Received: 16 April 2023 | Revised: 4 July 2023 | Accepted: 5 July 2023

DOI: 10.1002/pmic.202300188

Proteomics
Proteomics and Systems Biology

RAPID COMMUNICATION

Tissue-based absolute quantification using large-scale TMT and LFQ experiments

Hong Wang[1] | Chengxin Dai[1,2] | Julianus Pfeuffer[3] | Timo Sachsenberg[4,5] | Aniel Sanchez[6] | Mingze Bai[1,2] | Yasset Perez-Riverol[7]

[1]Chongqing Key Laboratory of Big Data for Bio Intelligence, Chongqing University of Posts and Telecommunications, Chongqing, China

[2]State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Life Omics, Beijing, China

[3]Algorithmic Bioinformatics, Freie Universität Berlin, Berlin, Germany

[4]Department of Computer Science, Applied Bioinformatics, University of Tübingen, Tübingen, Germany

[5]Institute for Biological and Medical Informatics, University of Tübingen, Tübingen, Germany

[6]Section for Clinical Chemistry, Department of Translational Medicine, Lund University, Skåne University Hospital Malmö, Malmö, Sweden

[7]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, UK

Correspondence
Yasset Perez-Riverol, European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, UK.
Email: yperez@ebi.ac.uk

Funding information
EMBL core funding; Wellcome grants, Grant/Award Numbers: 208391/Z/17/Z, 223745/Z/21/Z; EU H2020 project EPIC-XS, Grant/Award Number: 823839; Forschungscampus MODAL, Grant/Award Number: 3FO18501; National Key Research and Development Program of China, Grant/Award Numbers: 2017YFC0908404, 2017YFC0908405; Natural Science Foundation of Chongqing, China, Grant/Award Number: cstc2018jcyjAX0225; Wellcome Trust, Grant/Award Number: 208391/Z/17/Z

Abstract

Relative and absolute intensity-based protein quantification across cell lines, tissue atlases and tumour datasets is increasingly available in public datasets. These atlases enable researchers to explore fundamental biological questions, such as protein existence, expression location, quantity and correlation with RNA expression. Most studies provide MS1 feature-based label-free quantitative (LFQ) datasets; however, growing numbers of isobaric tandem mass tags (TMT) datasets remain unexplored. Here, we compare traditional intensity-based absolute quantification (iBAQ) proteome abundance ranking to an analogous method using reporter ion proteome abundance ranking with data from an experiment where LFQ and TMT were measured on the same samples. This new TMT method substitutes reporter ion intensities for MS1 feature intensities in the iBAQ framework. Additionally, we compared LFQ-iBAQ values to TMT-iBAQ values from two independent large-scale tissue atlas datasets (one LFQ and one TMT) using robust bottom-up proteomic identification, normalisation and quantitation workflows.

KEYWORDS
absolute protein expression, proteomics data reanalysis, public data, TMT, LFQ, big data

Abbreviations: CCC, Concordance correlation coefficient; CV, Coefficient of Variation; DDA, Data-Dependent Acquisition; DIA, Data-Independent Acquisition; emPAI, Exponentially Modified Protein Abundance Index; IBAQ, Intensity-Based Absolute Quantification; LFQ, Label-Free Quantification; MS, Mass Spectrometry; NSAF, Normalized Spectral Abundance Factor; rIBAQ, normalized intensity-Based Absolute Quantification; RNA, Ribonucleic Acid; SDRF, Sample and Data Relationship Format; SIn, Normalize Spectral index; TMT, Tandem Mass Tag; TOP3, Top 3 peptides.

Proteomics is a powerful tool for understanding the underlying biology of cells and tissues. Large-scale cell lines, tumour datasets or tissue atlases enable researchers to ask fundamental questions about the proteome, such as protein existence, expression location and correlation with RNA expression [1–3]. The number of publicly available datasets continues to expand every year [4], facilitating their reuse [5, 6] and integration into protein expression resources [7, 8]. Label-free intensity-based absolute quantification (iBAQ) is a robust and common method to estimate the expression of proteins without the need for a standard reference sample [9, 10]. This method measures relative protein abundances within a sample and can be converted to approximate absolute scales, like copy number when certain assumptions are met. iBAQ protein expression has been only explored for the label-free data-dependent (DDA) [9] and independent acquisition (DIA) methods using MS1 [10]. riBAQ is similar to iBAQ except that each protein's iBAQ value was normalised to the sum of all iBAQ values to obtain its riBAQ value [10, 11].

