio -- Tutorial

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Running Quantitation Tools - Please do the first three steps of the tutorial **before** the Quantitation lecture

- Using Petunia go to the “Analyze Peptides” tab. Add the following files to the analyses:
 - `c:\Inetpub\wwwroot\ISB\data\class\Quantitation\xtandem-k\OR20080317_S_SILAC-LH_I-I_01.pep.xml`
 - `c:\Inetpub\wwwroot\ISB\data\class\Quantitation\xtandem-k\OR20080317_S_SILAC-LH_I-I_11.pep.xml`
- In the “PeptideProphet Options” pane select to “Use accurate mass binning” and “Run ProteinProphet Afterwards”. In the “XPRESS Options” pane select “RUN XPRESS”. Change “XPRESS Mass Tolerance” to **0.1**, set “Change XPRESS residue mass difference:” with **K 8.0142** and **R 10.0083**. In the “ASAPRatio Options” pane select “RUN ASAPRatio”. Change “Labeled Residues” to **K** and **R**, set “m/z range to include in summation of peak:” to **0.05**. Set “Specified masses:” to **M 147.035**, **K 136.10916** and **R 166.10941**. Check the “Use fixed scan range” option.
- To run this analysis Click on “Run XInteract”. When the program is finished the results can be accessed through files “`c:\Inetpub\wwwroot\ISB\data\class\Quantitation\xtandem-k\semitryptic\interact.pep.shtml`” and “`c:\Inetpub\wwwroot\ISB\data\class\Quantitation\xtandem-k\semitryptic\interact.prot.shtml`”.

For the next part of this tutorial open the file: `c:\Inetpub\wwwroot\ISB\data\class\Quantitation\xtandem-k\semitryptic\interact.pep.shtml`

- In the viewer, in the “Pick Columns” tab add the “massdiff” column to “columns to display”. Next, on the “Summary” tab set a **minimum probability of 1**, and sort the spectra by “peptide” in “descending” order.
- Click on the XPRESS ratio for spectrum - **OR20080320_S_SILAC-LH_I-I_01.03002.03002.2** (index 855 near the bottom of page 1). The page displayed shows the reconstructed chromatogram for the identified peptide. The raw signal is shown as vertical triangles, the smoothed chromatogram is displayed as a dotted line, and the region of the chromatogram used for quantitation is highlighted in red on the raw signal and blue on the smoothing curve. By default the program quantitates the chromatogram of the charge state in which the peptide was identified. In the top panel select a different charge state (change the Z parameter) and click the “Quantitate”. What happens to the peak when you select charge state +3? What happens to the peak when you select charge state +1?
- Go back to the PepXML Viewer, and click on the ASAPRatio link for the same spectrum. What is the ratio for this peptide, according to ASAPRatio? How does it compare to the one reported by XPRESS Unlike XPRESS, ASAPRatio tries to quantitate using chromatograms from all charge

states where it can find a decent signal; in this case, it is using +2 and +3, even though the identification was made in the +2 charge state. Look at the data for the rest of the charge states; did ASAPRatio do a good job of rejecting bad signals? You may have also noticed that ASAPRatio used a much larger scan range to quantitate than Xpress; this is due to differences in the way these programs smooth the data to determine elution peaks. We'll learn how to adjust these boundaries below.

