

Precursor intensity-based label-free quantification software tools for proteomic and multi-omic analysis within the Galaxy Platform.

Authors: Subina Mehta, Caleb Easterly, Ray Sajulga, Robert J. Millikin, Andrea Argentini, Ignacio Eguino, Lennart Martens, Michael R. Shortreed, Lloyd M. Smith, Thomas McGowan, Praveen Kumar, James E. Johnson, Timothy J. Griffin and Pratik Jagtap

Affiliation:

Key words: Proteomics, label-free quantification, Galaxy framework, workflows

Abstract:

For mass spectrometry-based peptide and protein quantification, label-free quantification (LFQ) based on precursor mass peak (MS1) intensities is considered reliable due to its dynamic range, reproducibility, and accuracy. In LFQ workflows, protein abundance changes are inferred from peptide-level information, including microbial peptides (for metaproteomics) and peptides carrying post-translational modifications (for proteomics) and/or variant sequences (for proteogenomics). Multi-omics studies (such as proteogenomics and metaproteomics) rely on peptide detection and quantification to identify and quantify peptides that map to unique proteoforms and metaproteins. The Galaxy for proteomics (Galaxy-P) platform has proven useful for the development of accessible workflows to identify proteins in these complex multi-omic studies. However, proteomics workflows within the Galaxy platform have lacked well-tested label-free quantification tools.

In this study, our main goals were to evaluate two recently published open-source LFQ tools and to implement them within the Galaxy platform, enabling their easy integration with established workflows. These two tools, moFF and FlashLFQ, were selected based on their described peptide quantification capabilities and amenability to Galaxy implementation. Through rigorous testing and communication with the tools' developers, we gained insights into the

software features necessary for maximizing the performance of each tool. Software features evaluated included: a) match-between-runs (MBR); b) using both Thermo .raw and HUPO standards .mzML file formats as input for improved quantification; c) use of containers and/or conda packages; d) parameters needed for analyzing large input datasets; and e) optimization and validation of software performance. This work 1) establishes a process for software implementation, optimization and validation within Galaxy; and 2) makes powerful new tools for LFQ available which should prove highly useful for a variety of proteomics and multi-omics applications employing the Galaxy platform.

Introduction:

Peptide- and protein-level quantification (either labeled or label-free) is routinely used in mass spectrometry (MS)-based shotgun proteomics data analysis workflows to determine the relative abundance of peptides or proteins in a given sample [1], including post-translationally modified peptides [2] and amino acid sequence variants identified by proteogenomics[3,4]. In the field of metaproteomics, where protein samples obtained from environmental microbiomes are studied, the quantification of microbial peptides or ‘metapeptides’ (peptides obtained from shotgun sequencing of microbial communities) is essential to perform taxonomic and functional quantification of proteins expressed from the microbiome[5].

In the case of the label-free quantification (LFQ) methods, the peak intensity or area under the curve of a detected peptide ion allows relative quantification of peptides across different samples. LFQ [6,7] is a useful method for quantification when the introduction of stable isotopes is impractical (for example, in human or animal model studies) or for applications such as proteogenomics or metaproteomics which rely on peptide-level quantification. Currently, there are several software packages available for LFQ analysis [8]. LFQ analysis can be performed by public domain software suites such as MaxQuant [9] and Skyline [10], or by commercial software such as PEAKS [11], and Progenesis [12]. Although commercial and actively-supported software offers reliability and ease of use, its usage comes with a cost and usually includes canned features that are used for most standard datasets. Open-source software, on the other hand, has the benefit of being amenable to testing and optimization for emerging disciplines to offer economical options for data analysis.

In this study, through a rigorous testing and evaluation process, we have incorporated and optimized two established, open source tools – moFF [13] and FlashLFQ [14] in the Galaxy platform. In order to achieve this, we worked with the software developers of these tools and tested features using two benchmark datasets—ABRF Proteomics Research Group (PRG) 2015 dataset [15] and a Universal Proteomics Standard (UPS) dataset [1]—and compared the outputs with results from MaxQuant, a highly used standalone software platform capable of LFQ analysis. Based on feedback, the tool developers for moFF and FlashLFQ made changes to software capabilities which included a) using match-between-runs (MBR); b) ability to process and analyze large input datasets; c) compatibility with a variety of input file formats. After this rigorous evaluation and optimization, these tools were implemented in the accessible and reproducible Galaxy[16] platform. Galaxy tools are maintained and developed by an international community of developers (<https://galaxyproject.org/iuc/>) so as to facilitate ease of usage and maintain its contemporary status for any emerging software tools or applications. An additional advantage of having these tools available via the Galaxy platform is the ability to process the data in workflows, wherein multiple tools can be used in a sequential manner to generate processed outputs from the input data. The Galaxy for proteomics (Galaxy-P) team has developed workflows related to MS-based multi-omic studies such as, proteogenomics [17,18] and metaproteomics [19,20]. Addition of these precursor intensity-based LFQ tools to the existing workflows will facilitate peptide level quantification for multi-omics research studies, as well as more standard proteomics applications.

As a result of this study, we made two quantitative software tools available to researchers via the Galaxy platform. These software are available via the Galaxy Tool Shed (https://toolshed.g2.bx.psu.edu/view/galaxyp/proteomics_moff/7af419c90f5f, <https://toolshed.g2.bx.psu.edu/view/galaxyp/flashlfq/908ab13490dc>), GitHub and on Galaxy public instances.

Methods:

We used two datasets, a) an ABRF dataset [15] and b) a spiked-in benchmark UPS dataset [1] to determine the accuracy of each tool with regards to their calculated protein fold-changes.

We obtained the MS (.raw) data from publicly available repositories and converted them to MGF (Mascot generic format) files using MSConvert (vendor support) [21] to make it compatible with search algorithms within the Galaxy Platform. moFF and FlashLFQ processing were performed within the Galaxy platform.

A) ABRF dataset:

The spiked-in dataset from the ABRF PRG 2015 study was used to determine the accuracy of each software tool. This dataset, generated through the collaborative work of the ABRF Proteomics Research Group (<https://abrf.org/research-group/proteomics-research-group-prg>) contains four proteins added to human cell lysate samples: ABRF-1 (beta galactosidase from *Escherichia coli*), ABRF-2 (lysozyme from *Gallus gallus*), ABRF-3 (amylase from *Aspergillus niger*) and ABRF-4 (protein G from *Streptococcus*) [15]. Each sample contained the four proteins at the same concentration, while the concentrations varied across the four samples (0 [blank/negative control], 20, 100 and 500 fmol).

B) Spiked-In UPS benchmark dataset:

To evaluate these tools, we downloaded publicly available data [1] (PRIDE #5412; ProteomeXchange repository PXD000279), wherein UPS1 and UPS2 standards (Sigma Aldrich) were spiked into *E. coli* K12 strain samples. The UPS1 and UPS2 standards contain 48 human proteins at either the same (5000 fmol, UPS1) or varying concentration (50000 fmol to 0.5 fmol, UPS2), respectively.

Peptide Identification:

For both datasets, we used SearchGUI (SG) [22] (version 3.3.3.0) and Peptide Shaker (PS) [23] (version 1.16.26) to search the MS/MS spectra against respective protein FASTA databases along with contaminants from cRAP database (<https://www.thegpm.org/crap/>). Although SearchGUI has the option to use as many as 8 search algorithms, we used only four search algorithms (X!tandem, OMSSA, MSGF+, and Comet) for this evaluation study.

For the spiked-in ABRF PRG dataset, a protein FASTA file was generated by merging the UniProt human reference database with spiked-in proteins and contaminant proteins (73,737 protein sequences database generated on February 6th 2019). Search parameters used were trypsin enzyme for digestion, where two missed cleavages were allowed. Carbamidomethylation

of cysteine was selected as a fixed modification and methionine oxidation was selected as a variable modification. The precursor mass tolerance was set to 10 ppm and the fragment mass tolerance to 0.5 Da, with minimum charge as 2 and maximum charge of 6. For Peptide Shaker, the false discovery rate (FDR) was set at 1% at the PSM, peptide and protein level, along with filtering the peptide length ranging from 6-65 peptides.

For the spiked-in UPS dataset, the mass spectra were searched against a protein FASTA database provided by Cox. et al., 2014 [1] - (4,494 protein sequences database generated on July 25th 2019). The parameters for SearchGUI-Peptide Shaker analysis were as follows: precursor mass tolerance was set to 10 ppm and the fragment mass tolerance to 20 ppm with minimum and maximum charge as 2 and 6 respectively.

For MaxQuant analysis (version 1.6.7.0), the built-in Andromeda search engine [24] was used. The parameters for MaxQuant were matched with the SearchGUI-PeptideShaker search. The fixed modification was set for carbamidomethylation of cysteine and oxidation of methionine as a variable modification. The FDR was set at 1% and the MS/MS tolerance was set at 10 ppm. The tabular output data from Peptide Shaker (PSM.tab) and Andromeda (msms.txt) were used for protein quantification.

Quantification tools:

moFF and FlashLFQ, were initially tested outside of the Galaxy platform. We tested various releases for moFF (versions 1.2.0 to 2.0.2) and FlashLFQ (versions 0.1.99 to 1.0.3) and provided developers with feedback to improve software stability and data quality. We then implemented these updated tools within Galaxy. The results from moFF (version 2.0.2) and FlashLFQ (version 1.0.3) were then compared with MaxQuant (version 1.6.0.16), a widely used LFQ quantification software suite. For testing, all the quantification tools were set at monoisotopic tolerance of 10 ppm and run with or without MBR, where indicated.

Normalization and Protein Quantification:

After peptide-level precursor intensity values were generated, normalization was performed using limma [25] and peptides were summarized into protein-level abundances with Protein Expression Control Analysis (PECA) [26]. Specifically, the “normalizeBetweenArrays” limma function was used for most normalization methods (*i.e.*, scale, cyclic loess, and quantile). For VSN (variance stabilizing normalization), the “normalizeVSN” limma function was used

[27,28]. After normalization, PECA was used to combine the peptide-level measurements to protein-level values for detection of differentially expressed proteins. These two tools were run via custom R scripts, which can be accessed via the Supplementary Document 2 .

Results:

Both moFF and FlashLFQ are established software tools and contain useful features such as amenability to Galaxy implementation, compatibility with existing Galaxy upstream and downstream tools, ability to read .mzML and Thermo .raw file formats, open-source code, MBR functionality, and results that can be easily evaluated with performance metrics.

moFF is an extensible quantification tool amenable to any operating system. The input for moFF is peptide search engine output and Thermo .raw files and/or .mzML files; it performs both MS/MS as well as MBR quantification. moFF tool also has a novel filtering option for MBR peak intensities [29] . moFF has been wrapped in Galaxy (**Figure 1A**) using a Bioconda package [30]. The Galaxy version of moFF is available via Galaxy toolshed (https://toolshed.g2.bx.psu.edu/repos/galaxyp/proteomics_moff), GitHub (<https://github.com/compomics/moFF>), and Galaxy public instances (proteomics.usegalaxy.eu, usegalaxy.be and z.umn.edu/metaproteomicsgateway).

