ASAPRatio Exercise

0. If you have any questions or get stuck on anything, please raise your hand and we will help you.

1. Start TPP interface for "Analyze Peptides". Follow instructions in Lecture to analyze the 4 datasets (haloICAT2_30.xml, haloICAT2_31.xml, haloICAT2_32.xml, and haloICAT2_33.xml) under directory:
   c:\Inetpub\wwwroot\ISB\data\class\ASAPRatio\haloICAT\Semitryptic\

2. Once the analysis is completed, follow instructions in Lecture to view results stored in
   c:\Inetpub\wwwroot\ISB\data\class\ASAPRatio\haloICAT\Semitryptic\interact-prot.shtml

3. The first protein (Chr_ORF1447) has a protein ratio of 4.00±4.50. This is a typical case where the quantification result is not reliable. Click on the ratio to review how this ratio was calculated.

   Two peptides were assigned to the protein. But the ratio of the first peptide RTADCVVASNLYQEFLR was NOT used in calculating protein ratio. Click on "Show|Hide" and "Expand ALL". Notice the PeptideProphet probability for the identification was only 0.1330. When calculating protein ratios, ASAPRatio only uses ratios of peptides whose probabilities are no less than 0.5.

   The ratio of the second peptide TADCVVASNLYQEFLRR was the only data used in calculating protein ratio and but had a large error. Click on "Show|Hide" and "Expand ALL". Notice the ratio of the third Experiment (11.18±0.80) was significantly different from ratios of others. Click on the ratio to see why. This is a case where the peptide eluted from the RP column over a long period of time (>100 scans). In this case, ASAPRatio had trouble in setting the scan range for the heavy peptide. To correct the mistake, change the scan range of the heavy peptide from 2048-2096 to 1984-2108 for +3 charge state. Click on "Evaluate_Ratio". The Interim Ratio is now 3.69±4.07. Switch to +2 charge state. Again, change the scan range of the heavy peptide from 2052-2096 to 1984-2108 for the +2 charge state and click on "Evaluate_Ratio". The Interim Ratio is now 2.16±0.07. Click on "Interim_Ratio" under "Set Accepted Ratio to". The changes we just made are now stored in the pepXML file. To confirm this, go back to the Protein Ratio interface and click on "Evaluate_Ratio". The new interim ratio for the protein is now 2.14±0.28.

   Now click on "Show|Hide" beside the second peptide TADCVVASNLYQEFLRR and click "Expand ALL". Notice ratios of the first Experiment and the last

Experiment still have large errors (they are in RED). Change their Acceptance from "YES" to "NO" and click on "Evaluate_Ratio". The new interim ratio for the protein is now 2.18±0.07. Click on "Interim_Ratio" under "Set Accepted Ratio to". The changes we just made are now stored in the protXML file. To confirm this, go back to interact-prot.shtml and click on "Recompute p-value". When it is done, the new ratio for the protein is 2.18±0.28.

4. Now click on the p-value. This page shows the distribution (and fit) of all protein ratios as originally calculated by ASAPRatio on this dataset. What is the average protein ratio of this dataset?[ANS: 1.385] ASAPRatio uses this average as the background ratio (assuming that this ratio is the true 1:1 ratio on this dataset) and calculates the adjusted ratio based on this average background. What is the background adjusted protein ratio of this protein?

   Go back to the interact-prot.xml file and refresh the view. Make sure that the ratio shown matches the adjusted ratio you saved.

5. To become familiar with ASAPRatio, manually validate ratios of the next 2 proteins in the output "interact-prot.shtml" file. Make changes on every field that is changeable and observe what happens. Ask instructors questions that you may have.

6. Write down ratios of the next 7 proteins (no. 4-10). Then manually validate those ratios. What is the average change in ratios before and after manual validation? What is the largest change?

7. Continue to manually validate as many protein ratios as you want.