7. Close the ASAPRatio screen, as well as the Xpress one if still open, without saving.
8. Using Petunia file browser open the file: **c: \ Inetpub \ wwwroot \ ISB \ data \ class \ Quantitation \ xtandem-k \ semitryptic \ interact.prot.shtml**.
9. Click on the Xpress ratio for protein **YAL044C** (the 4th entry). How many peptide ratios contribute to the ratio of this protein? [3] Adjust the mass tolerance and the peak boundaries for the spectrum IDs that don't have a reasonable quantitation peaks displayed. For spectra that don't show reasonable elution profiles, set the ratios to unknown by clicking on the question mark button below the lower left corner of the chromatogram image. Do not close the Xpress pages, after changing each Peptide Ratio, refresh the Protein Ratio page and make sure the change gets correctly recorded, then go on to the next peptide ratio. What is the Xpress Protein Ratio and Error after you've corrected the peaks?
10. Click "Update ProteinProphet ratio" button on the Protein Ratio page. Close the Protein Ratio page and refresh the ProteinProphet page.
11. Now let's look at the ASAPRatio analysis; click on the ASAPRatio link for this protein. What is the protein ratio as evaluated by ASAPRatio? What is the normalized protein ratio? What is the protein p-value? How many peptide sequences contribute to the protein ratio?
12. Click on the p-value link. What is the computed mean peptide ratio in this dataset? What is the standard deviation?
13. Click on "[Expand All]" under the peptide sequence "LGEGVNVEQVEGLMSLEQYEK." How many independent LC peaks were detected for the peptide? How many times was the peptide identified? In what isotopic forms and charge states was the peptide identified? What are the peptide ratios at those identifications? Why are the last two peptide ratios so similar? What is the unique peptide ratio? What is the CV? How was the CV calculated?
 - a. Click on the Peptide Ratio link of the identification **OR20080317_S_SILAC-LH_I-I_01.09309.09309.2**. In what isotopic form and charge state was the peptide identified? In what charge states were signals of the peptide detectable? What were the charge states that contributed to the calculation of peptide ratio? Adjust the peaks and charge states used to compute the peptide ratio to reduce the error and save the Interim Ratio you are happy with.
 - b. Click on the Peptide Ratio link of the identification **OR20080320_S_SILAC-LH_I-I_11.09396.09396.2**. In what isotopic form and charge state was the peptide identified? In what charge states were signals of the peptide detectable? What were the charge states that contributed to the calculation of peptide ratio?

- c. Refresh the “ASAPRatio: Protein Ratio” page, what happens to the error in the Interim Protein Ratio? [becomes smaller]
14. Now let's look at the ASAPRatio analysis for protein **YAL012W**. What is the protein ratio as evaluated by ASAPRatio? How many independent peptides contribute to the evaluation? What is the normalized protein ratio? What is the protein p-value?
15. Which peptides contributed to the evaluation of protein ratio? What are their ratios?
16. Click on “[Show | Hide]” next to the 2nd peptide, “ISVGIEDTDDLLEDIKQALK”, of the protein, and then click on “[Expand All]”. How many independent LC peaks were detected for the peptide? How many times was the peptide identified? In what isotopic forms and charge states was the peptide identified?
 - a. Click on the Peptide Ratio link of the identification OR20080320_S_SILAC-LH_I-I_11.10488.10488.3. In what isotopic form and charge state was the peptide identified? In what charge states were signals of the peptide detectable? What were the charge states that contributed to the calculation of peptide ratio? What went wrong with this quantitation? Adjust this peak and save the ratio.
 - b. Refresh (reload) the Protein Ratio interface in order to view the recent changes. What is the new (“Interim”) protein ratio?
17. The 4th peptide (QFLQNAIGAIPSPFDWLTHR) has a ratio that is quite higher than the others; “show” the peptide section, and click on the ratio link therein. What is the peptide ratio? Now look at the areas that ASAPRatio picked for quantifying; what could be throwing off the ratio?
 - a. Modify the peaks contributing to this peptide ratio to get a reasonable ratio or invalidate the ratio.
 - b. Go back to the Protein Ratio interface. Click on “Evaluate_Ratio”. What is the new protein ratio?
18. The 6th peptide (YINGHSDVVLGVLATNNKPLYER) has an error that is high “show” the peptide section, and click on the ratio link therein. What is the peptide ratio? Now look at the areas that ASAPRatio picked for quantifying; what could be throwing off the ratio?
 - c. Modify the peaks contributing to this peptide ratio to get a reasonable ratio or invalidate the ratio.
 - d. Go back to the Protein Ratio interface. Click on “Evaluate_Ratio”. What is the new protein ratio?
19. Click on “Interim_Ratio” under “Set Accepted Ratio to”. What is the Accepted Ratio now?
20. Go back to the “interact-prot.shtml” file and refresh the browser. What the ratio of the protein now?