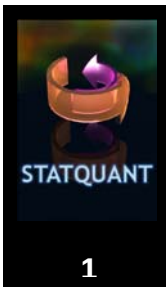
How to use StatQuant



Installation

- Make sure you have JAVA 1.6 or higher installed on your machine (http://www.java.com)
- Download the StatQuant-xxx.zip file and unzip this in a folder (or on the Desktop).
- Inside this .zip file you will find the complete MAVEN2 project of StatQuant and another .zip file (StatQuant executables). Unzip this file on your desktop (or other folder). You can now either keep the source codes or delete the initial StatQuant -xxx folder.
- Go into the extracted StatQuant executables folder. You should now find the following files/folders
    - Lib (folder containing all libraries)
    - StatQuant-x.x.x.jar
- Double click on the StatQuant-x.x.x.jar file

Troubleshooting

- StatQuant does not respond after loading large amounts of data

If you notice that when loading large amount of datafiles StatQuant doesn't show anything anymore, you probably need to assign more memory to Java:

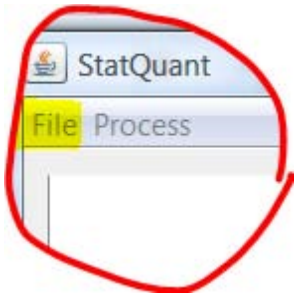
make a BATCH file (eg. Start.bat) and put the following line in it: (make sure to replace the x.x.x with your current StatQuant version number.

```
java –Xms512m –Xmx1024m –jar StatQuant-x.x.x.jar
```

and save this file. Please note: Java needs to be on the windows PATH.

Using StatQuant

StatQuant starts with a Splash screen showing the StatQuant logo.



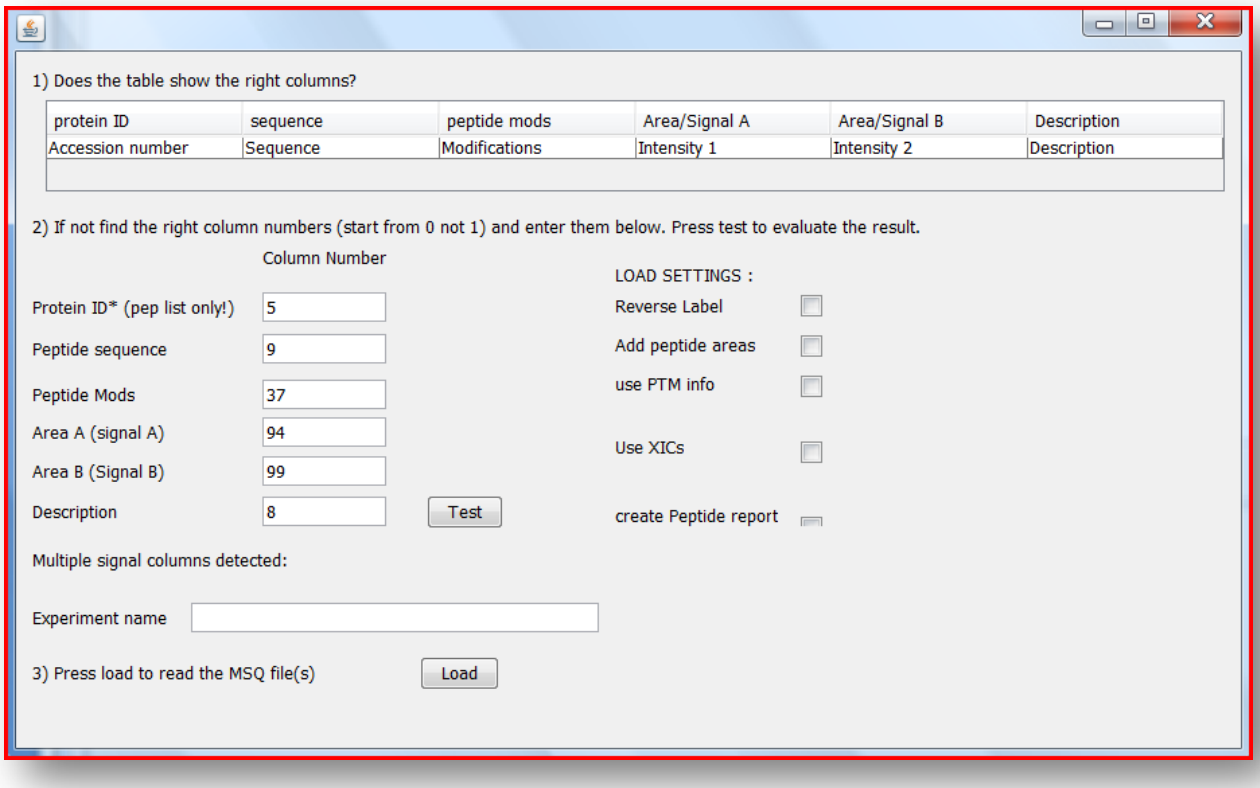
If you have downloaded the Example files you can start with the MSQuant file. Open the file menu and select open MSQuant file.