MS2 methods [12, 13], such as spectral counting, can serve as a proxy for absolute quantification in bottom-up proteomics experiments. Spectral-counting algorithms offer some advantages because they can be applied directly to the data commonly collected for identification purposes including tandem mass tags (TMT, multiplex) experiments. In 2011, Colaert et al. [13] explored three MS2-based quantitative methods: Exponentially modified Protein Abundance Index (EmPAI) [14], Normalised Spectral Abundance Factor (NSAF) [15] and normalised Spectral Index (SIn) [16]. Their findings indicated that the NSAF method outperformed both EmPAI and SIn in terms of accuracy and precision [13]. However, spectral counting-based quantification has limitations because it does not use chromatography peak attributes such as height or area potentially limiting its accuracy and dynamic range [17, 18]. Ahrné et al. [19] undertook a distinct intensity-based strategy to calculate iBAQ values in TMT datasets, treating them as label-free datasets. This involved distributing MS1 intensities of all TMT-labelled features among the individual samples based on the relative reporter ion intensities. However, this approach is more complex, as the datasets need to be analysed as label-free experiments and precursor ion intensities must be extracted. Furthermore, this approach has not been applied to a large-scale dataset or benchmarked across different datasets.

Here, we explored an alternative approach to perform absolute protein expression analysis on TMT datasets using the direct reporter ion intensities. To assess the accuracy of this method, we employed a gold-standard mix-proteome dataset (PXD007683) [20] analysed with both LFQ and TMT methods. We then calculated iBAQ values based on either MS1 feature or reporter ion intensities (respectively) and compared the correlation for all quantified proteins. Additionally, we applied robust normalisation and quantitation workflows to analyse two large-scale tissue datasets from Jian et al. (TMT – PXD016999) [1] and Wang et al. (LFQ – PXD010154) [2].

iBAQ values were estimated using the MS1 intensities for label-free experiments, and the reporter ion intensities in the case of TMT datasets. Feature intensity tables for all analysed datasets were generated using the quantms (https://quantms.readthedocs.io/) workflow which enables the analysis of DDA, DIA label-free and TMT datasets [21, 22]. quantms is a novel workflow that allows performing cloud and HPC data analysis in a distributed manner [23] and has been already benchmarked with popular tools such as MaxQuant and ProteomeDiscover [24]. Each generated feature was the combination of a peptide sequence, modifications, charge state, sample, fraction, and technical or biological replicate. Feature intensities were normalised using quantile normalisation, the highest intensity for each feature was selected across replicates (Supplementary Note 1). Then feature intensities were added together across replicates of the same sample. Finally, feature intensities were averaged (median) at the peptide sequence level. iBAQ is computed by dividing the sum of peptide intensities by the number of theoretically observable peptides of the protein. Each iBAQ value was normalised to the sum of all iBAQ values for the same sample (riBAQ) [11, 25]. All analysis steps are included in a Python package (https://github.com/bigbio/ibaqpy , Supplementary Note 2).

We tested the TMT-iBAQ approach using a mix-proteome dataset comprising both Human and Yeast samples in multiple concentrations [20]. The primary objective of the dataset and the original study was to evaluate the capability of TMT and LFQ approaches in accurately quantifying fold changes of 3-, 2- and 1.5-fold across the entire dataset. All parameters for the reanalysis were annotated using the SDRF file format [26] (Supplementary Note 3). In the present study, we did not explore the differential expression across samples (as originally designed by O'Connell et al. [20]) but compared the expression of the human proteins when using TMT-iBAQ or LFQ-iBAQ.

