5-day Course XPress Exercises

1. Using Petunia file browser open file: **c: \ Inetpub \ wwwroot \ ISB \ data \ class \ XPress \ haloICAT \ Prerun \ interact.shtml**, the results of running XPress quantitation software are captured in a column entitled “XPRESS”. In the viewer, select to show all entries on one page and sort the spectra by probability in descending order.
2. Click on the first XPress ratio in the list (spectrum - haloICAT2_32.1358.1358.2). 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 right panel select a different charge state and click the “Quantitate”. What happens to the ratio when you select charge state +3? [ANS: ratio changes slightly] What happens to the ratio when you select charge state +1? [ANS: no ratio reported] What is the explanation for this behavior? [ANS: the m/z value for 1+ is outside the range recorded by the instrument].
3. Click on the next Xpress ratio in the original PepXML Viewer window (spectrum haloICAT2_31.1068.1068.1). Compare the peaks for the various charge states of this peptide. How do the peaks/ratios for the charge states of this peptide compare to each other? [ANS: they should all be similar]
4. Close the XPress window without saving.
5. Using Petunia file browser open the file: **c: \ Inetpub \ wwwroot \ ISB \ data \ class \ XPress \ haloICAT \ Prerun \ interact-prot.shtml**.
6. Click on the first XPress ratio in the file (protein Chr_ORF1447). How many peptide ratios contribute to the ratio of this Protein? [ANS: 8] Which spectra seem to have the most reasonable looking quantitation peaks (only considering spectra having a probability of 0.5 or above)? [ANS: haloICAT2_31.2072.2072.3, haloICAT2_31.2068.2068.3] For the peptides that don’t have a reasonable quantitation peak 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 Protein Ratio after you’ve eliminated the bad ones? [ANS: 1.69 +- 0.1]
7. Click “Update ProteinProphet ratio” button on the Protein Ratio page. Close the Protein Ratio page and refresh the ProteinProphet page.