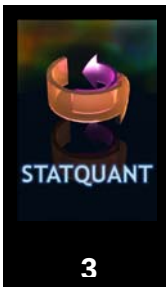
Exporting from MSQuant

When exporting a file from an MSQuant parse (protein window, file menu, export peptide and protein information), it is enough to export peptide information. Protein information can be read by StatQuant, but this information will be re-calculated on the fly. StatQuant cannot read in the spectrum information from the MSQuant files and will give erroneous results if this is attempted.

The import screen

You now have several options: On top you see the columns StatQuant could find/match in the file. On the left are the column numbers (starting from 0 for the 1rst column). On the right are additional options:





- Set the **column numbers** to other values if you need different columns to be selected. If you have more than two signal columns StatQuant will tell you the other column numbers containing the signals: (behind the line: Multiple signal columns detected: xx xx). You can manually set these columns to be loaded.
- The **Load settings** column allow you to: select if this is a reverse label experiment: StatQuant will now divide signal 2/signal 1 (normally signal 1/ signal 2).
- **Add peptide areas** will (when multiple files were loaded) add up the signal values (or XIC values) of identical peptides, found in defferent files. Result is only one peptide with one cumulative peptide signal (most often this LOWERS the variation).
- **Use PTM info** is for MSQuant files only (grey when Generic is loaded) and shows the PTM info as well. Use XICs (also MSQuant only) will select the XIC columns instead of the Signal columns.
- **Create Peptide report**: Changes the protein ID to peptide ID, which will create a peptide centric view later on.
- **Experiment name**: Sets the name of the experiment tab in StatQuant.

When loading multiple datasets you have multiple tabs which you can cross compare. Please note always load ALL files from one experiment at once. You cannot add files to a dataset later on (it creates a new experiment).

Press the load button to start loading your files.

The main panel will now show (can take some time depending on the amount of data).

You can click on a feature in the top table, which will make the detailed info appear in the table below. You can either click or use the arrow buttons to go through the data.

If you click on a column header it will sort on this column.