In the PXD007683 dataset, we quantified a total of 94,804 peptides and 8401 proteins. There were 33,321 peptides and 6273 proteins commonly identified using TMT and LFQ approaches; while 18,524 peptides from 392 proteins and 42,959 peptides from 1736 proteins were quantified using only LFQ or TMT approaches, respectively. The peptide intensity between both approaches is statistically significantly correlated for all samples ($R > 0.4$, Lin's concordance correlation coefficient [CCC] > 0.02 – Supplementary Note 4) and the protein intensity between both approaches shows a correlation coefficient higher than 0.8 ($R > 0.86$, Lin's CCC > 0.1 – Supplementary Note 5). The log-scale iBAQ values for both TMT and LFQ approaches of the PXD007683 dataset were compared, as shown in Figure 1A,B. First, we evaluated the reproducibility of the two methods across all 11 sample replicates for both approaches (Figure 1A). Samples analysed with the label-free method showed a higher coefficient of variation (average CV = 14%), while TMT samples had an average CV = 10%. The iBAQ values displayed a similar distribution across the 11 samples, with a higher median intensity observed for TMT experiments than LFQ in all samples (Figure 1A). The iBAQ Pearson correlation and Lin's CCC between the TMT and LFQ approaches is remarkably high ($R > 0.84$, Lin's CCC > 0.74). These results demonstrate that the iBAQ values obtained from both LFQ and TMT approaches in this benchmark dataset are highly consistent and reliable. In fact, this result is supported by the long use of MS2 (based on fragment ion intensities) data for quantification in proteomics experiments by using MRM, DIA or having found good correlations between precursors and their reporters in DDA experiments [27].