FlashLFQ is a peptide and protein LFQ algorithm developed for proteomics data analysis. It was developed to quantify peptides and proteins from any search tool, including MetaMorpheus, which also performs PTM identification from MS/MS data. It uses Bayesian statistics to estimate the difference in the abundance of inferred proteins between samples, though this feature was not evaluated here. FlashLFQ can normalize fractionated datasets by using a bounded Nelder-Mead optimizer [31] to find a normalization coefficient for each fraction, similar to MaxLFQ. FlashLFQ was implemented in Galaxy (**Fig 1B**) within a Singularity container [32] as FlashLFQ is a Windows application requiring the .NET core framework for deployment in the Unix-based Galaxy environment. Singularity provides a secure means of running such tools in Galaxy. The Galaxy version of FlashLFQ is available via Galaxy toolshed (<https://toolshed.g2.bx.psu.edu/repos/galaxyp/flashlfq>), GitHub

(<https://github.com smith-chem-wisc/FlashLFQ>) and via Galaxy public instances (proteomics.usegalaxy.eu and z.umn.edu/metaproteomicsgateway).

moFF extracts MS1 intensities from spectrum files (Galaxy Version 2.0.2.0) ☆ Favorite Versions Options

Choose which module to run
All (match-between-runs followed by quantitation)

Choose the format for the identification file:
Peptide Shaker PSM report (standard, not extended)

Peptide Shaker PSM report
329: UPS_PSM_All_edited.tabular ↗

Choose the format for the MS/MS file
Thermo RAW file

RAW file(s)
134: UPS_raw

(--raw_list)

Tolerance parameter
10.0
Specify the tolerance parameter in ppm. (--tol)

moFF workflow
on
select the moFF workflow (--mbr)

retention time windows for XIC
3.0
Specify rt window for xic in minutes. (--xic_length)

retention time window for apex detection
1.0
Specify the retention time window for the peak in minutes. (--rt_peak_win)

retention time window for the matched peak
1.2
Specify the retention time window for the matched peak in minutes. (--rt_peak_win_match)

Activate filtering of matched peptides
Filter by flags to exclude or require

sample_size
0.2
percentage of MS2 peptide used to estimated the threshold. Default value: 0.20 (--sample_size)

-quantile_thr_filtering
0.75
quantile value used to compute the filtering threshold for the matched peak (--quantile_thr_filtering)

ptm_file
Nothing selected
load your ptm file in order to overwrite internal method (--ptm_file)

Output the peptide summary?
Yes No
(--peptide_summary)

✓ Execute

FlashLFQ ultrafast label-free quantification for mass-spectrometry proteomics
(Galaxy Version 1.0.3.0)

identification file
329: UPS_PSM_All_edited.tabular

MetaMorpheus,Morpheus,PeptideShaker PSM Report,MaxQuant (--idt)

spectrum files
134: UPS_raw
19: mzml_UPS

monoisotopic ppm tolerance
10
(--ppm)

isotopic distribution tolerance in ppm
5
(--iso)

number of isotopes required to be observed
2
(--nis)

integrate peak areas (not recommended)
Yes No
(--int)

use only precursor charge state
Yes No
(--chg)

match between runs
Yes No
(--mbr)

maximum MBR window in minutes
2.5
(--mrt)

Use experimental design for normalization or protein fold-change analysis
Yes
ExperimentalDesign.tsv
20: ExperimentalDesign.tsv

normalize intensity results
Yes No
(--nor)

Perform Bayesian protein fold-change analysis
Yes
control condition for Bayesian protein fold-change analysis
S1
(--ctr)

fold-change cutoff
1
Leave blank to determine empirically from data. (--fcc)

use shared peptides for protein quantification
Yes No
(--sha)

require MS/MS ID in condition
Yes No
(--rmc)

number of markov-chain monte carlo iterations
3000
(--mcm)

random seed
(--rns)

Execute

Figure 1): Galaxy interface of moFF and FlashLFQ: A) Bioconductor package of moFF is wrapped within Galaxy and available via Galaxy toolshed (https://toolshed.g2.bx.psu.edu/view/galaxyp/proteomics_moff/7af419c90f5f), and Galaxy public instances (proteomics.usegalaxy.eu).

B) A docker/singularity container of FlashLFQ is wrapped within Galaxy and available via Galaxy toolshed (<https://toolshed.g2.bx.psu.edu/view/galaxyp/flashlfq/908ab13490dc>), and Galaxy public instances (proteomics.usegalaxy.eu).

Relevant features of moFF and FlashLFQ, as well as the design of the evaluation study are summarized in **Figure 2**. An essential aspect to this study was both of these tools being in active development by groups amenable to collaboration, which greatly helped optimization based tests and feedback from the Galaxy-P team members.

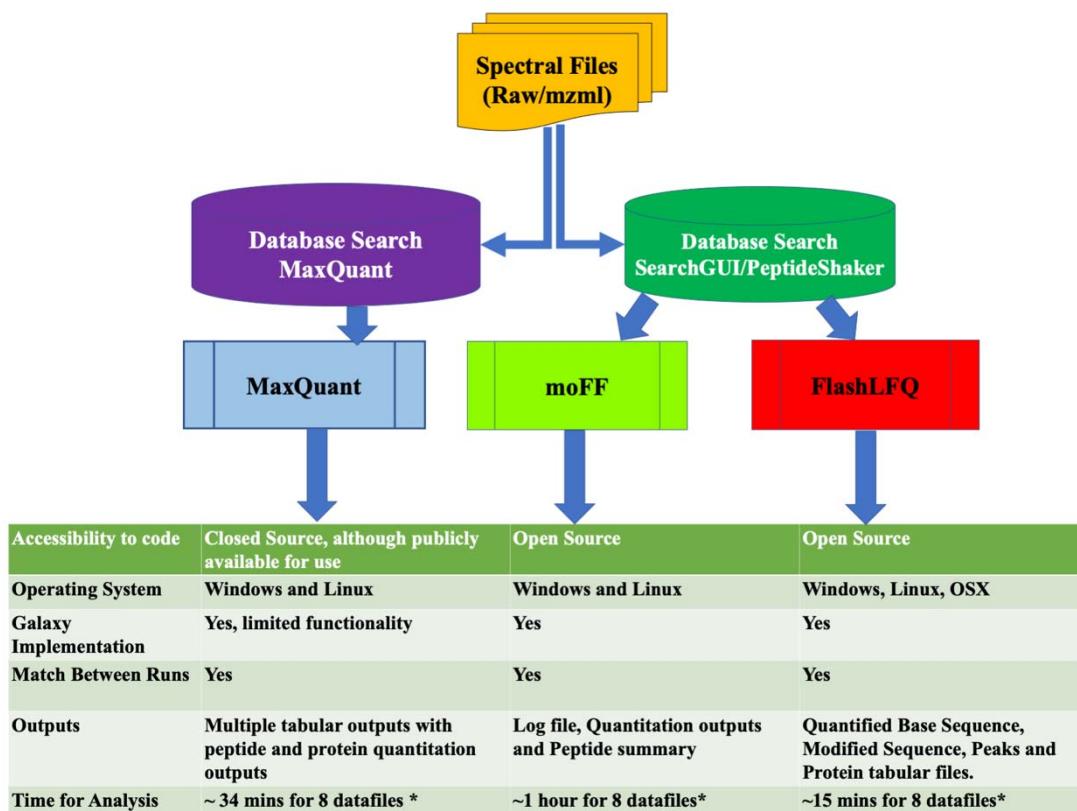
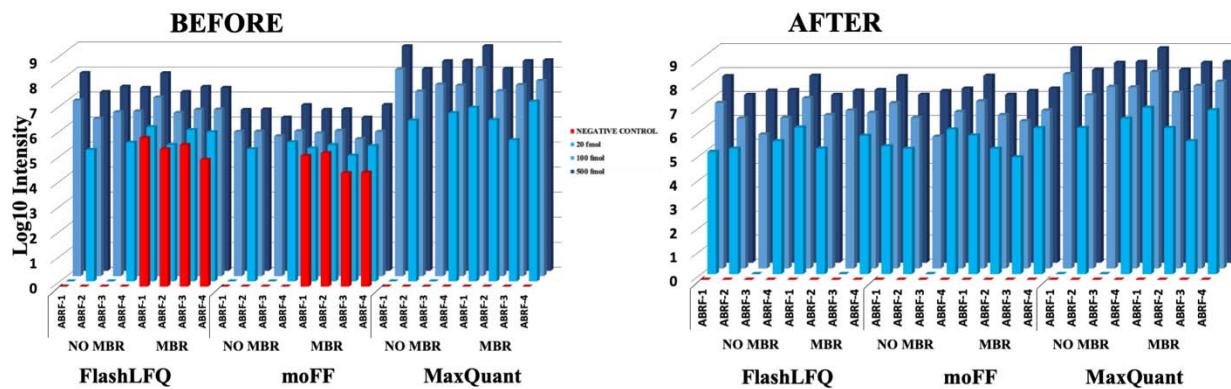


Figure 2. Experimental Design of the evaluation study: Spectra files are converted to MGF before mass spectra are matched with peptides using respective search engines. Each of the quantification tools use RAW files and the peptide identification tabular output as inputs. The figure also shows features of each tool. The outputs from all of the tools were then compared against each other.

To generate peptide identification inputs for moFF and FlashLFQ, datasets were searched against appropriate protein databases using SearchGUI/PeptideShaker. The speed and accuracy of FlashLFQ and moFF were evaluated in comparison to MaxQuant, a popular software tool used for LFQ. For MaxQuant, searches were performed by MaxQuant's built-in Andromeda search algorithm. All three software programs have a MBR feature, where unidentified peaks are “matched” to identify peaks in other runs based on similar m/z and retention time. MaxLFQ, an algorithm within MaxQuant, normalizes raw intensities and also aggregates them into protein groups [1]. For moFF and FlashLFQ, limma was used to normalize peptide intensities and PECA was used to determine protein fold-changes and associated p-values. The limma tool within Galaxy implements different normalization techniques such as quantile, VSN, cyclicLOESS, and scale normalization. Users can choose between these normalization methods. FlashLFQ also has built-in normalization and protein quantification functions which we have used in this study.

A)



B)

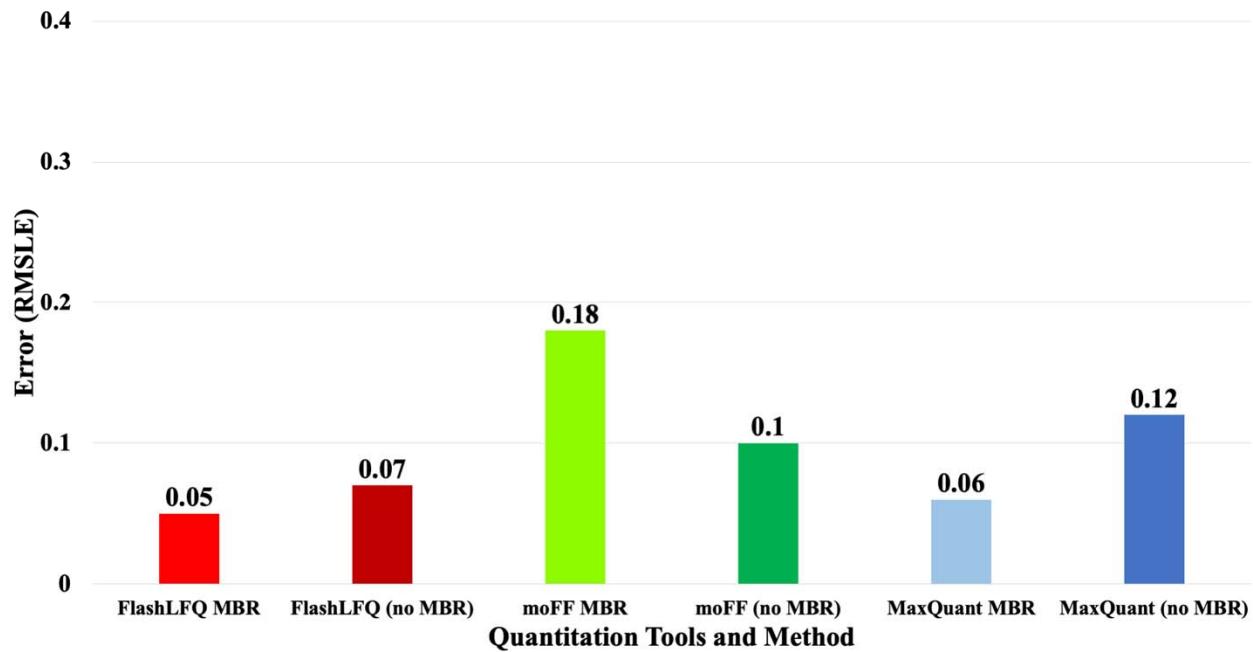


Figure 3A) Effect of MBR after software version updates: The log10 values of the intensities (Blue bars) from each of the four ABRF spiked-in proteins (ABRF-1: Beta Galactosidase from *E. coli*, ABRF-2: Lysozyme from *Gallus gallus*, ABRF-3: Amylase from *Aspergillus*, ABRF-4: Protein G *Streptococcus*) were plotted. The results from prior versions of moFF (v1.2.1) and FlashLFQ (v0.1.99) (before) shows that MBR detects ABRF proteins (shown in red) in the negative control sample in both software. The results from the current versions of moFF (v2.0.2) and FlashLFQ (v1.0.3.0) implemented in Galaxy (after), shows that the MBR feature does not detect ABRF proteins in the negative control.

