

FLEXIQuant-LF GUI

Overview

Improvements in LC-MS/MS methods and technology have enabled the identification of thousands of modified peptides in a single proteomics experiment. However, to fully understand the role of such post-translational modifications (PTMs), robust quantification methods are crucial to identify and monitor the modification extent. Here, we introduce FLEXIQuant-LF, a software tool for large-scale identification of differentially modified peptides and quantification of their modification extent without prior knowledge of the type of modification. We developed FLEXIQuant-LF using label-free quantification of unmodified peptides and random sample consensus (RANSAC)-based robust linear regression to quantify the modification extent of peptides. The unbiased approach of FLEXIQuant-LF to assess the modification extent in quantitative proteomics data provides a novel platform to better understand the function and regulation of PTMs in new experiments and reanalyzed data.

Download and Installation

Requirements

Windows 10 and 800MB of free disk space

Download

FLEXIQuant LF GUI executable for Windows 10 systems as well as the Python source code can be downloaded [here](#).

Installation

No installation needed. Just move the folder to your location of choice. To start the program, double-click FLEXIQuant_LF_GUI.exe

Getting Started

Input format

FLEXIQuant LF requires a comma separated file (.csv) containing peptide intensities in the following format as input:

Column	Column name	Description
1	Sample	Name of sample (unique sample name)
2	Group	Name experimental group (will be used to identify reference samples)
3-n	*	Peptide intensities

* can be anything, e.g. peptide sequence

For example:

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sample	Group	ASVLFANEK	DAVFLAER	DLTDMDK	DVALSVLSK	ESLVYFLIGK	FSLAEMHFQK	LDSSIIEGK	LFTSDSSTTK	LNLESSNSK	SALQELEELK	SVFSQSGNSR
2	1	0h	1781.15	8790.31	235.07	6726.29	5045.48	2226.83	4308.14	303.91	170.34	5197.41	197.05
3	2	4h	1359.80	7789.56	190.18	7473.36	6175.01	2704.16	4084.10	254.43	168.39	6277.81	185.20
4	3	8h	1244.86	7708.35	210.38	7421.61	7888.01	2700.19	2036.16	126.62	81.30	6464.44	100.45
5	4	10h	1264.15	6601.78	227.80	6730.52	6415.72	2350.32	1483.58	114.59	72.52	6175.84	105.80

The example input file can be downloaded [here](#).

Output

FLEXIQuant LF creates the following outputs:

- **_FQ-LF-output_raw_scores.csv:**
This file contains the calculated raw scores for all peptides.
- **__FQ-LF-output_RM_scores.csv:**
This file contains the calculated RM scores for all peptides that were removed by raw score filtering. Additionally, the following columns are included:
 - Slope: Slope of the regression line of the best linear regression model
 - R² model: Coefficient of determination of the best linear regression model
(calculated only using inliers as determined by RANSAC)
 - R² data: Coefficient of determination of the best linear regression model
(calculated using all data points)
- **_FQ-LF-output_diff_modified.csv:**
This file states for each peptide if it was classified as differentially modified based on the chosen modification cutoff (True) or not (False)
- **_FQ-LF-output_removed_peptides.csv:**
This file lists the peptides that were removed by raw score filtering i.e. that resulted in a raw score higher than the median raw score of the sample plus three times the median absolute deviation of all raw scores of the sample
- **_FQ-LF-output_regression_plots.pdf (optionally):**
Contains a linear regression plot for each sample. Can be used as quality control to check if the linear regression line was fitted correctly.