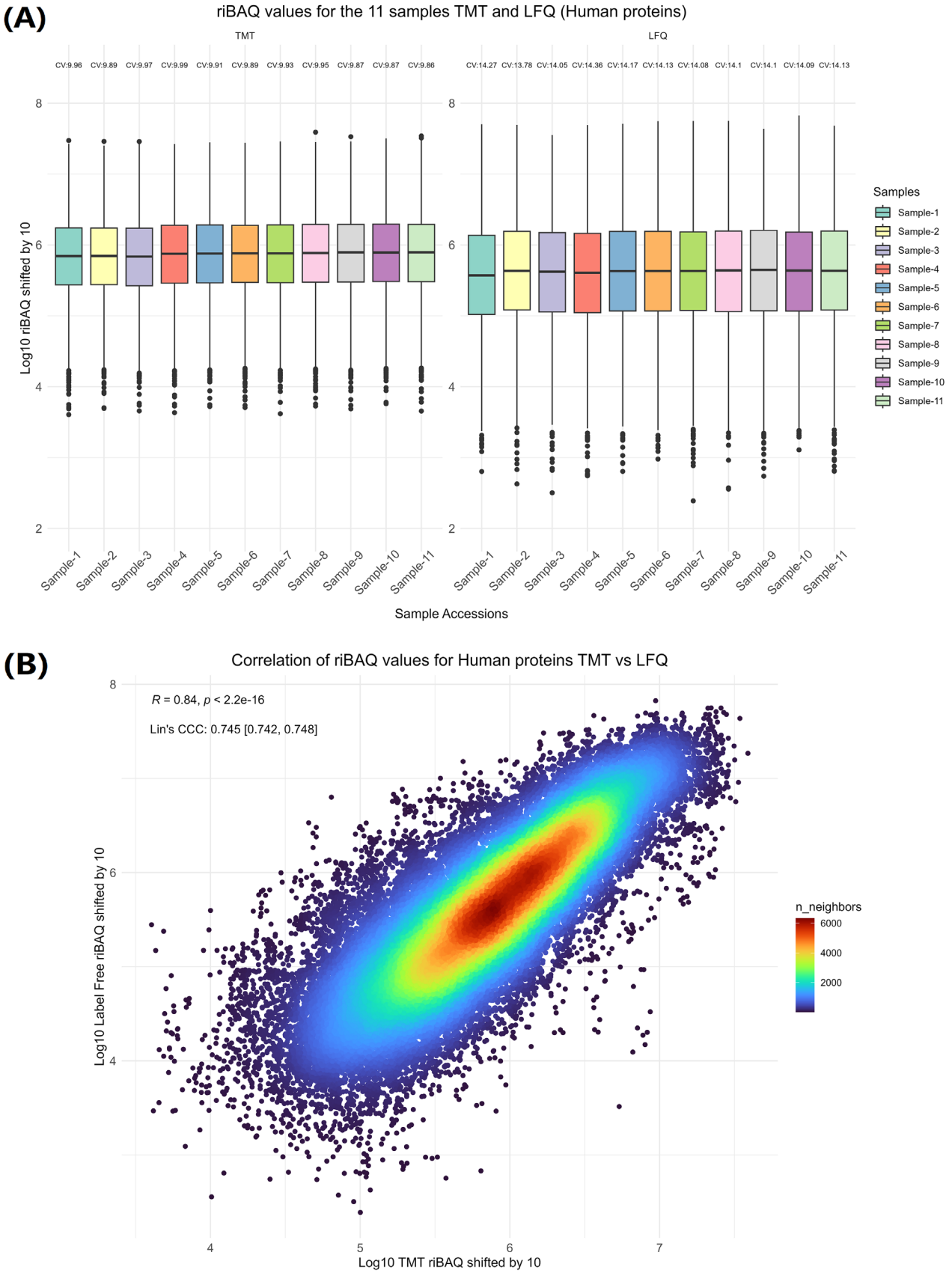

FIGURE 1 (A) Boxplot of riBAQ Log-transformed for the 11 samples dataset PXD007683, for both TMT and LFQ approaches. (B) Correlation between riBAQ values for all quantified proteins between the TMT and LFQ approaches, for dataset PXD007683. iBAQ, intensity-based absolute quantification; LFQ, label-free quantitative; TMT, tandem mass tags.