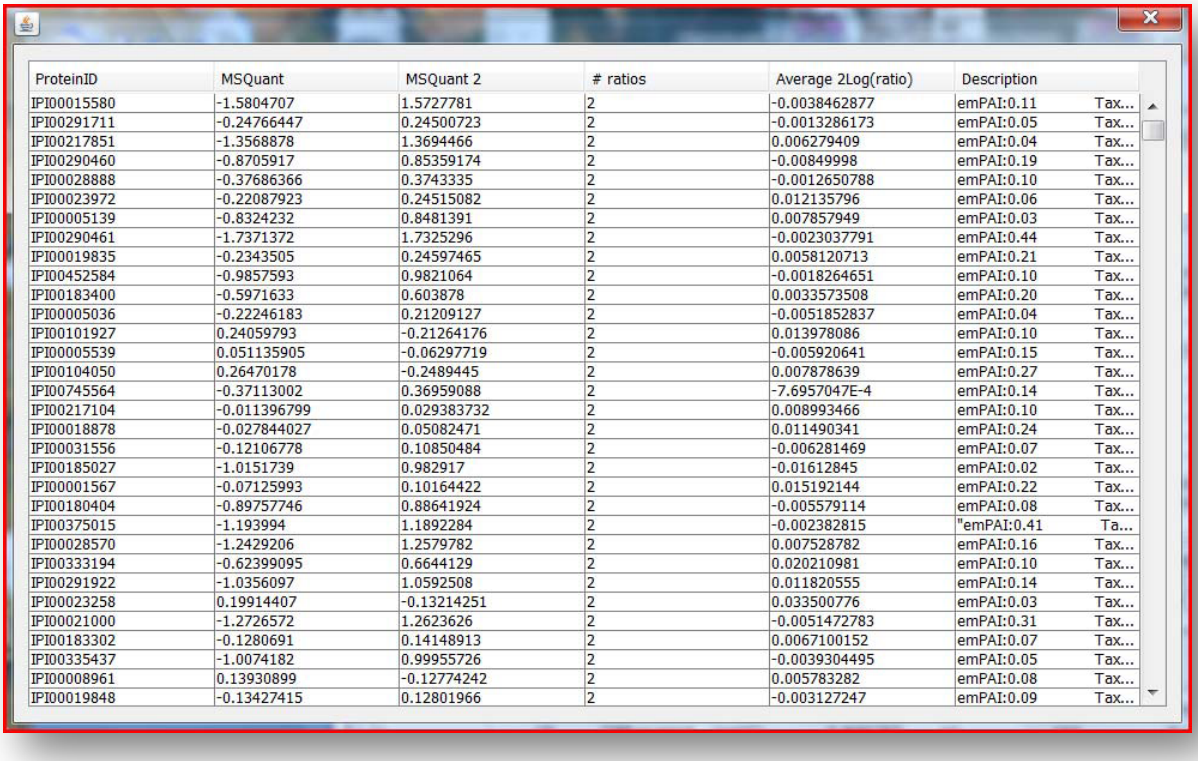
Click on a checkbox in the bottom panel below (include) to include or exclude peptides.

In the process menu all processing options are available. Here you can choose to normalize, do outlier detection (do this, you will see that it will automatically screen for outliers and exclude them). You can perform conversion correction from here and plot the results on a MA plot.

In the file menu you will find options to store the tables (views) as TAB delimited files. (readable by excel).

Multiple experiments

When multiple experiments have been loaded (you can try this by loading the MSQuant file multiple times (for fun select some different checkboxes like XIC or reverse label)), you can compare cross experiments. You will get a table with all proteins from all experiments, and their ratios as found in 1 or more experiments, an average etc.



