SILAC Ratio Analyzer:

http://proteome.moffitt.org/proteomics/silacratioanalyser/silacratioanalyser.jnlp

1. Open SilacRatioAnalyser
    Here are some requirements for the program to run correctly:
2. Put all the proteinGroup files exported from MaxQuant to a folder and don't include any other files in this folder.
3. Use the same naming method as in the example files, that is, xxx_xxx_proteinGroup.txt
4. Click on Ratio Analysis tab.
5. Highlight the folder that contains the text files you like to compare. Click open
6. Choose your own FASTA formatted IPI database by clicking "Analysis→Load IPI data base"
7. Click on "Analysis→Show Protein Info. This will activate the protein info window on bottom left." You will see 4 windows once you arrange them by dragging the windows.
    Top Left window lists IPI protein names, Counts (number of data sets the protein is common to), Gene name and protein description (Figure 1).
    Top Right is plots for $\log_{10}$ Ratio H/L vs. Proteins Identified
    Bottom Left is protein information for selected protein. Internet connection is needed to display protein information.
    Bottom Right is bar graph showing Ratio H/L normalized for selected protein in each sample.
    The bar chart use individual sample's cut-off value to color the bars:
   -   RED color is for values > cut-off High;
    -   BLUE color is for values < cut-off Low;
    -   BLACK color is the any value in between.

The cut-off values are calculated as follows:
In order to determine significantly up- and down-regulated proteins, median and standard deviation (σ) of "normalized protein H/L ratio" is calculated using log transformed data. A cut-off of median ± 2σ is also calculated in log space which is then transformed back into linear space.

8. To export data to excel, click "Save Ratio" and name and save the file. The very last line in the excel sheet is the calculated H/L Normalized "cut-off high" and "cut-off low" values for each data set. Because for some proteins, there is more than one IPI entry in Protein IDs column, each IPI name is split in a new row carrying all the information for the corresponding row. Once the excel sheet is created, collapsing on H/L ratio will simplify this file.

Figure 1. SILAC Ratio Analyser screen-shot.