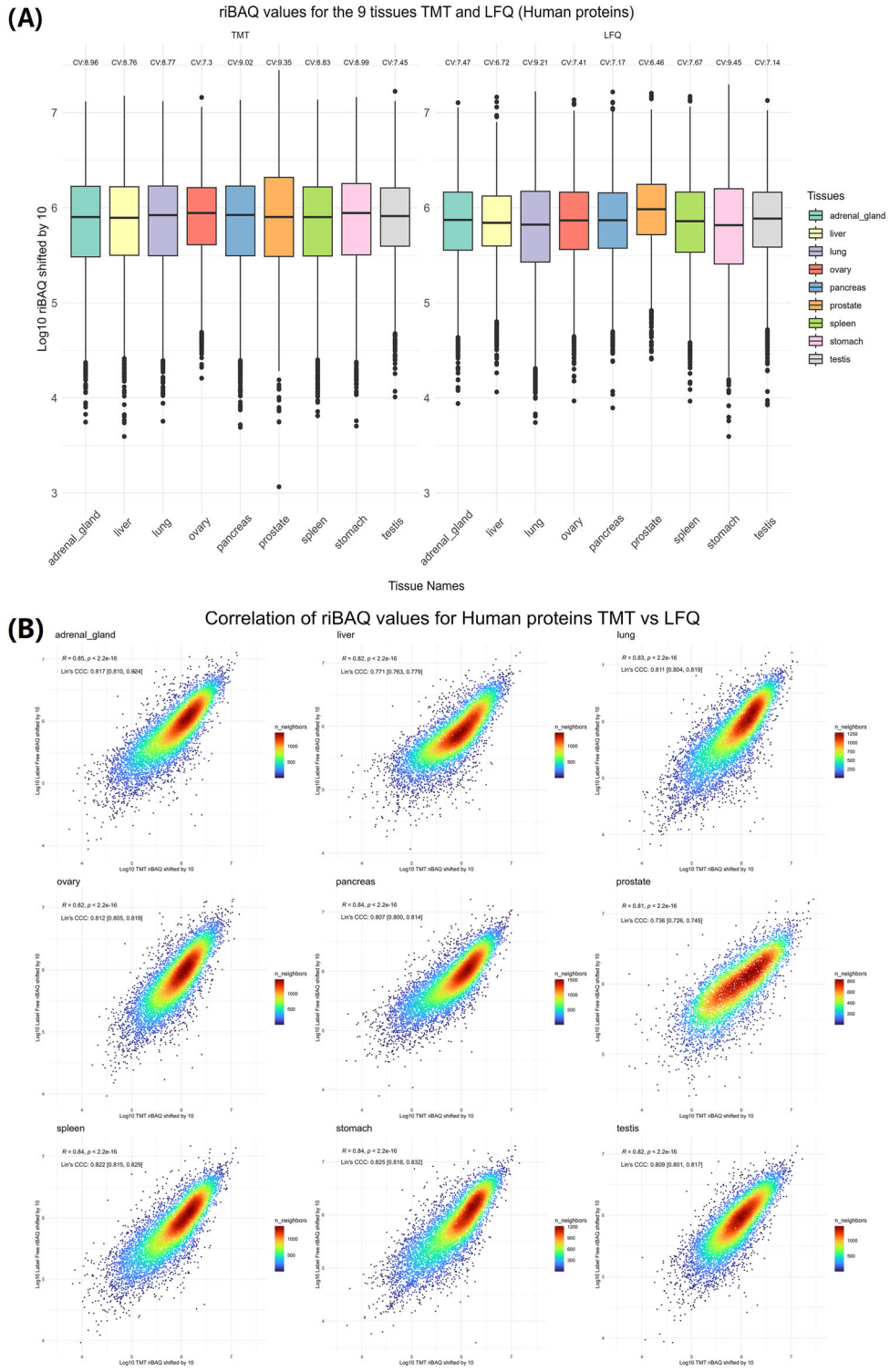

FIGURE 2 (A) Boxplot of riBAQ log-transformed for all tissues shared between datasets PXD016999 and PXD010154. (B) Correlation between riBAQ values for all quantified proteins between PXD016999 and PXD010154 datasets. iBAQ, intensity-based absolute quantification.

While previous authors [17, 20, 28] have found that LFQ and TMT methods offer similar performance in terms of accuracy when analysing the same sample, comparisons of these methods for proteome characterisation between different studies with similar tissue remain unexplored. We tested this in the reanalysis of two large-scale human tissue datasets from Jian et al. (TMT – PXD016999) [1] and Wang et al. (LFQ – PXD010154) [2] (Supplementary Note 3). Both datasets were analysed using the same database (UniProt human Swiss-Prot 092022), the quantms workflow and the corresponding datasets parameters (Supplementary Note 3). For PXD010154, a total

number of 340,306 peptides and 14,602 proteins were quantified, while the number of quantified peptides and proteins for PXD016999 were 173,678 and 10,351, respectively. Figure 2A shows the distribution of iBAQ values for all shared tissues between both datasets (adrenal gland, liver, lung, ovary, pancreas, prostate, spleen, stomach and testis), while median intensity is higher for TMT experiments compared with LFQ for all tissues except prostate. Figure 2B shows the iBAQ correlation between both experiments for the shared tissues, and all tissues show a correlation coefficient higher than 0.80 and a Lin's CCC higher than 0.7. The iBAQ values obtained by LFQ and TMT of these nine tissues had a strong correlation and high consistency. Previously, Betancourt et al. [29] integrated TMT results with LFQ using the three most abundant peptides for each protein quantified (TOP3), but the reproducibility and the correlation between both technologies were never explored. Using the transformed normalised intensities as suggested by Jiang et al. [1], instead of the iBAQ values from reporter ion intensities (as suggested in this research), could negatively affect the correlation between relative proteome abundances obtained with LFQ or TMT.

In summary, iBAQ, as previously reported, is a robust and common method for estimating the relative/absolute expression of proteins. This study explored and extended the capabilities of the LFQ-iBAQ approach to perform proteome-wide quantification in TMT datasets using direct reporter ion intensities. The results showed that the iBAQ correlation between the TMT and LFQ approaches in different datasets is high, indicating the potential of the direct reporter ion intensity method for relative protein abundance analyses in TMT datasets. This new approach can enable the future integration of public TMT and LFQ proteomics datasets using intensity-based methods instead of less accurate spectral counting which could improve the accuracy and reproducibility of proteomics meta-analyses.