B) Accuracy of fold-change estimation: For evaluating the accuracy of quantified results, we estimated the fold change of the spiked-in proteins in the 500 fmol sample as compared to 100 fmol sample. The Root Mean Squared Log Error (RMSLE) was calculated for fold change estimation. For this dataset, moFF with MBR displayed significantly higher RMSLE value, whereas FlashLFQ's MBR performed similarly to MaxQuant's MBR.

After ascertaining that moFF and FlashLFQ results correlate well with MaxQuant results (**Supplementary Figure S1**), we set out to evaluate the MBR feature of the software tools. For this, we used the ABRF PRG datasets, with four spiked-in proteins at 3 different concentrations (20 fmol, 100 fmol and 500 fmol) and a negative control (see methods). The spiked-in proteins should not be detected in the negative control, either with or without MBR. We observed that moFF and FlashLFQ outputs showed non-zero intensity values for the spiked-in proteins in

blank control samples if MBR was enabled (**Figure 3A**, left). We worked with the developers to improve their MBR algorithms so that intensity values for these proteins in the blank control samples were correctly reported as zero (**Figure 3A**, right).

The 500 fmol and 100 fmol datasets from the ABRF dataset were used to determine the fold-change accuracy (**Fig 3B**). In order to determine the accuracy of the fold-change, root mean squared log error (RMSLE) [33] was calculated.

$$RMSLE = \sqrt{\frac{\sum_{i=1}^N (\log_{10} r_i - \log_{10} \hat{r}_i)^2}{N}}$$

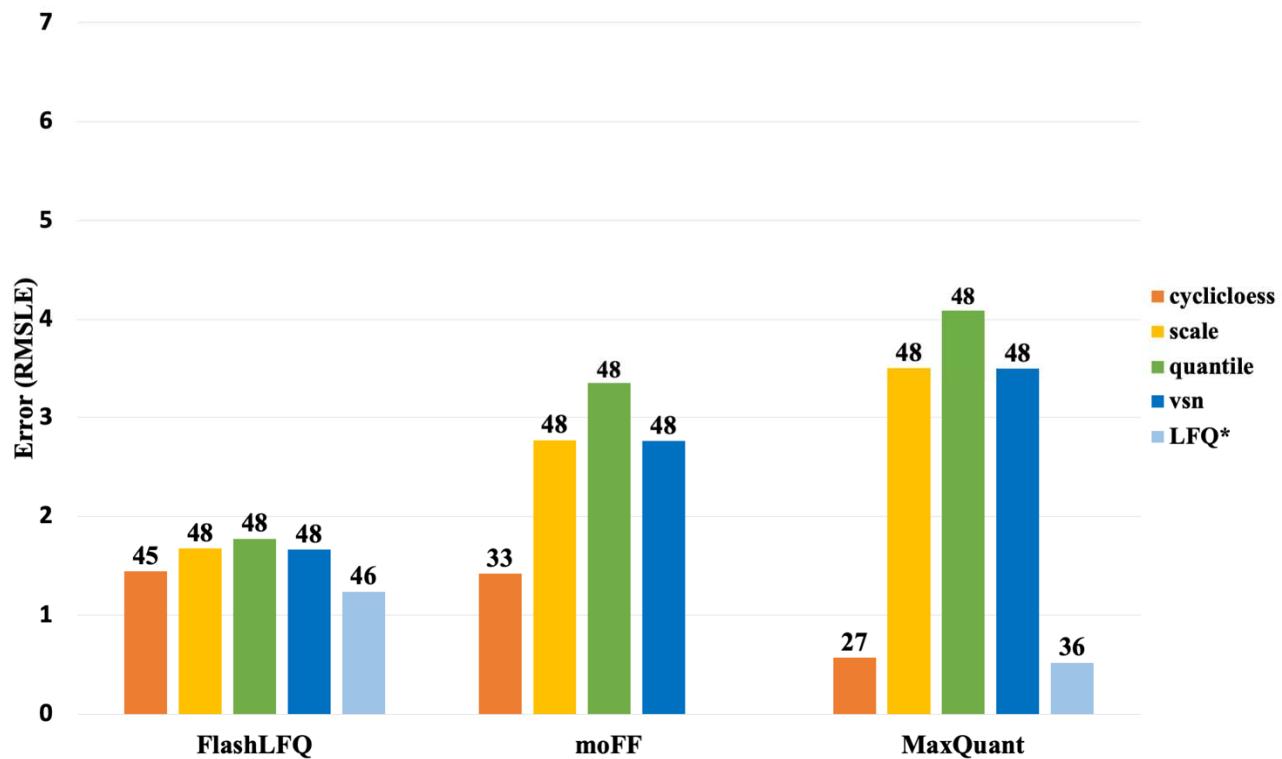
Where, r_i is the true ratio, \hat{r}_i is the estimated ratio and N is the number of proteins identified via sequence database searching of the sample

Root mean squared log error is a metric to evaluate the difference between predicted and observed values. In this case, the values being compared are the predicted (known) fold-changes and the observed fold-changes. RMSLE being an error based metric provides the true picture of prediction quality, however, deciding a suitable threshold value is challenging. The objective was to obtain a RMSLE value closer to zero for all the tools. The RMSLE values for the three tools are shown in **Figure 3B**, with the MBR feature enabled. We observed that moFF with MBR had slightly higher error compared to the other tools. MaxQuant's MBR and FlashLFQ's MBR perform quite similarly, though all three tools show low error when the MBR is enabled.

Although MaxLFQ and FlashLFQ have their own in-built methods for normalizing peptide abundance, for a more direct comparison of moFF and FlashLFQ performance we normalized the peptide intensity levels using limma and obtained differentially expressed proteins through the PECA bioconductor package. Normalized peptide intensity values from moFF and FlashLFQ were input into PECA, wherein, the tool calculates the p-values of the peptide level data and then groups the values into protein level data. The PECA output was then compared with MaxQuant values using the UPS benchmark dataset. For this, quantitative information for the 48 proteins from the UPS dataset was extracted using an R-script to generate a tabular output with fold-change values.

The fold-change accuracy of all quantified UPS proteins after normalization was calculated by comparing the estimated protein fold-change with the true fold-change using the RMSLE (Figure 4A).

A)



B)

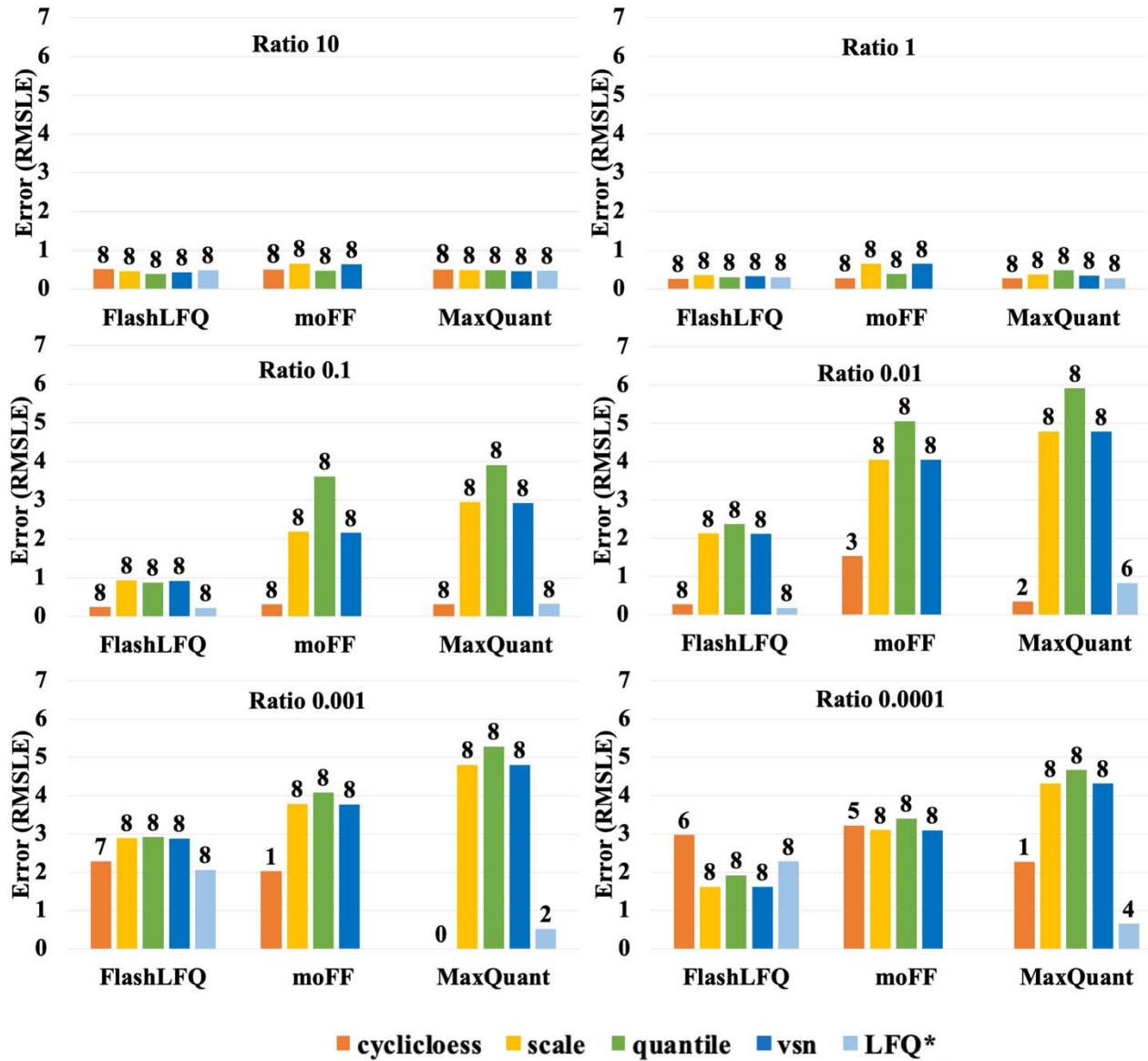


Figure 4A) Fold-change accuracy (MBR) of all proteins: After normalization, the estimated protein abundance ratios for all the identified UPS proteins were compared to the true abundance ratios, using the Root mean squared log error (RMSLE). The plot represents the RMSLE values using different normalization methods. Note that LFQ values represent FlashLFQ's and MaxQuant's inbuilt normalization value. The value on the top of the bars denotes the number of proteins that were quantified.

B) Fold change accuracy (MBR) of proteins with similar estimated ratios: In total there are 48 UPS proteins, we classified the UPS proteins into different groups based on the UPS2/UPS1 ratio estimation, the true ratios run from 10 to 10^{-4} . The value on the top of the bars denotes the

number of proteins that were quantified using each normalization method. The RMSLE of the intensity ratio was used to measure the accuracy of the estimated fold change.

