proteiNorm – A user-friendly tool for normalization and analysis of TMT and label-free protein/proteome quantification

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KEYWORDS: Proteomics, Normalization, Differential analysis

1. **Introduction**

With the latest technological advances in the development of mass spectrometer, the detection accuracy of the instruments has increased as well as the speed with which data is being produced. The amount of data generated by these high-throughput devices increases rapidly and the need for tools that allow to streamline analyses becomes more apparent.

The peptide level of thousands of proteins are being measured for up to several patients at once. However, data generated from mass spectrometry is often affected by systemic bias, variation caused by non-biological sources, which can lead to incorrect conclusions. This can include, but is not limited to, sample preparation and handling, device calibration, changes in temperature and other unknown sources. Unlike the effect of batching, these sources of variation are either not measured or simply cannot be measured, and therefore cannot be controlled for in statistical modelling. Normalization is a techniques that aims to account for these systematic biases and make samples more comparable, while preserving the signal.

Many methods

Evaluation of methods

structured normalization evaluation

Appropriate method depend on data set

Tools for choosing proper method (normalyzer)

General introduction; What is the problem/ impact of said problem; What has been done; What are the gaps; What did we do/ How did we fill gaps

1. **Methods**
   1. **Implementation**

proteiNorm implemented in R statistical language (<http://r-project.org>, version 3.6) using the package “shiny” to provide a user-friendly user interface. proteiNorm can be downloaded from GitHub (<https://github.com/ByrumLab/proteiNorm>).

* 1. **Data Requirements**

As input, proteiNorm expects tab-separated raw peptide and/or protein data (not on logarithmic scale) as produced by software such as XXX (where is the data coming from), where the column names of the measured intensities start with “Reporter intensity corrected” followed by an integer and an optional label (e.g. “Reporter intensity corrected 5 TMT2”,), and each row represents a peptide or protein. Data from both quantitation method, Tandem Mass Tag (TMT) and label-free, are supported.

Due to the detection limit of mass spectrometry instruments many peptide/protein measurements have an intensity measurement of zero. These values will be considered as missing values (denoted as NA) and can be imputed with precaution.

* 1. **Normalization Methods**

The current implementation of proteiNorm includes several popular normalization methods, including log2 normalization, median normalization, mean normalization, variance stabilizing normalization (VSN), quantile normalization, cyclic loess normalization, global robust linear regression normalization, and global intensity normalization. The individual performance of each methods can be evaluated in comparisons of the following metrics: total intensity, Pooled intragroup Coefficient of Variation (PCV), Pooled intragroup Median Absolute Deviation (PMAD), Pooled intragroup estimate of variance (PEV), intragroup correlation, sample correlation heatmap (Pearson), and log2-ratio distributions.

* 1. **Differential Analysis**

DAtest is an available R packages that allows to compare different differential abundance and expression methods. As the DAtest package requires complete data (no missing values), the user can user can select appropriate methods for normalization and imputation for the differential analysis, based on the comparison of the normalization methods and a heatmap visualizing patterns of missing values (missing at random (MAR) or missing not at random (MNAR)). The current implementation of proteiNorm provides the following imputation methods: k-nearest neighbors (KNN), Quantile Regression Imputation of Left-Censored data (QRILC), deterministic minimal value imputation (MinDet), stochastic minimal value imputation (MinProb), minimal value imputation, and zero imputation.

In addition, proteiNorm provides the opportunity to adjust DAtest-specific parameters (i.e., number of times to run the tests, effect size for the spike-ins (on log2 scale), and number of cores to use for parallel computing) and to exclude certain statistical tests. This latter option can be useful, as the exclusion of test with high computation demand (such as the permutation-based test), will drastically reduce the run-time.

DAtest will automatically select appropriate statistical tests from the pool of tests provided by DAtest and evaluate their performance. Therefore, the data is shuffled and spiked-in for randomly chosen features. DAtest then assesses if the spiked-in features are correctly identified by each test and whether the false discovery rate is controlled. The results are summarized in a table and figures comparing and ranking the performance of each individual test. Additionally, proteiNorm provides the opportunity to investigate the performance of each individual test by estimating statistical power (probability of correctly detecting a true signals) over a range of effect sizes (around the previously specified effect size for the spike-ins), utilizing the “powerDA” function from the “DAtest” package.

1. **Results**
2. **Discussion**
3. **Conclusion**
4. **References**

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally. (match statement to author names with a symbol)

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ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography.

REFERENCES

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