ACKNOWLEDGEMENTS

Yasset Perez-Riverol would like to acknowledge funding from EMBL core funding, Wellcome grants (208391/Z/17/Z, 223745/Z/21/Z) and the EU H2020 project EPIC-XS [823839]. Julianus Pfeuffer would like to acknowledge Forschungscampus MODAL (project grant 3FO18501). Chengxin Dai and Mingze Bai are supported by the National Key Research and Development Program of China (2017YFC0908404, 2017YFC0908405) and the Natural Science Foundation of Chongqing, China (cstc2018jcyjAX0225). The authors acknowledge Dr. Phillip Wilmarth for helpful discussions and assistance with manuscript preparation.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The Raw data of the three reanalysed datasets can be found in ProteomeXchange with the original accessions: PXD016999, PXD010154, and PXD007683. The iBAQ values for all samples can be found on GitHub: https://github.com/ypriverol/quantms-research/tmt-lfq-ibaq.

ORCID

Mingze Bai https://orcid.org/0000-0002-9782-2056
Yasset Perez-Riverol https://orcid.org/0000-0001-6579-6941

REFERENCES

1. Jiang, L., Wang, M., Lin, S., Jian, R., Li, X., Chan, J., Dong, G., Fang, H., Robinson, A. E., Snyder, M. P., Aguet, F., Anand, S., Ardlie, K. G., Gabriel, S., Getz, G., Graubert, A., Hadley, K., Handsaker, R. E., Huang, K. H., … Volpi, S. (2020). A quantitative proteome map of the human body. *Cell*, *183*(1), 269–283.e19. https://doi.org/10.1016/j.cell.2020.08.036
2. Wang, D., Eraslan, B., Wieland, T., Hallström, B., Hopf, T., Zolg, D. P., Zecha, J., Asplund, A., Li, L.-H., Meng, C., Frejno, M., Schmidt, T., Schnatbaum, K., Wilhelm, M., Ponten, F., Uhlen, M., Gagneur, J., Hahne, H., & Kuster, B. (2019). A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Molecular Systems Biology*, *15*(2), e8503. doi: 10.15252/msb.20188503
3. Di Meo, A., Sohaei, D., Batruch, I., Alexandrou, P., Prassas, I., & Diamandis, E. P. (2021). Proteomic profiling of the human tissue and biological fluid proteome. *Journal of Proteome Research*, *20*(1), 444–452. https://doi.org/10.1021/acs.jproteome.0c00502
4. Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D. J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., & Vizcaíno, J. A. (2022). The PRIDE database resources in 2022: A hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Research*, *50*(D1), D543–D552. https://doi.org/10.1093/nar/gkab1038
5. Claeys, T., Menu, M., Bouwmeester, R., Gevaert, K., & Martens, L. (2022). Machine learning on large-scale proteomics data identifies tissue- and cell type-specific proteins. bioRxiv. https://doi.org/10.1021/acs.jproteome.2c00644
6. Prakash, A., García-Seisdedos, D., Wang, S., Kundu, D. J., Collins, A., George, N., Moreno, P., Papatheodorou, I., Jones, A. R., & Vizcaíno, J. A. (2023). Integrated view of baseline protein expression in human tissues. *Journal of Proteome Research*, *22*(3), 729–742. https://doi.org/10.1021/acs.jproteome.2c00406
7. Lautenbacher, L., Samaras, P., Muller, J., Grafberger, A., Shraideh, M., Rank, J., Fuchs, S. T., Schmidt, T. K., The, M., Dallago, C., Wittges, H., Rost, B., Krcmar, H., Kuster, B., & Wilhelm, M. (2022). ProteomicsDB: Toward a FAIR open-source resource for life-science research. *Nucleic Acids Research*, *50*(D1), D1541–D1552. https://doi.org/10.1093/nar/gkab1026
8. Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D., & Mering, C. (2015). Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics*, *15*(18), 3163–3168. https://doi.org/10.1002/pmic.201400441
9. Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., & Selbach, M. (2011). Global quantification of mammalian gene expression control. *Nature*, *473*(7347), 337–342. https://doi.org/10.1038/nature10098
10. He, B., Shi, J., Wang, X., Jiang, H., & Zhu, H.-J. (2019). Label-free absolute protein quantification with data-independent acquisition. *Journal of Proteomics*, *200*, 51–59. https://doi.org/10.1016/j.jprot.2019.03.005
11. Jarnuczak, A. F., Najgebauer, H., Barzine, M., Kundu, D. J., Ghavidel, F., Perez-Riverol, Y., Papatheodorou, I., Brazma, A., & Vizcaíno, J. A. (2021). An integrated landscape of protein expression in human cancer. *Scientific Data*, *8*(1), 115. https://doi.org/10.1038/s41597-021-00890-2