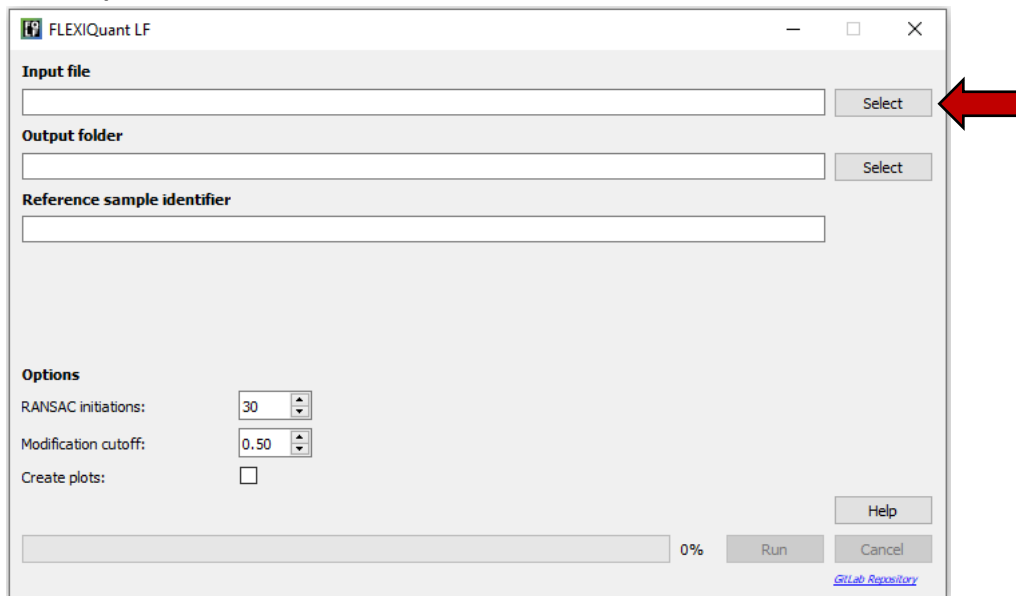
Parameters

- **Input file:** Path to input file
- **Output folder:** Path to output folder

- **Reference sample identifier:** Based on this FLEXIQuant LF determines which samples to choose as reference samples. This needs to match exactly the value in the Group column for all samples that shall be taken as reference.
- **RANSAC initiations:** Number of times FLEXIQuant LF fits a new RANSAC linear regression model to each sample to choose the best model. Between 5 and 100 initiations can be selected. The more initiations, the higher the reproducibility and the probability that FLEXIQuant LF finds the optimal model. However, choosing a high number can significantly increase the run time and more than 50 initiations rarely provide additional benefit. Default: 30
- **Modification cutoff:** RM score cutoff used to classify peptides as differentially modified. Default: 0.5
- **Create plots:** If checked, a linear regression plot for each sample is created. All plots will be saved in one pdf file (_FQ-LF-output_regression_plots.pdf)

Step by step

1. Select input file



2. Select output fold

Folder of the selected input file will be selected automatically as output folder. If a different location is preferred, select a different location.

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier

Options
RANSAC initiations: 30
Modification cutoff: 0.50
Create plots: ☐

0% Run Cancel Help

[GitHub Repository](#)

3. Enter reference sample identifier

(Needs to match group column value of reference samples)

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier
0h

Options
RANSAC initiations: 30
Modification cutoff: 0.50
Create plots: ☐

0% Run Cancel Help

[GitHub Repository](#)

4. Select number of RANSAC initiations

The default setting is 30

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier
0h

Options

RANSAC initiations: 30

Modification cutoff: 0.50

Create plots: ☐

0% Run Cancel Help

[GitHub Repository](#)

5. Select modification cutoff

The default setting is 0.5

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier
0h

Options

RANSAC initiations: 30

Modification cutoff: 0.50

Create plots: ☐

0% Run Cancel Help

[GitHub Repository](#)

6. Check “Create plots” checkbox if you want FLEXIQuant LF to create linear regression plots

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier
0h

Options
RANSAC initiations: 30
Modification cutoff: 0.50
Create plots: ☒

0% Run Cancel Help

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7. Click "Run"

FLEXIQuant LF

Input file
D:/Data/CDC27/CDC27.csv Select

Output folder
D:/Data/CDC27 Select

Reference sample identifier
0h

Options
RANSAC initiations: 30
Modification cutoff: 0.50
Create plots: ☒

0% Run Cancel Help

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