ProteinID	MSQuant	MSQuant 2	# ratios	Average 2Log(ratio)	Description
IPI00015580	-1.5804707	1.5727781	2	-0.0038462877	emPAI:0.11 Tax...
IPI00291711	-0.24766447	0.24500723	2	-0.0013286173	emPAI:0.05 Tax...
IPI00217851	-1.3568878	1.3694466	2	0.006279409	emPAI:0.04 Tax...
IPI00290460	-0.8705917	0.85359174	2	-0.00849998	emPAI:0.19 Tax...
IPI00028888	-0.37686366	0.3743335	2	-0.0012650788	emPAI:0.10 Tax...
IPI00023972	-0.22087923	0.24515082	2	0.012135796	emPAI:0.06 Tax...
IPI00005139	-0.8324232	0.8481391	2	0.007857949	emPAI:0.03 Tax...
IPI00290461	-1.7371372	1.7325296	2	-0.0023037791	emPAI:0.44 Tax...
IPI00019835	-0.2343505	0.24597465	2	0.0058120713	emPAI:0.21 Tax...
IPI00452584	-0.9857593	0.9821064	2	-0.0018264651	emPAI:0.10 Tax...
IPI00183400	-0.5971633	0.603878	2	0.0033573508	emPAI:0.20 Tax...
IPI00005036	-0.22246183	0.21209127	2	-0.0051852837	emPAI:0.04 Tax...
IPI00101927	0.24059793	-0.21264176	2	0.013978086	emPAI:0.10 Tax...
IPI00005539	0.051135905	-0.06297719	2	-0.005920641	emPAI:0.15 Tax...
IPI00104050	0.26470178	-0.2489445	2	0.007878639	emPAI:0.27 Tax...
IPI00745564	-0.37113002	0.36959088	2	-7.6957047E-4	emPAI:0.14 Tax...
IPI00217104	-0.011396799	0.029383732	2	0.008993466	emPAI:0.10 Tax...
IPI00018878	-0.027844027	0.05082471	2	0.011490341	emPAI:0.24 Tax...
IPI00031556	-0.12106778	0.10850484	2	-0.006281469	emPAI:0.07 Tax...
IPI00185027	-1.0151739	0.982917	2	-0.01612845	emPAI:0.02 Tax...
IPI00001567	-0.07125993	0.10164422	2	0.015192144	emPAI:0.22 Tax...
IPI00180404	-0.89757746	0.88641924	2	-0.005579114	emPAI:0.08 Tax...
IPI00375015	-1.193994	1.1892284	2	-0.002382815	"emPAI:0.41 Ta...
IPI00028570	-1.2429206	1.2579782	2	0.007528782	emPAI:0.16 Tax...
IPI00333194	-0.62399095	0.6644129	2	0.020210981	emPAI:0.10 Tax...
IPI00291922	-1.0356097	1.0592508	2	0.011820555	emPAI:0.14 Tax...
IPI00023258	0.19914407	-0.13214251	2	0.033500776	emPAI:0.03 Tax...
IPI00021000	-1.2726572	1.2623626	2	-0.0051472783	emPAI:0.31 Tax...
IPI00183302	-0.1280691	0.14148913	2	0.0067100152	emPAI:0.07 Tax...
IPI00335437	-1.0074182	0.99955726	2	-0.0039304495	emPAI:0.05 Tax...
IPI00008961	0.13930899	-0.12774242	2	0.005783282	emPAI:0.08 Tax...
IPI00019848	-0.13427415	0.12801966	2	-0.003127247	emPAI:0.09 Tax...

You can copy paste the contents of this table into excel.

This is in short how StatQuant functions.

If you have any questions remaining, you can contact me at: b.vanbreukelen@uu.nl

Refererring to StatQuant

If you used StatQuant for your work, please refer to it as follows:

> Bas van Breukelen, Madalina Drugan, Henk WP van den Toorn and Albert JR Heck **StatQuant: A post quantification analysis toolbox for improving quantitative mass spectrometry**, Bioinformatics (In press, tba, current status: accepted with minor revision)

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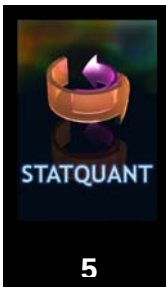

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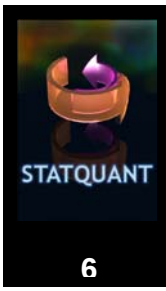

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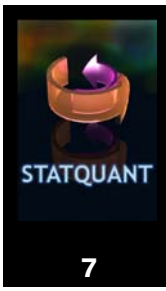

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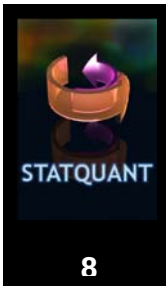

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