12. Arike, L., & Peil, L. (2014). Spectral counting label-free proteomics. *Methods in Molecular Biology*, *1156*, 213–222. https://doi.org/10.1007/978-1-4939-0685-7_14
13. Colaert, N., Vandekerckhove, J., Gevaert, K., & Martens, L. (2011). A comparison of MS2-based label-free quantitative proteomic techniques with regards to accuracy and precision. *Proteomics*, *11*(6), 1110–1113. https://doi.org/10.1002/pmic.201000521
14. Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., & Mann, M. (2005). Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Molecular & Cellular Proteomics*, *4*(9), 1265–1272. https://doi.org/10.1074/mcp.M500061-MCP200
15. Paoletti, A. C., Parmely, T. J., Tomomori-Sato, C., Sato, S., Zhu, D., Conaway, R. C., Conaway, J. W., Florens, L., & Washburn, M. P. (2006). Quantitative proteomic analysis of distinct mammalian Mediator complexes using normalized spectral abundance factors. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(50), 18928–18933. https://doi.org/10.1073/pnas.0606379103
16. Griffin, N. M., Yu, J., Long, F., Oh, P., Shore, S., Li, Y., Koziol, J. A., & Schnitzer, J. E. (2010). Label-free, normalized quantification of complex mass spectrometry data for proteomic analysis. *Nature Biotechnology*, *28*(1), 83–89. https://doi.org/10.1038/nbt.1592
17. Old, W. M., Meyer-Arendt, K., Aveline-Wolf, L., Pierce, K. G., Mendoza, A., Sevinsky, J. R., Resing, K. A., & Ahn, N. G. (2005). Comparison of label-free methods for quantifying human proteins by shotgun proteomics. *Molecular & Cellular Proteomics*, *4*(10), 1487–1502. https://doi.org/10.1074/mcp.M500084-MCP200
18. Chen, Y.-Y., Chambers, M. C., Li, M., Ham, A.-J. L., Turner, J. L., Zhang, B., & Tabb, D. L. (2013). IDPQuantify: Combining precursor intensity with spectral counts for protein and peptide quantification. *Journal of Proteome Research*, *12*(9), 4111–4121. https://doi.org/10.1021/pr400438q
19. Ahrné, E., Martinez-Segura, A., Syed, A. P., Vina-Vilaseca, A., Gruber, A. J., Marguerat, S., & Schmidt, A. (2015). Exploiting the multiplexing capabilities of tandem mass tags for high-throughput estimation of cellular protein abundances by mass spectrometry. *Methods (San Diego, Calif.)*, *85*, 100–107. https://doi.org/10.1016/j.ymeth.2015.04.032
20. O'connell, J. D., Paulo, J. A., O'brien, J. J., & Gygi, S. P. (2018). Proteome-wide evaluation of two common protein quantification methods. *Journal of Proteome Research*, *17*(5), 1934–1942. https://doi.org/10.1021/acs.jproteome.8b00016
21. Dai, C., Pfeuffer, J., Wang, H., Sachsenberg, T., Demichev, V., Kohlbacher, O., & Perez-Riverol, Y. (2023). Quantms: A cloud-based pipeline for proteomics reanalysis enables the quantification of 17521 proteins in 9,502 human samples. https://doi.org.10.21203/rs.3.rs-3002027/v1
22. Umer, H. M., Audain, E., Zhu, Y., Pfeuffer, J., Sachsenberg, T., Lehtiö, J., Branca, R. M., & Perez-Riverol, Y. (2022). Generation of ENSEMBL-based proteogenomics databases boosts the identification of non-canonical peptides. *Bioinformatics*, *38*(5), 1470–1472. https://doi.org/10.1093/bioinformatics/btab838