Figure 4A shows the comparison of different normalizations using the MBR values. Although the bar graph shows that MaxQuant's MaxLFQ performs the best compared to all, it does so at the cost of the number of proteins quantified. However, we noticed that FlashLFQ's MBR with its in-built normalization (light blue bar in Figure 4A) performed better overall in terms of quantification and the number of proteins identified. Meanwhile, moFF and FlashLFQ provide higher numbers of quantified proteins while still maintaining low RMSLE values. We also performed comparison studies of MBR vs. no MBR, the results of which are shown in **Supplementary Figure S3A**. We also found that moFF and FlashLFQ quantify similar numbers of peptides across the ABRF and UPS datasets (**Supplementary Figures S2**).

After evaluating the RMSLE for all proteins, we estimated the accuracy for similarly abundant UPS proteins (**Figure 4B**). We categorized the UPS standards by their concentration ratio (UPS2/UPS1) which resulted in 6 different categories (i.e. ratios of 1 to 0.0001). The results showed that MaxQuant quantification works optimally for high and medium abundant proteins. However, for low abundance proteins the fold-accuracy is lower, presumably because of missing intensity values. Another important observation is that MaxLFQ denotes a smaller error compared to the other tools in the low abundance samples, but quantifies fewer proteins. An evaluation study for MBR vs noMBR was also performed showing a similar trend represented in **Supplementary figure S3B**.

After evaluation of the moFF and FlashLFQ tools, we worked with the developers of these tools to implement their optimized software in Galaxy, enabling integration into diverse MS-based proteomics workflows and promoting their usage by the Galaxy community. Our implementation will allow the users to choose their choice of tool and normalization (**Fig S4**) which will benefit their research.

Discussion:

Protein and peptide-level quantification has been used by proteomics researchers to determine how the proteome responds to biological perturbation [34]. In particular, precursor-

intensity based LFQ has enabled researchers to perform quantitative proteogenomics analyses [35]. Quantitative changes in the proteome can also be correlated with transcript abundance changes [36] to get a more complete picture of how an organism responds to a stimulus. For example, in cancer proteogenomics studies, these abundance measurements help identify differential expression patterns of variant peptides that may have functional significance in cancer [37,38].

Peptide-level quantification also aids in functional studies of microbial communities and microbiomes using metaproteomics. For example, in metaproteomics studies, metapeptides or metaproteins detected from environmental [39] or host-derived samples [40] can be quantified to shed light on the dynamics of the taxa, biological function, and their abundance [41]. Our group has developed and optimized Galaxy-based tools and workflows for proteogenomics [42] and metaproteomics analyses [19,20]. Tools implemented through this study will extend these workflows to enable quantification of metapeptides and/or metaproteins.

In our analyses, the three LFQ tools – moFF, FlashLFQ, and MaxQuant - correlate well in their results according to our evaluation. In this study, we have added moFF and FlashLFQ to the Galaxy framework, which not only facilitates the dissemination of these tools, but also enables automated data analysis by using them within workflows [16]. We also highlight the importance of the process of careful user evaluation, feedback to developers and optimization of the tools and workflows. Preliminary testing was performed on the command line or GUI versions of the tool. These tools were then packaged into the Galaxy platform, where results were compared to the command-line/GUI versions and also optimized more usage in automated workflows.

Open-source software usage has faced challenges due to dependencies such as operating system (Windows, Linux, OSX), language or platforms (Python, C++, Java), lack of adhering to HUPO standards [43] , and installation or usability issues [44]. To overcome these issues, the Galaxy-P project, as well as others in the Galaxy community, have sought collaborations with many research groups that have developed these tools, following a protocol which includes defining key input and output data types, establishing key operating parameters for the Galaxy tool, overcoming operating system compatibility issues (*e.g.*, Singularity containers for Windows

tools), along with rigorous testing and optimization. This collaborative and iterative process of development and optimization ensures the software performs accurately and efficiently within the Galaxy platform.

Ideally, software tools which are UNIX based – such as moFF - are easier for deployment within Galaxy. We also demonstrate here that tools such as FlashLFQ can be packaged within a Singularity container to enable easy and secure implementation within Galaxy. MaxQuant, which is a popular, public-domain proteomics software package, is available in both Windows and Linux-compatible versions [45]. Although in early development and testing phase, the LFQ module that uses MS1 precursor intensity data within MaxQuant (MaxLFQ) was made available within Galaxy toolshed (<https://toolshed.g2.bx.psu.edu/view/galaxyp/maxquant/175e062b6a17>). Once fully tested and evaluated, accessibility to this software via the Galaxy platform will offer even more choices for precursor-intensity based quantification. Offering users a choice of multiple validated software tools also highlights benefits of a workflow engine such as Galaxy, where users can easily develop parallel workflows using different combinations of tools to determine methods that provide optimal results based on user requirements.

In our tests, we observed that FlashLFQ has a faster runtime as compared to the other two tools. MaxQuant processing time is longer, presumably since it performs peptide identification and quantitation simultaneously. For example, on the same computing device, the UPS dataset was processed by FlashLFQ in approximately 15 mins for quantification only, whereas MaxQuant and moFF took 34 minutes and 3 hours respectively. Again, our evaluation and availability of these tools within a unified platform such as Galaxy offers users a choice for their workflows where speed of analysis can also be considered.

This study demonstrates a successful collaborative effort in software tool development and dissemination, which is a hallmark of the Galaxy community and the Galaxy-P project [19]. This community-driven approach brings together users and software developers who work together to validate and make the tool accessible and usable for other researchers across the world. The study described here provides a model of success for the process used to ultimately provide optimized, well-validated tools for community use. We did not seek a goal to determine the single best tool for LFQ use, but rather focused on offering users a choice of validated

quantification tools amenable to customizable analytical workflows. In addition to our work here, others from the Galaxy community are also working on integrating tools within the MaxQuant suite <http://www.mol-med.uni-freiburg.de/mom-en/schilling/pub>), which will extend the choices for LFQ quantification available. As a result of this study, Galaxy users can now confidently use two rigorously validated LFQ software tools (moFF and FlashLFQ) for their quantitative proteomic studies. We are currently working on incorporating the quantitative capabilities of moFF and FlashLFQ within existing metaproteomics and proteogenomics workflows, so that they can be used by the research community in their quantitative multi-omics studies.

Acknowledgements

We would like to thank European Galaxy team for the help in the support during Galaxy implementation. We would also like to thank Carlo Horro (from Barnes Group, University of Bergen) and Björn A. Grüning (University of Freiburg, Germany) for helping us during the quantification tools analysis. We thank Emma Leith for proofreading the manuscript.

We acknowledge funding for this work from the grant National Cancer Institute - Informatics Technology for Cancer Research (NCI-ITCR) grant 1U24CA199347 and National Science Foundation (U.S.) grant 1458524 to T.G. We would also like to acknowledge the Extreme Science and Engineering Discovery Environment (XSEDE) research allocation BIO170096 to P.D.J. and use of the Jetstream cloud-based computing resource for scientific computing (<https://jetstream-cloud.org/>) maintained at Indiana University. We also acknowledge the support from the Minnesota Supercomputing Institute for maintenance and update of the Galaxy instances. The European Galaxy server that was used for some calculations is in part funded by Collaborative Research Centre 992 Medical Epigenetics (DFG grant SFB 992/1 2012) and German Federal Ministry of Education and Research (BMBF grants 031 A538A/A538C RBC, 031L0101B/031L0101C de.NBI-epi, 031L0106 de.STAIR (de.NBI)). Part of the work was performed by the Belgian ELIXIR node, also hosting the tools at the Belgian Galaxy instance which is funded by the Research Foundation - Flanders (FWO) grant I002919N.

Data Availability

All the data files used for this study are uploaded onto a Zenodo repository at <https://doi.org/10.5281/zenodo.3733904>, we have provided the input and output files of our data analysis. Supplementary 2 (<https://github.com/jraysajulga/quant-tools-analysis>) is the GitHub repository of the Rscripts. The original dataset for UPS study is available via ProteomeXchange identifier-PXD000279 (spiked-in Universal Proteomic Standard).

References:

- [1] J. Cox, M.Y. Hein, C.A. Luber, I. Paron, N. Nagaraj, M. Mann, Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ., *Mol. Cell. Proteomics.* 13 (2014) 2513–26.
<https://doi.org/10.1074/mcp.M113.031591>.
- [2] C. Zhang, Y. Liu, Retrieving Quantitative Information of Histone PTMs by Mass Spectrometry, in: *Methods Enzymol.*, Academic Press Inc., 2017: pp. 165–191.
<https://doi.org/10.1016/bs.mie.2016.10.017>.
- [3] J.A. Alfaro, A. Ignatchenko, V. Ignatchenko, A. Sinha, P.C. Boutros, T. Kislinger, Detecting protein variants by mass spectrometry: a comprehensive study in cancer cell-lines, *Genome Med.* 9 (2017) 62. <https://doi.org/10.1186/s13073-017-0454-9>.
- [4] J. Yeom, M.H. Kabir, B. Lim, H.S. Ahn, S.Y. Kim, C. Lee, A proteogenomic approach for protein-level evidence of genomic variants in cancer cells, *Sci. Rep.* 6 (2016).
<https://doi.org/10.1038/srep35305>.
- [5] C.W. Easterly, R. Sajulga, S. Mehta, J. Johnson, P. Kumar, S. Hubler, B. Mesuere, J. Rudney, T.J. Griffin, P.D. Jagtap, metaQuantome: An Integrated, Quantitative Metaproteomics Approach Reveals Connections Between Taxonomy and Protein Function in Complex Microbiomes, *Mol. Cell. Proteomics.* 18 (2019) S82–S91.
<https://doi.org/10.1074/mcp.ra118.001240>.
- [6] J.F. Krey, P.A. Wilmarth, J.B. Shin, J. Klimek, N.E. Sherman, E.D. Jeffery, D. Choi, L.L. David, P.G. Barr-Gillespie, Accurate label-free protein quantitation with high- and low-resolution mass spectrometers, *J. Proteome Res.* 13 (2014) 1034–1044.
<https://doi.org/10.1021/pr401017h>.

- [7] T. Välikangas, T. Suomi, L.L. Elo, A comprehensive evaluation of popular proteomics software workflows for label-free proteome quantification and imputation, *Brief. Bioinform.* 19 (2017) 1344–1355. <https://doi.org/10.1093/bib/bbx054>.
- [8] A. Chawade, M. Sandin, J. Teleman, J. Malmström, F. Levander, Data processing has major impact on the outcome of quantitative label-free LC-MS analysis, *J. Proteome Res.* 14 (2015) 676–687. <https://doi.org/10.1021/pr500665j>.
- [9] S. Tyanova, T. Temu, J. Cox, The MaxQuant computational platform for mass spectrometry-based shotgun proteomics, *Nat. Protoc.* 11 (2016) 2301–2319. <https://doi.org/10.1038/nprot.2016.136>.
- [10] B. Schilling, M.J. Rardin, B.X. MacLean, A.M. Zawadzka, B.E. Frewen, M.P. Cusack, D.J. Sorensen, M.S. Bereman, E. Jing, C.C. Wu, E. Verdin, C.R. Kahn, M.J. MacCoss, B.W. Gibson, Platform-independent and label-free quantitation of proteomic data using MS1 extracted ion chromatograms in skyline: Application to protein acetylation and phosphorylation, in: *Mol. Cell. Proteomics*, 2012: pp. 202–214. <https://doi.org/10.1074/mcp.M112.017707>.
- [11] B. Ma, K. Zhang, C. Hendrie, C. Liang, M. Li, A. Doherty-Kirby, G. Lajoie, PEAKS: Powerful software for peptide de novo sequencing by tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 17 (2003) 2337–2342. <https://doi.org/10.1002/rcm.1196>.
- [12] Progenesis QI for Proteomics□: Waters, (n.d.).
https://www.waters.com/waters/en_US/Progenesis-QI-for-Proteomics/nav.htm?locale=en_US&cid=134790665 (accessed October 8, 2019).
- [13] A. Argentini, L.J.E. Goeminne, K. Verheggen, N. Hulstaert, A. Staes, L. Clement, L. Martens, MoFF: A robust and automated approach to extract peptide ion intensities, *Nat. Methods.* 13 (2016) 964–966. <https://doi.org/10.1038/nmeth.4075>.
- [14] R.J. Millikin, S.K. Solntsev, M.R. Shortreed, L.M. Smith, Ultrafast Peptide Label-Free Quantification with FlashLFQ, *J. Proteome Res.* 17 (2018) 386–391. <https://doi.org/10.1021/acs.jproteome.7b00608>.
- [15] S. van Riper, E. Chen, H. Remmer, A. Chien, P. Stemmer, Y. Wang, P. Jagtap, Identification of Low Abundance Proteins in a Highly Complex Protein Mixture, (2016). <https://doi.org/10.5281/ZENODO.3563207>.
- [16] E. Afgan, D. Baker, M. van den Beek, D. Blankenberg, D. Bouvier, M. Čech, J. Chilton,

D. Clements, N. Coraor, C. Eberhard, B. Grüning, A. Guerler, J. Hillman-Jackson, G. Von Kuster, E. Rasche, N. Soranzo, N. Turaga, J. Taylor, A. Nekrutenko, J. Goecks, The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update, *Nucleic Acids Res.* 44 (2016) W3–W10. <https://doi.org/10.1093/nar/gkw343>.