SILAC Viewer: http://proteome.moffitt.org/proteomics/silacviewer/silacviewer.jnlp
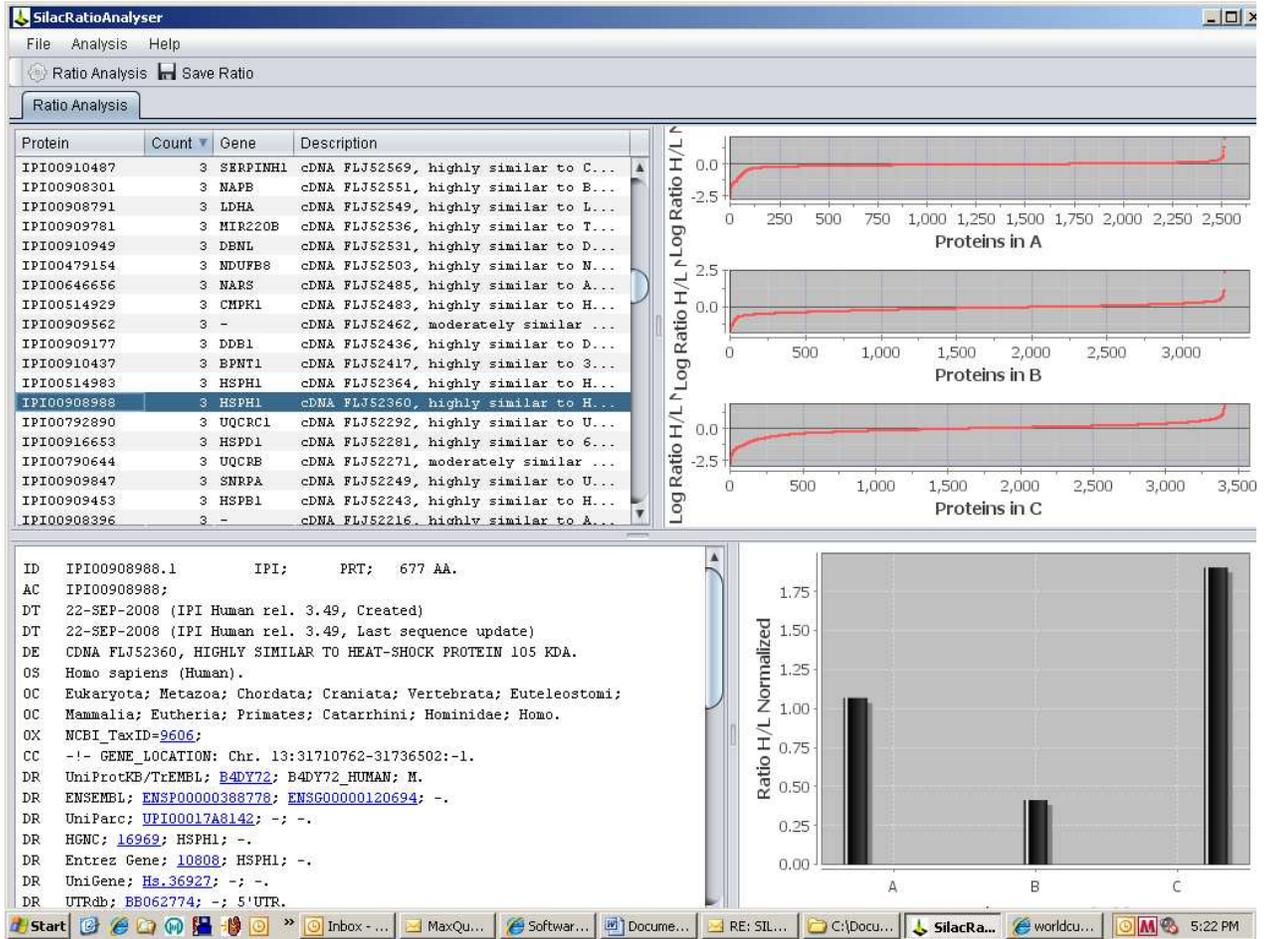
1. Open SILACViewer
2. Click Run and select the "combined" folder for the data of interest. The combined folder must be the combined folder generated by MaxQuant and include all the necessary protein and peptide files.
3. There will be 4 windows (Figure 2).
   Top Left window lists IPI protein names, Ratio H/L, and Standard Deviation (Standard deviation = "Ratio H/L Normalized * Ratio H/L Variability [%]/100 (Column AX)")
   Top Right lists all the peptides (Sequence) used to identify this protein and the related information for this peptide.
   Bottom Left is protein information for selected protein. Need internet connection to display the protein information
   Bottom Right displays all the samples that the selected peptide is found once a peptide is selected by clicking on in the Top Right window.
4. **Save Peptides** will export all the peptides identified for selected protein. **Save Scan Numbers** will export all the samples the selected peptide is identified in.

Figure 2. SILAC Viewer screen-shot.

Mass Error Analysis: http://proteome.moffitt.org/proteomics/

1. Click on Mass Error Analysis tab on SILAC Viewer
2. Click Analysis → Mass Error Analysis and select evidence.txt file to be analyzed.
3. There will be 4 windows (Figure 3). You can drag the lines to arrange the window sizes.

Top Left Mass Deviation of All Identified peptides

Top Right Intensity vs. Mass Error Deviation of All Identified peptides. x-axis is Mass Error (ppm) y-axis is Intensity of peptides identified grouped for their intensities.

Bottom Left Mascot Score vs. Mass Error Deviation of All identified Peptides. x-axis is Mass Error (ppm) y-axis is mascot score of peptides identified in groups.

Bottom Right Intensity vs. Mascot Score for All Identified peptides. x-axis is Peptide Intensity y-axis is peptide Counts/bin

Figure 3. SILAC Viewer Mass Error Analysis screen-shot.