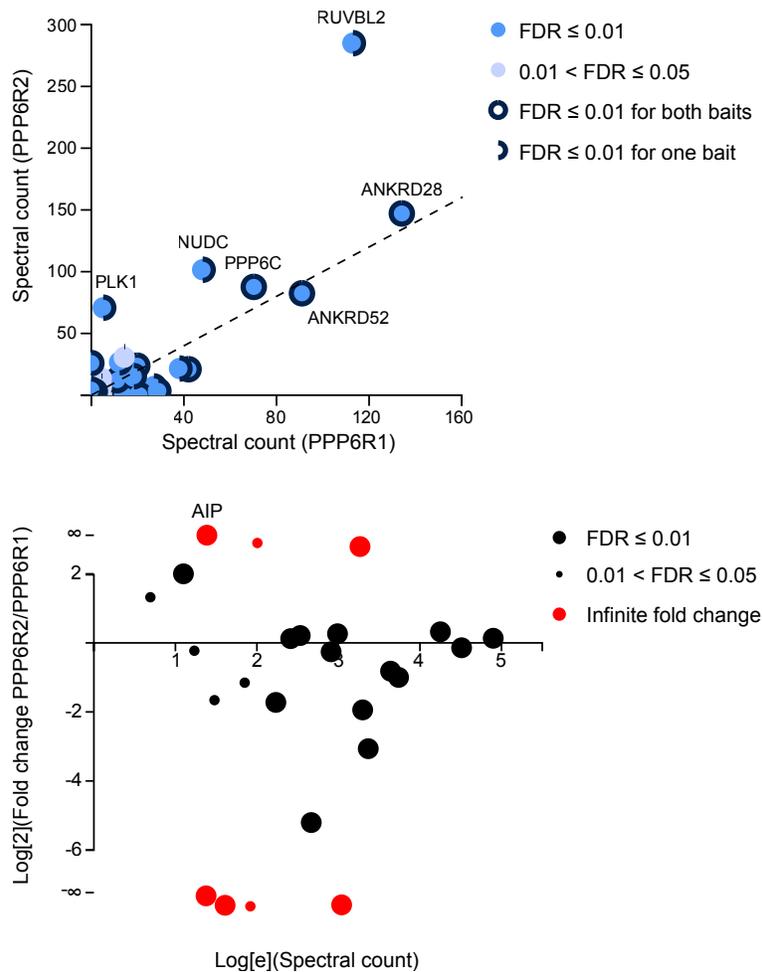




Bait vs bait comparison

This tool will take quantitative protein-protein interaction data for two baits and generate a scatterplot summarizing the spectral counts, relative change and confidence information. The image produced can be in one of two formats, as shown below. The first (called versus) displays the prey abundances for each bait relative to each other, while the second shows the prey fold change of one bait relative to a reference bait. These types of images are particular helpful for a pair of baits when there are a large number of preys between them, as the information is displayed in a much smaller area than what would be generated with a dot plot or heat map.



A static image and a file compatible with our interactive viewer will be available upon completion of the task. Alternatively, interactively images can be directly viewed by clicking on the appropriate link.

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Help is available directly on the page by selecting the "help" links next to the section headers. Any additional questions jknight@lunenfeld.ca. For more information, see [PMID: 25422071](#).

Inputting data

Specify the file for upload and the file type, then hit "Process". Explicit support is provided for data files output from SAINT and CRAPome (CRAPome matrix format is not supported, however). Datasets from other tools or pipelines can be input by selecting the "Other" option. Files must be in tabular format as tab-delimited text. At a minimum, the file must contain four columns specifying the bait/condition, prey/readout, abundance measure (spectral count, intensity, etc) and a confidence metric (e.g. FDR). Sample input files are available for download by clicking the "help" link and contain BioID published in [PMID: 24255178](#).

For detailed information on tools that generate compatible input for ProHits-viz, see the references and links below.