[17] P.D. Jagtap, J.E. Johnson, G. Onsongo, F.W. Sadler, K. Murray, Y. Wang, G.M. Shenykman, S. Bandhakavi, L.M. Smith, T.J. Griffin, Flexible and accessible workflows for improved proteogenomic analysis using the galaxy framework, *J. Proteome Res.* 13 (2014) 5898–5908. <https://doi.org/10.1021/pr500812t>.

[18] K.J. Anderson, K.L. Vermillion, P. Jagtap, J.E. Johnson, T.J. Griffin, M.T. Andrews, Proteogenomic Analysis of a Hibernating Mammal Indicates Contribution of Skeletal Muscle Physiology to the Hibernation Phenotype, *J. Proteome Res.* 15 (2016) 1253–1261. <https://doi.org/10.1021/acs.jproteome.5b01138>.

[19] C. Blank, C. Easterly, B. Gruening, J. Johnson, C.A. Kolmeder, P. Kumar, D. May, S. Mehta, B. Mesuere, Z. Brown, J.E. Elias, W.J. Hervey, T. McGowan, T. Muth, B.L. Nunn, J. Rudney, A. Tanca, T.J. Griffin, P.D. Jagtap, Disseminating metaproteomic informatics capabilities and knowledge using the galaxy-P framework, *Proteomes*. 6 (2018). <https://doi.org/10.3390/proteomes6010007>.

[20] P.D. Jagtap, A. Blakely, K. Murray, S. Stewart, J. Kooren, J.E. Johnson, N.L. Rhodus, J. Rudney, T.J. Griffin, Metaproteomic analysis using the Galaxy framework, *Proteomics*. 15 (2015) 3553–3565. <https://doi.org/10.1002/pmic.201500074>.

[21] R. Adusumilli, P. Mallick, Data conversion with proteoWizard msConvert, in: *Methods Mol. Biol.*, Humana Press Inc., 2017: pp. 339–368. https://doi.org/10.1007/978-1-4939-6747-6_23.

[22] H. Barsnes, M. Vaudel, SearchGUI: A Highly Adaptable Common Interface for Proteomics Search and de Novo Engines, *J. Proteome Res.* 17 (2018) 2552–2555. <https://doi.org/10.1021/acs.jproteome.8b00175>.

[23] M. Vaudel, J.M. Burkhardt, R.P. Zahedi, E. Oveland, F.S. Berven, A. Sickmann, L. Martens, H. Barsnes, PeptideShaker enables reanalysis of MS-derived proteomics data sets: To the editor, *Nat. Biotechnol.* 33 (2015) 22–24. <https://doi.org/10.1038/nbt.3109>.

[24] J. Cox, N. Neuhauser, A. Michalski, R.A. Scheltema, J. V. Olsen, M. Mann, Andromeda: A peptide search engine integrated into the MaxQuant environment, *J. Proteome Res.* 10

(2011) 1794–1805. <https://doi.org/10.1021/pr101065j>.

[25] M.E. Ritchie, B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, G.K. Smyth, Linear Models for Microarray and RNA-Seq Data, *Nucleic Acids Res.* 43 (2015) e47. <https://doi.org/10.1093/nar/gkv007>.

[26] T. Suomi, G.L. Corthals, O.S. Nevalainen, L.L. Elo, Using peptide-level proteomics data for detecting differentially expressed proteins, *J. Proteome Res.* 14 (2015) 4564–4570. <https://doi.org/10.1021/acs.jproteome.5b00363>.

[27] T. Välikangas, T. Suomi, L.L. Elo, A systematic evaluation of normalization methods in quantitative label-free proteomics, *Brief. Bioinform.* 19 (2018) 1–11. <https://doi.org/10.1093/bib/bbw095>.

[28] M.E. Ritchie, B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, G.K. Smyth, Limma powers differential expression analyses for RNA-sequencing and microarray studies, *Nucleic Acids Res.* 43 (2015) e47. <https://doi.org/10.1093/nar/gkv007>.

[29] A. Argentini, A. Staes, B. Grüning, S. Mehta, C. Easterly, T.J. Griffin, P. Jagtap, F. Impens, L. Martens, Update on the moFF Algorithm for Label-Free Quantitative Proteomics, *J. Proteome Res.* 18 (2019) 728–731. <https://doi.org/10.1021/acs.jproteome.8b00708>.

[30] R. Dale, B. Grüning, A. Sjödin, J. Rowe, B.A. Chapman, C.H. Tomkins-Tinch, R. Valieris, B. Batut, A. Caprez, T. Cokelaer, D. Yusuf, K.A. Beauchamp, K. Brinda, T. Wollmann, G. Le Corguillé, D. Ryan, A. Bretaudeau, Y. Hoogstrate, B.S. Pedersen, S. van Heeringen, M. Raden, S. Luna-Valero, N. Soranzo, M. De Smet, G. Von Kuster, R. Kirchner, L. Pantano, Z. Charlop-Powers, K. Thornton, M. Martin, M. van den Beek, D. Maticzka, M. Miladi, S. Will, K. Gravouil, P. Unneberg, C. Brueffer, C. Blank, V.C. Piro, J. Wolff, T. Antao, S. Gladman, I. Shlyakhter, M. de Hollander, P. Mabon, W. Shen, J. Boekel, M. Holtgrewe, D. Bouvier, J.R. de Ruiter, J. Cabral, S. Choudhary, N. Harding, R. Kleinkauf, E. Enns, F. Eggenhofer, J. Brown, P.J.A. Cock, H. Timm, C. Thomas, X.O. Zhang, M. Chambers, N. Turaga, E. Seiler, C. Brislaw, E. Pruesse, J. Fallmann, J. Kelleher, H. Nguyen, L. Parsons, Z. Fang, E.B. Stovner, N. Stoler, S. Ye, I. Wohlers, R. Farouni, M. Freeberg, J.E. Johnson, M. Bargull, P.R. Kensche, T.H. Webster, J.M. Eppley, C. Stahl, A.S. Rose, A. Reynolds, L.B. Wang, X. Garnier, S. Dirmeyer, M. Knudsen, J. Taylor, A. Srivastava, V. Rai, R. Agren, A. Junge, R.V. Guimera, A. Khan, S.

Schmeier, G. He, L. Pinello, E. Hägglund, A.S. Mikheyev, J. Preussner, N.R. Waters, W. Li, J. Capellades, A.T. Chande, Y. Pirola, S. Hiltemann, M.L. Bendall, S. Singh, W.A. Dunn, A. Drouin, T. Di Domenico, I. de Bruijn, D.E. Larson, D. Chicco, E. Grassi, G. Gonnella, J. B, L. Wang, F. Giacomoni, E. Clarke, D. Blankenberg, C. Tran, R. Patro, S. Laurent, M. Gopez, B. Sennblad, J.A. Baaijens, P. Ewels, P.R. Wright, O.M. Enache, P. Roger, W. Dampier, D. Koppstein, U.K. Devisetty, T. Rausch, M. Cornwell, A.E. Salatino, J. Seiler, M. Jung, E. Kornobis, F. Cumbo, B.K. Stöcker, O. Moskalenko, D.R. Bogema, M.L. Workentine, S.J. Newhouse, F. da V. Leprevost, K. Arvai, J. Köster, Bioconda: Sustainable and comprehensive software distribution for the life sciences, *Nat. Methods.* 15 (2018) 475–476. <https://doi.org/10.1038/s41592-018-0046-7>.

[31] M.A. Luersen, R. Le Riche, F. Guyon, A constrained, globalized, and bounded Nelder-Mead method for engineering optimization, *Struct. Multidiscip. Optim.* 27 (2004) 43–54. <https://doi.org/10.1007/s00158-003-0320-9>.

[32] G.M. Kurtzer, V. Sochat, M.W. Bauer, Singularity: Scientific containers for mobility of compute, *PLoS One.* 12 (2017) e0177459. <https://doi.org/10.1371/journal.pone.0177459>.

[33] B.A. Walther, J.L. Moore, The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance, *Ecography (Cop.).* 28 (2005) 815–829. <https://doi.org/10.1111/j.2005.0906-7590.04112.x>.

[34] A. Bensimon, A.J.R. Heck, R. Aebersold, Mass Spectrometry-Based Proteomics and Network Biology, *Annu. Rev. Biochem.* 81 (2012) 379–405. <https://doi.org/10.1146/annurev-biochem-072909-100424>.

[35] K.L. Vermillion, P. Jagtap, J.E. Johnson, T.J. Griffin, M.T. Andrews, Characterizing cardiac molecular mechanisms of mammalian hibernation via quantitative proteogenomics, *J. Proteome Res.* 14 (2015) 4792–4804. <https://doi.org/10.1021/acs.jproteome.5b00575>.

[36] A. Ori, B.H. Toyama, M.S. Harris, T. Bock, M. Iskar, P. Bork, N.T. Ingolia, M.W. Hetzer, M. Beck, Integrated Transcriptome and Proteome Analyses Reveal Organ-Specific Proteome Deterioration in Old Rats, *Cell Syst.* 1 (2015) 224–237. <https://doi.org/10.1016/j.cels.2015.08.012>.

[37] T.I. Roumeliotis, S.P. Williams, E. Gonçalves, C. Alsinet, M. Del Castillo Velasco-

Herrera, N. Aben, F.Z. Ghavidel, M. Michaut, M. Schubert, S. Price, J.C. Wright, L. Yu, M. Yang, R. Dienstmann, J. Guinney, P. Beltrao, A. Brazma, M. Pardo, O. Stegle, D.J. Adams, L. Wessels, J. Saez-Rodriguez, U. McDermott, J.S. Choudhary, Genomic Determinants of Protein Abundance Variation in Colorectal Cancer Cells, *Cell Rep.* 20 (2017) 2201–2214. <https://doi.org/10.1016/j.celrep.2017.08.010>.

[38] C.N. Schlaffner, G.J. Pirklbauer, A. Bender, J.A.J. Steen, J.S. Choudhary, A fast and quantitative method for post-translational modification and variant enabled mapping of peptides to genomes, *J. Vis. Exp.* 2018 (2018). <https://doi.org/10.3791/57633>.

[39] J. Gans, M. Wolinsky, J. Dunbar, Microbiology: Computational improvements reveal great bacterial diversity and high toxicity in soil, *Science* (80-.). 309 (2005) 1387–1390. <https://doi.org/10.1126/science.1112665>.