23. Perez-Riverol, Y., & Moreno, P. (2020). Scalable data analysis in proteomics and metabolomics using BioContainers and workflows engines. *Proteomics*, *20*(9), e1900147. https://doi.org/10.1002/pmic.201900147
24. Bai, M., Deng, J., Dai, C., Pfeuffer, J., Sachsenberg, T., & Perez-Riverol, Y. (2023). LFQ-based peptide and protein intensity differential expression analysis. *Journal of Proteome Research*, 2114–2123. https://doi.org/10.1021/acs.jproteome.2c00812
25. Shin, J.-B., Krey, J. F., Hassan, A., Metlagel, Z., Tauscher, A. N., Pagana, J. M., Sherman, N. E., Jeffery, E. D., Spinelli, K. J., Zhao, H., Wilmarth, P. A., Choi, D., David, L. L., Auer, M., & Barr-Gillespie, P. G. (2013). Molecular architecture of the chick vestibular hair bundle. *Nature Neuroscience*, *16*(3), 365–374. https://doi.org/10.1038/nn.3312
26. Dai, C., Füllgrabe, A., Pfeuffer, J., Solovyeva, E. M., Deng, J., Moreno, P., Kamatchinathan, S., Kundu, D. J., George, N., Fexova, S., Grüning, B., Föll, M. C., Griss, J., Vaudel, M., Audain, E., Locard-Paulet, M., Turewicz, M., Eisenacher, M., Uszkoreit, J., … Perez-Riverol, Y. (2021). A proteomics sample metadata representation for multiomics integration and big data analysis. *Nature Communications*, *12*(1), 5854. https://doi.org/10.1038/s41467-021-26111-3
27. Krey, J. F., Wilmarth, P. A., Shin, J.-B., Klimek, J., Sherman, N. E., Jeffery, E. D., Choi, D., David, L. L., & Barr-Gillespie, P. G. (2014). Accurate label-free protein quantitation with high- and low-resolution mass spectrometers. *Journal of Proteome Research*, *13*(2), 1034–1044. https://doi.org/10.1021/pr401017h
28. Wang, Z., Karkossa, I., Großkopf, H., Rolle-Kampczyk, U., Hackermüller, J., Von Bergen, M., & Schubert, K. (2021). Comparison of quantitation methods in proteomics to define relevant toxicological information on AhR activation of HepG2 cells by BaP. *Toxicology*, *448*, 152652. https://doi.org/10.1016/j.tox.2020.152652
29. Betancourt, L. H., Gil, J., Sanchez, A., Doma, V., Kuras, M., Murillo, J. R., Velasquez, E., Çakir, U., Kim, Y., Sugihara, Y., Parada, I. P., Szeitz, B., Appelqvist, R., Wieslander, E., Welinder, C., De Almeida, N. P., Woldmar, N., Marko-Varga, M., Eriksson, J., … Marko-Varga, G. (2021). The human melanoma proteome atlas—Complementing the melanoma transcriptome. *Clinical and Translational Medicine*, *11*(7), e451. https://doi.org/10.1002/ctm2.451

SUPPORTING INFORMATION

Additional supporting information may be found online https://doi.org/10.1002/pmic.202300188 in the Supporting Information section at the end of the article.

How to cite this article: Wang, H., Dai, C., Pfeuffer, J., Sachsenberg, T., Sanchez, A., Bai, M., & Perez-Riverol, Y. (2023). Tissue-based absolute quantification using large-scale TMT and LFQ experiments. *Proteomics*, *23*, e2300188. https://doi.org/10.1002/pmic.202300188