References: [SAINT - PMID:21131968](#), [SAINTexpress - PMID:24513533](#), [SAINT-MS1 - PMID:22352807](#), [ProHits - PMID:20944583](#), [ProHits Protocol - PMID:22948730](#), [ProHits 4.0 - PMID:27132685](#), [CRAPome - PMID:23921808](#)

Links: [SAINT-APMS.sourceforge.net](#), [ProHits.com](#), [CRAPome.org](#), [GalaxyP](#)

Data columns

Up to five columns will be used when processing the input file: a column listing the 1) baits, 2) preys, 3) abundance measure (spectral count, intensity or simply a non-negative number), 4) score for filtering (for unknown filter types, the direction of the filter must be specified, i.e. is a smaller number better, or vice versa), and 5) control values for performing control subtraction, i.e. subtracting the average control value from the observed prey abundance (this is optional and can either be a pipe-separated list of values or a single averaged number).

Bait selection

Two baits will be compared against one another and they should be specified here. The reference bait would be the control/wild-type/untreated bait against which the other bait (secondary) will be compared.

Parameters

1. Output type: Bait-bait comparisons can be output in two different types. Select the type here. By default, images will be output as the "versus" plot type.
2. Primary filter value: All preys that satisfy this cutoff for the control bait will be displayed in the scatter plot. For the "fold-change" plot type, these preys will be displayed at the largest circle size, while in the "versus" plot type, they will be shown in [blue](#).
3. Secondary filter value: All preys that satisfy this filter will also be displayed on the scatter

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plot. For the "fold-change" plot type, these preys will be shown at a smaller size than those that passed the primary filter, while in the "versus" plot type, they will be shown in light blue.

4. Control subtraction: The average value of a prey across control samples will be subtracted from the detected value for the bait if this is set to "yes". The quantitative value for the prey becomes the value above and beyond what is seen across the control samples. Specify the column to use for controls in the adjacent "Control column" field.

5. Normalization (none by default): No normalization is applied by default, but when baits in the same dataset have been run on instruments with varying sensitivity or dynamic range, normalization should be applied. The options for normalization are to divide by the total abundance for all proteins identified in the run or normalize based on a specific prey. Normalization will be applied after control subtraction if both are specified.

6. Log transform (default no): If desired, data can be log-transformed by base 2, base 10 or natural on the "versus" plot type. Log transformation will be performed after control subtraction and/or normalization if these are also specified.

Output

After the data has been processed, the user will be prompted to download a BaitvBait_results.zip file that contains the results in a folder. By default the unzipped folder will be named "RESULTS", but this name can be modified as desired. There are four files in this folder.

1. baitvbait.pdf: This is the scatter plot which can be opened and edited in Adobe Illustrator or a similar program. In some cases the following warning may appear on opening the file in Illustrator: "The font AdobePiStd is missing. Affected text will be displayed using a substitute font." If this occurs, the image will not display correctly.

To fix this issue on a Mac, copy the file AdobePiStd.otf from /Library/Application Support/Adobe/PDFL/*Current Version*/Fonts/ and transfer it to the folder /Library/Fonts/. The *Current Version* folder refers to your version of Adobe. On Windows, the font file is located in C:\Program Files\Common Files\Adobe\PDFL*Current Version*\Fonts\ and needs to be placed in C:\Program Files\Adobe\Adobe Illustrator CS5\Support Files\Required\Fonts\. If the AdobePiStd.otf file is missing, it can be freely downloaded from a number of sites on the web in either Mac or Windows format.

2. legend.pdf: This is an FDR legend for the scatter plot and it can be opened and edited in Adobe Illustrator or a similar program.

3. BaitVsBait_data.txt: This file is a tab-delimited table that contains information for each point on the scatter plot. Often in the case of crowded scatter plots, data points and labels may overlap. This table is provided to assist the user in finding preys of interest in such cases, and to help resolve issues as to which points and labels belong to one another.

4. baitvbait_df.tsv: Plain text file formatted for our interactive scatter plot viewer found at ProHits-viz that allows navigation of images. This file should be loaded directly into that tool without modification.

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5. process.log: This file contains information on the input parameters that were selected for the user's future reference (e.g. to assist with writing the Methods section for a manuscript).

Troubleshooting

Problems generally result from errors in the input file format, and we encourage users to compare their input files against the samples provided on the web page.

Any other issues should be sent to jknight@lunenfeld.ca.