[40] X. Li, J. LeBlanc, A. Truong, R. Vuthoori, S.S. Chen, J.L. Lustgarten, B. Roth, J. Allard, A. Ippoliti, L.L. Presley, J. Borneman, W.L. Bigbee, V. Gopalakrishnan, T.G. Graeber, D. Elashoff, J. Braun, L. Goodlick, A metaproteomic approach to study human-microbial ecosystems at the mucosal luminal interface, *PLoS One.* 6 (2011). <https://doi.org/10.1371/journal.pone.0026542>.

[41] K. Cheng, Z. Ning, X. Zhang, L. Li, B. Liao, J. Mayne, A. Stintzi, D. Figeys, MetaLab: an automated pipeline for metaproteomic data analysis, *Microbiome.* 5 (2017) 157. <https://doi.org/10.1186/s40168-017-0375-2>.

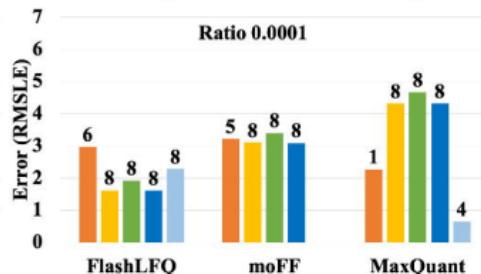
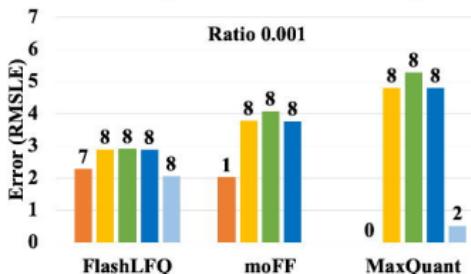
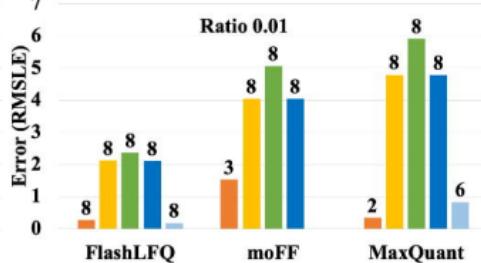
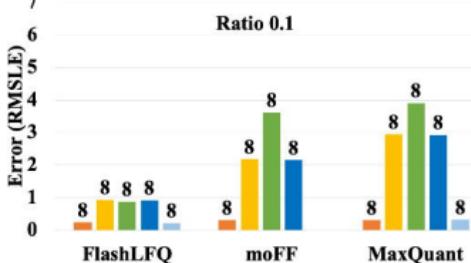
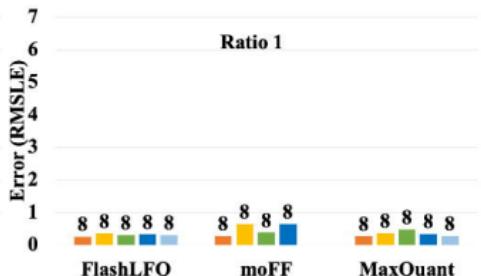
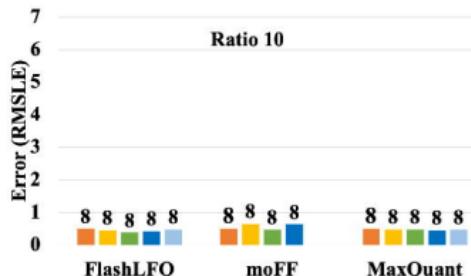
[42] M.C. Chambers, P.D. Jagtap, J.E. Johnson, T. McGowan, P. Kumar, G. Onsongo, C.R. Guerrero, H. Barsnes, M. Vaudel, L. Martens, B. Grüning, I.R. Cooke, M. Heydarian, K.L. Reddy, T.J. Griffin, An Accessible Proteogenomics Informatics Resource for Cancer Researchers., *Cancer Res.* 77 (2017) e43–e46. <https://doi.org/10.1158/0008-5472.CAN-17-0331>.

[43] G. Mayer, L. Montecchi-Palazzi, D. Ovelleiro, A.R. Jones, P.A. Binz, E.W. Deutsch, M. Chambers, M. Kallhardt, F. Levander, J. Shofstahl, S. Orchard, J.A. Vizcaíno, H. Hermjakob, C. Stephan, H.E. Meyer, M. Eisenacher, The HUPO proteomics standards initiativemass spectrometry controlled vocabulary, *Database.* 2013 (2013). <https://doi.org/10.1093/database/bat009>.

[44] R. Smith, Conversations with 100 Scientists in the Field Reveal a Bifurcated Perception of the State of Mass Spectrometry Software, *J. Proteome Res.* 17 (2018) 1335–1339.

<https://doi.org/10.1021/acs.jproteome.8b00015>.

[45] S. Tyanova, T. Temu, J. Cox, The MaxQuant computational platform for mass spectrometry-based shotgun proteomics, *Nat. Protoc.* 11 (2016) 2301–2319.
<https://doi.org/10.1038/nprot.2016.136>.



■ cyclocoess ■ scale ■ quantile ■ vsn ■ LFQ*

MaxLFQ ultrafast label-free quantification for mass-spectrometry proteomics (Saville Version 1.2.5.0)

Identification file
 329_UPS_PSM_AltMethod.txtvwr

MetaMorpheus/Morpheus/ProteoShaker PSM Report, MaxQuant (- id)

spectrum files
 1341_UPS.txt
10_ms2m_UPS

monoisotopic ppm tolerance
10
(--open)

isotopic distribution tolerance in ppm
0
(--open)

number of isotopes required to be observed
2
(--open)

integrate peak areas (not recommended)
 Yes
 No
(--open)

use early precursor charge state
 Yes
 No
(--open)

match between runs
 Yes
 No
(--open)

maximum MBR window in minutes
2.5
(--open)

use experimental design for normalization or protein fold-change analysis
Yes
Experimental Design.txt
 20_ExperimentalDesign.txt
normalize Intensity results
 Yes
 No
(--open)

Perform Bayesian protein fold-change analysis
Yes
control condition for Bayesian protein fold-change analysis
SI
(--open)

fold-change cut-off
1
Leave bins to determine empirically from data. (--763)

use shared peptides for protein quantification
 Yes
 No
(--open)

require MS/MS ID in conditions
 Yes
 No
(--open)

number of markov-chain monte carlo iterations
3000
(--open)

random seed
(-160)

ms2FF extracts MS1 intensities from spectrum files
(Galaxy Version 2.0.2.0)

[O Favorite](#) [Versions](#) [Options](#)

Choose which module to run

AE (inbatch-between-run followed by quantitation)

Choose the format for the identification file:

Peptide Shaker PSM report (standard, not extended)

Peptide Shaker PSM report

32P_UPS_PSM_A1_edited.tabular

Choose the format for the MS/MS file

Thermo RAW file

RAW file(s)

134: UPS.raw

Tolerance parameter

10.0

Specify the tolerance parameter in ppm. (—tol)

ms2FF workflow

select the ms2FF workflow (—mwf)

retention time windows for XIC

1.0

Specify rt window for xic in minutes. (—xic_length)

retention time window for apex detection

1.0

Specify the retention time window for the peak in minutes. (—rt_peak_win)

retention time window for the matched peaks

1.2

Specify the retention time window for the matched peak in minutes. (—rt_peak_win_match)

Activate filtering of matched peptides

Filter by flags to exclude or require

sample_size

0.2

percentage of MS2 peptide used to estimate the threshold. Default value 0.20 (—sample_size)

—quantile_thr_filtering

0.75

quantile value used to compute the filtering threshold for the matched peak (—quantile_thr_filtering)

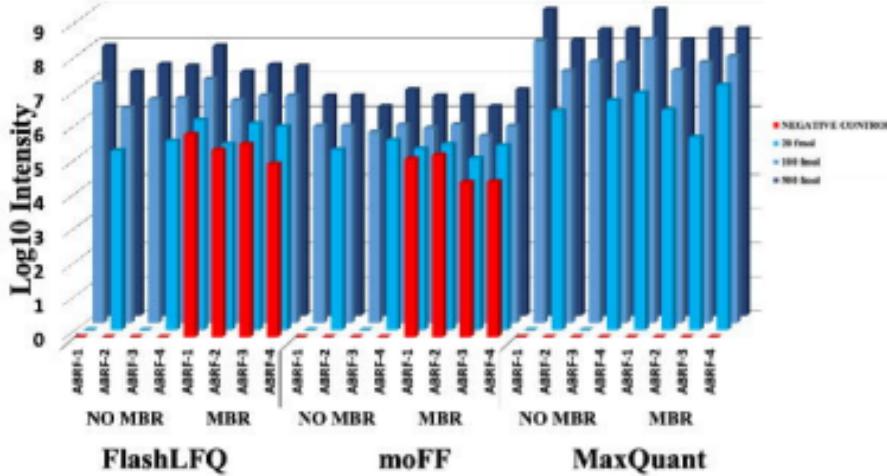
prin_file

load your pml file in order to override internal method (—pml_file)

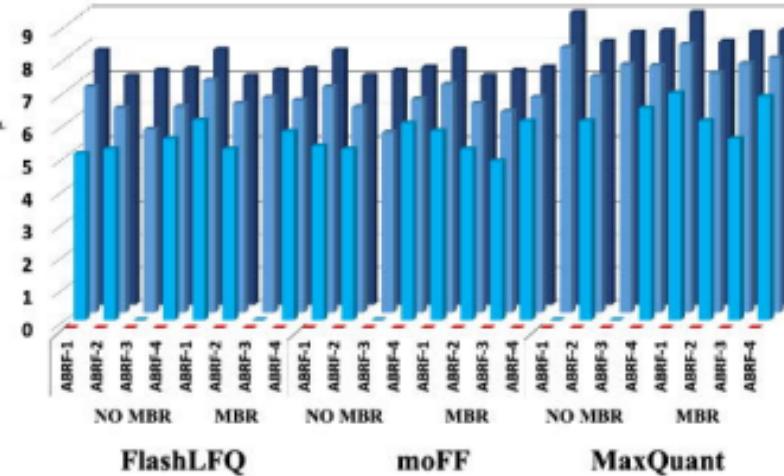
Output the peptide summary?

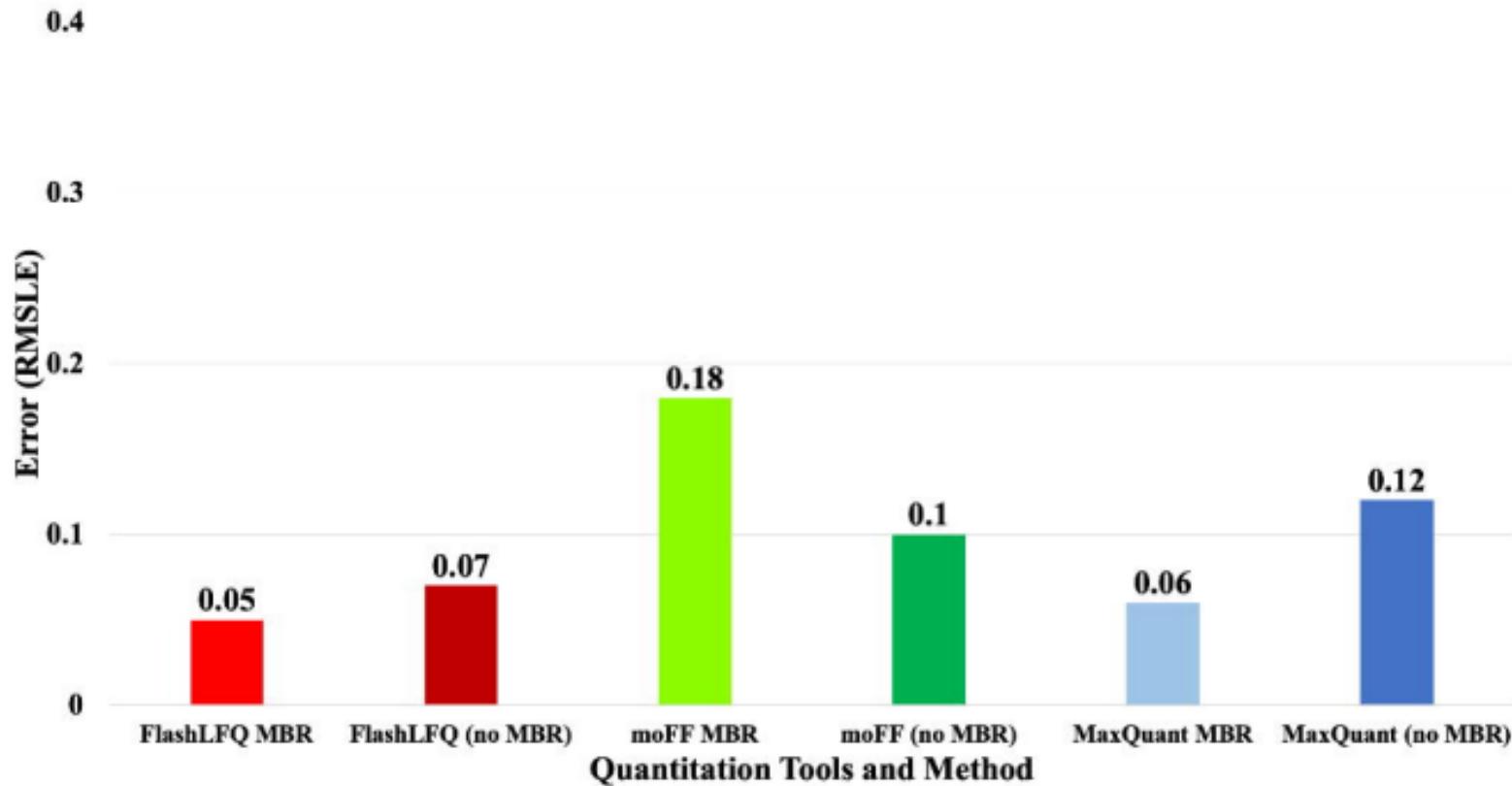
(—peptide_summary)

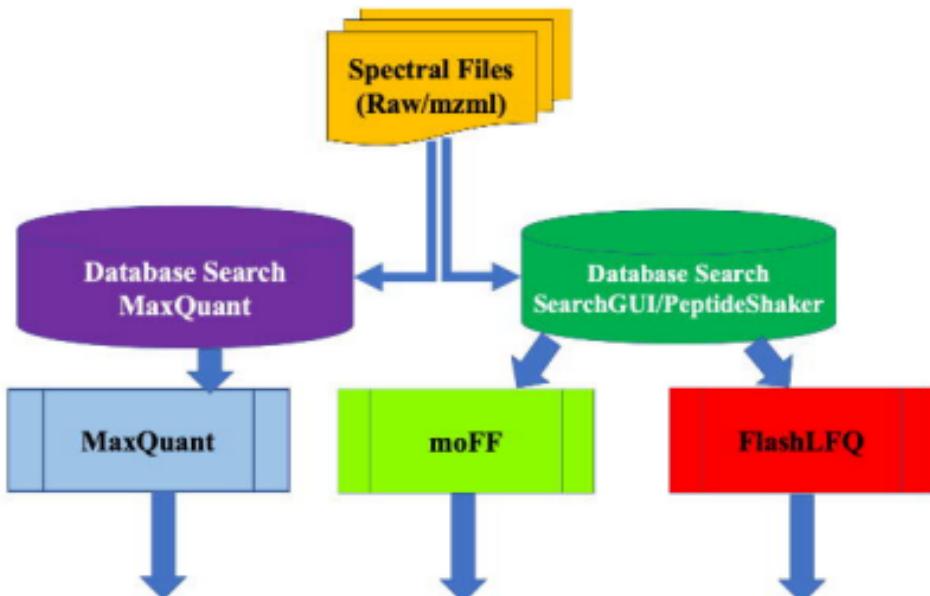
BEFORE



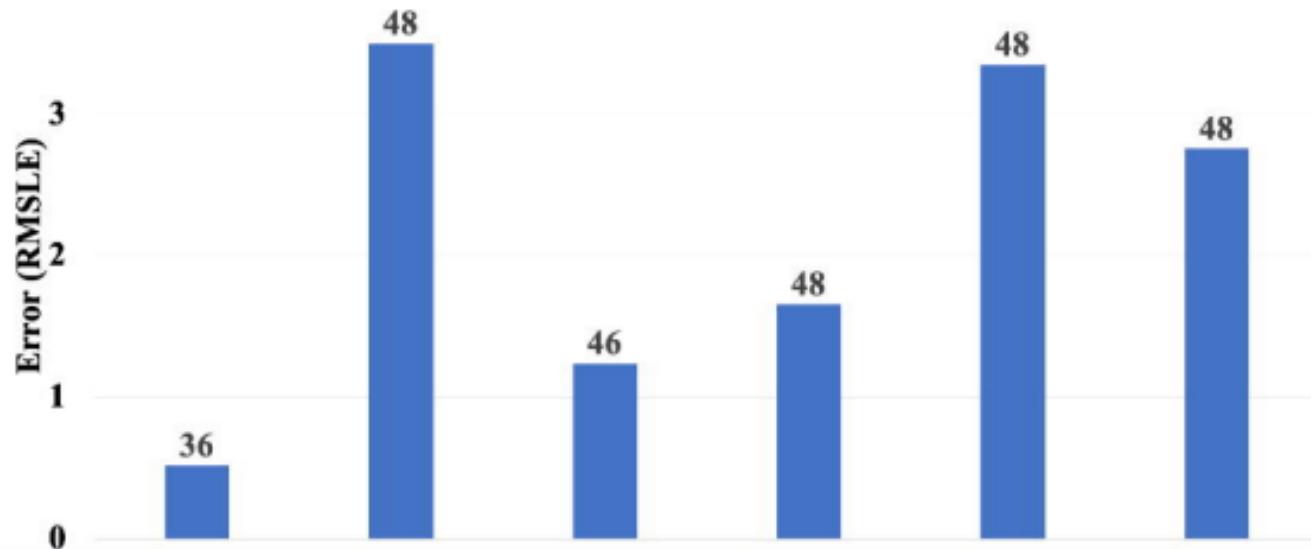
AFTER



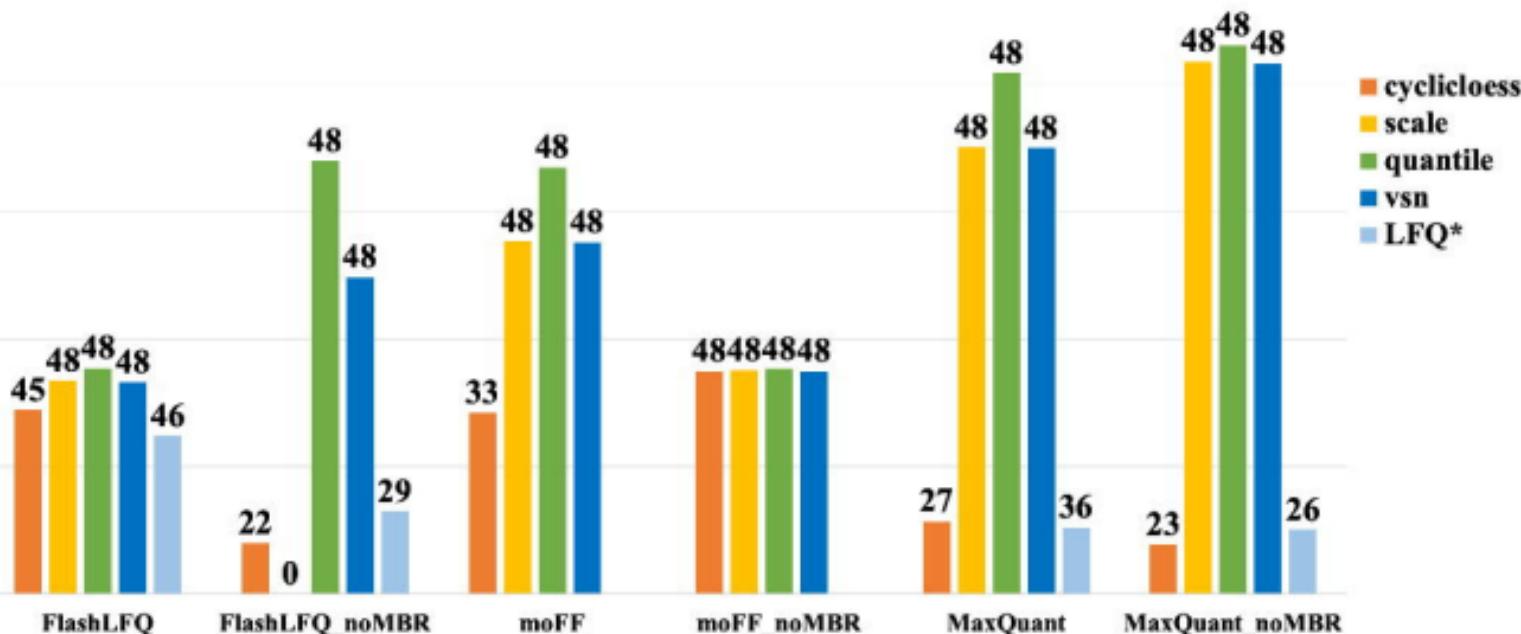




Accessibility to code	Closed Source, although publicly available for use	Open Source	Open Source
Operating System	Windows and Linux	Windows and Linux	Windows, Linux, OSX
Galaxy Implementation	Yes, limited functionality	Yes	Yes
Match Between Runs	Yes	Yes	Yes
Outputs	Multiple tabular outputs with peptide and protein quantitation outputs	Log file, Quantitation outputs and Peptide summary	Quantified Base Sequence, Modified Sequence, Peaks and Protein tabular files.
Time for Analysis	~ 34 mins for 8 datafiles *	~1 hour for 8 datafiles*	~15 mins for 8 datafiles*

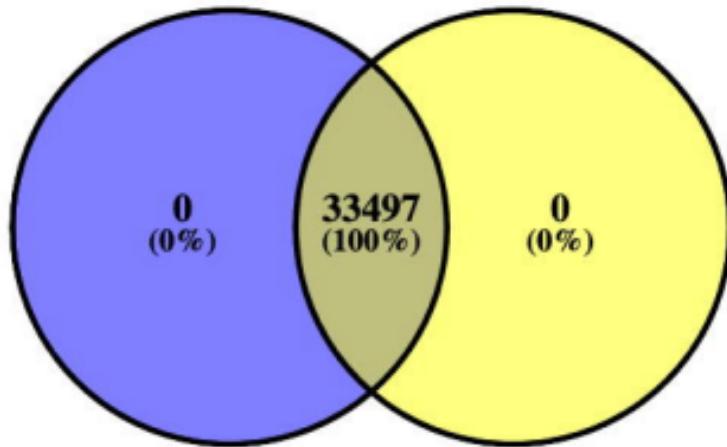


Search Engine	MaxQuant	MaxQuant	SG/PS*	SG/PS*	SG/PS*	SG/PS*
Peptide Quantification	MaxQuant	MaxQuant	FlashLFQ	FlashLFQ	moFF	moFF
Normalization	MaxQuant	vsn	FlashLFQ	vsn	quantile	vsn
Protein Quantification	MaxQuant	PECA	FlashLFQ	PECA	PECA	PECA



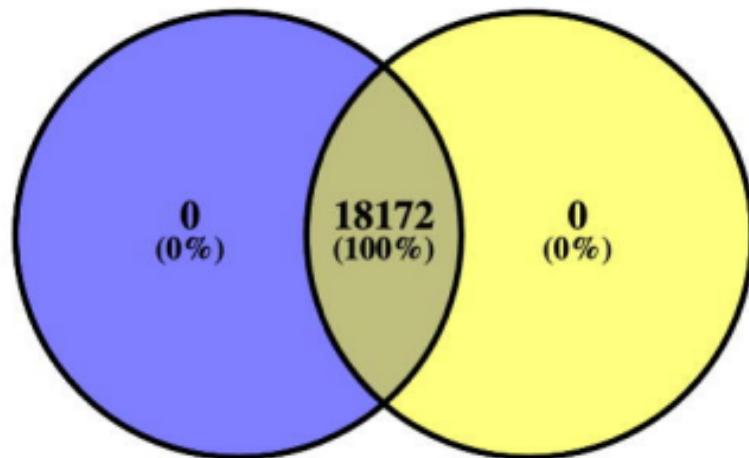
FlashLFQ

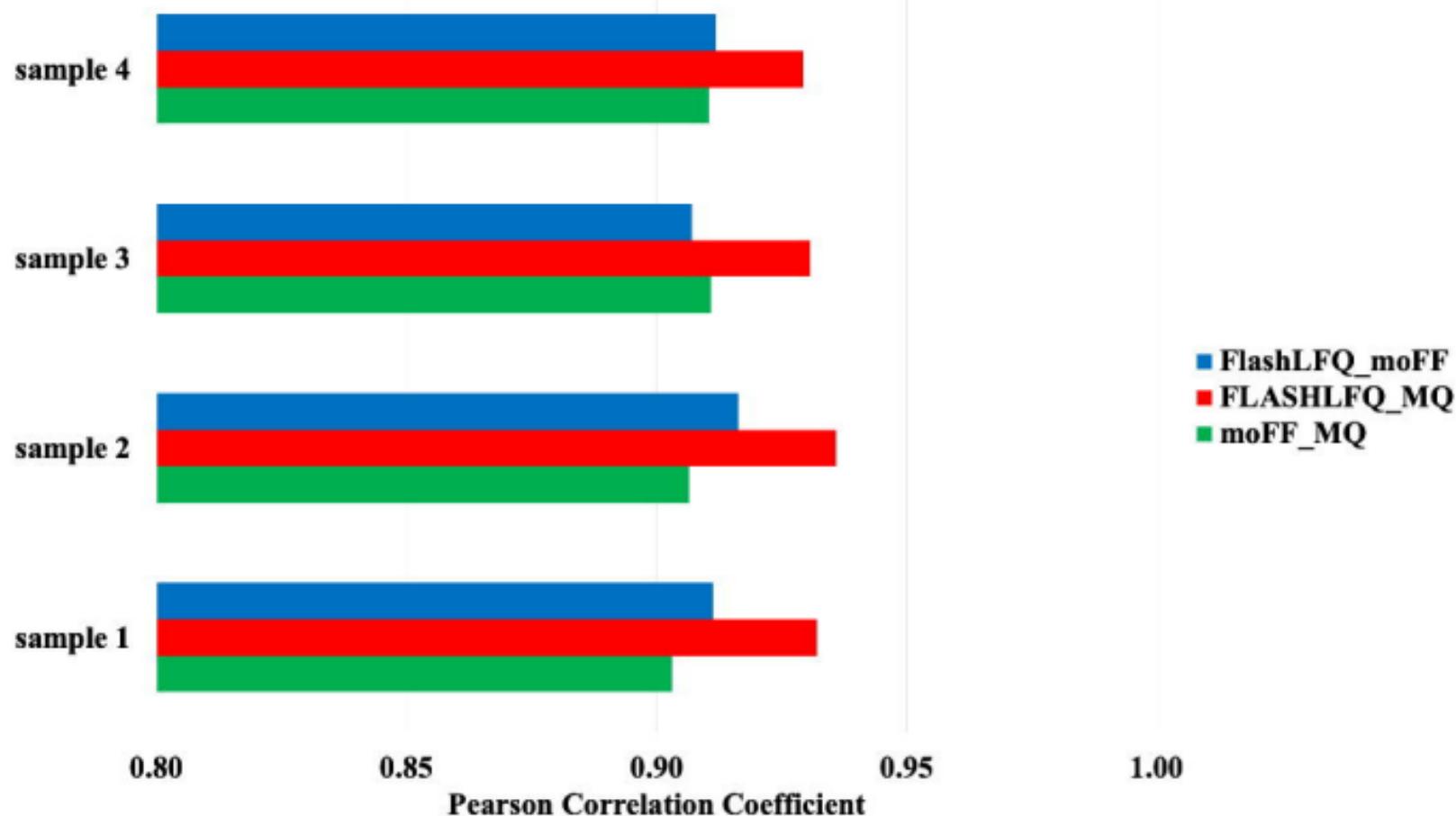
moFF

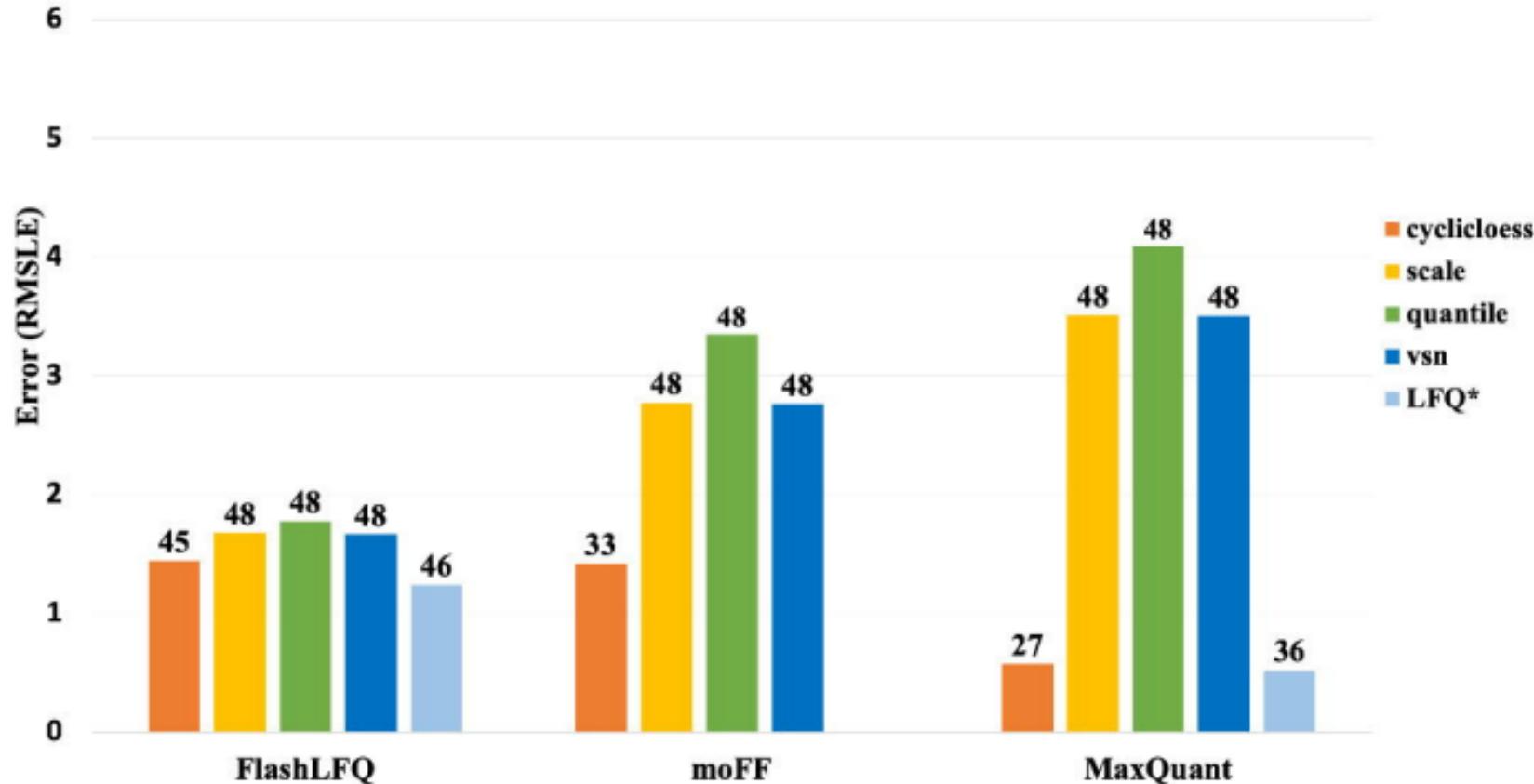


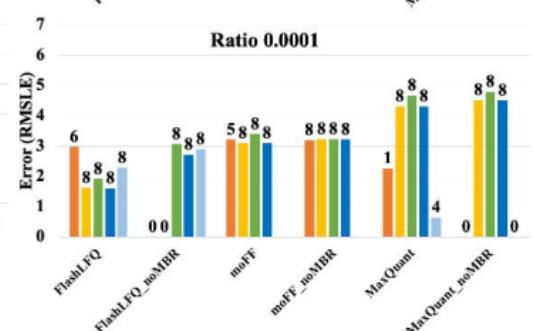
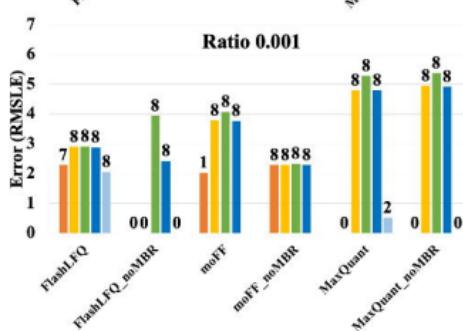
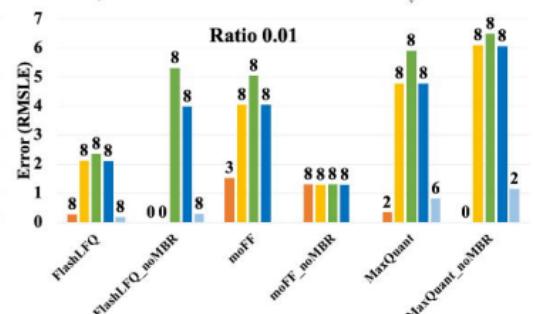
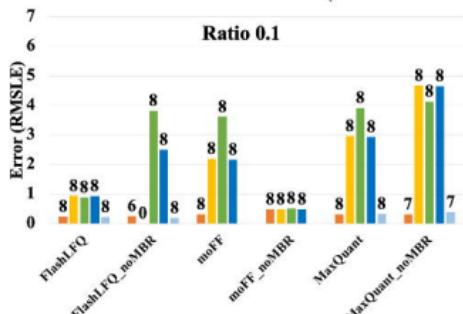
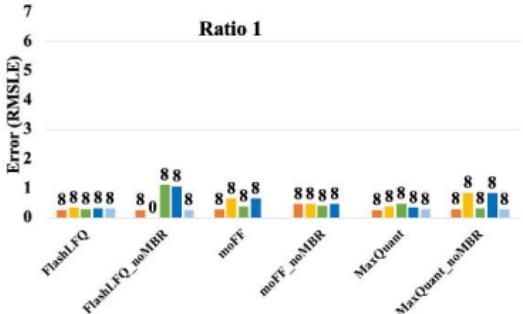
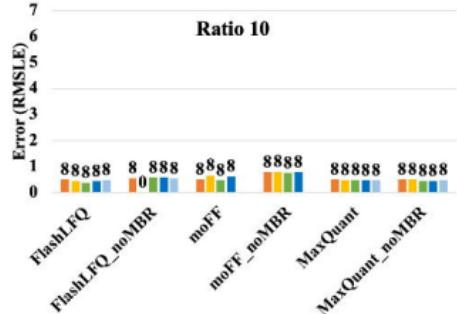
FlashLFQ

moFF









■ cycloless ■ scale ■ quantile ■ vsn ■ LFQ*