Editorial Summary: MassIVE.quant is a data repository and data resource for reproducible quantitative mass spectrometry-based proteomics

MassIVE.quant: a community resource of quantitative mass spectrometry-based proteomics datasets

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Abstract

MassIVE.quant is a repository infrastructure and data resource for reproducible quantitative mass spectrometry-based proteomics, which is compatible with all mass spectrometry data acquisition types and computational analysis tools. A branch structure enables MassIVE.quant to systematically store raw experimental data, metadata of the experimental design, scripts of the quantitative analysis workflow, intermediate input and output files, as well as alternative reanalyses of the same dataset.

Main

Quantitative mass spectrometry data analysis currently has multiple unmet reproducibility goals¹. At minimum, the mass spectrometry-based workflows must provide enough information to enable its full independent replication². Beyond that, conclusions of data analysis should not be dependent upon particular tuning parameters or software tools. Data analysis should demonstrate that alternative and equally appropriate parameter settings or software lead to qualitatively similar conclusions.

In mass spectrometry-based proteomics, data analysis is broadly categorized into peptide ion identification and quantification. Much progress in identification has been made in terms of open availability of tools and transparency of their algorithms. Archival resources MassIVE, PRIDE³, Panorama^{4,5}, PASSEL⁶ component of Peptide Atlas, and jPOST in ProteomeXchange^{7,8} store raw data, peak lists, search engine output, identification results, and corresponding mass spectra.

Unfortunately, reproducibility and transparency of data analysis for relative protein quantification is less satisfactory. First, given the great diversity of biological objectives and experiments, quantitative analyses require richer metadata describing experimental design and biological samples. Second, quantitative experiments require many data processing steps, which are distinct from similar steps in quantitative transcriptomic investigations. These include detection and identification of chromatographic peaks and reporter ions, and propagating those identities across multiple runs. Finally, existing analysis tools (such as Skyline⁹, MaxQuant¹⁰, OpenMS¹¹, OpenSWATH¹², DIA-Umpire¹³, Proteome Discoverer, or Spectronaut¹⁴) integrate, in their own unique ways, diverse functionalities for identification and quantification. These tools offer various

parameters and options, and output different details in various storage formats. Many tools also offer graphical user interfaces, for which analyses are difficult to document.

Benchmarking of individual analysis strategies and tools for quantitative proteomics workflows has become increasingly prevalent¹⁵, but lack infrastructure to store, document, annotate and reanalyze the full diversity of analyses.

To meet these reproducibility needs, we implemented MassIVE.quant, an infrastructure that supports quantitative mass spectrometry-based proteomics experiments. MassIVE.quant is integrated with an existing repository, the Mass Spectrometry Interactive Virtual Environment (MassIVE).

MassIVE.quant systematically stores the intermediate output files of every tool and workflow in a way which allows the user to easily inspect, reproduce or modify any component of the workflow, beginning with well-defined intermediate files. To accomplish this, we first developed a series of steps which represent a quantitative proteomics experiment with any experimental design, data acquisition, and data analysis tools (**Figure 1**). These steps consist of (1) annotations of experimental design; in particular, descriptors of biological samples and conditions; (2) strategies of sample preparation and data acquisition; (3) peptide ions identification; (4) quantification; and (5) statistical analysis. At each step, MassIVE.quant provides the infrastructure to store all intermediate descriptions, annotations, analysis scripts, and results.

MassIVE.quant does not prescribe a standard format, but meets scientists where they are by

directly accommodating the diverse nature of existing workflows. Each dataset contains links to the original publications or to metadata, which can be used to gain deeper insight into the biological context of the experiment. While analyte identification is represented by existing standard formats (such as mzTab, thousands of examples available for MassIVE datasets) and some of these can represent some quantitative information (such as the quant section in mzTab files, e.g., the [mzTab file for RMSV000000249.18](#)), the output files produced by quantitative analyses tools can be of any nature. None of the formats mentioned above support reporting of the results of statistical analyses of quantitative data. We chose a tabular format (i.e., csv) as a common representation of the output of quantitative and statistical analyses for all tools. This format emphasizes biologically relevant aspects of the output, such as the identity of differentially abundant peptides or proteins, the magnitudes of fold changes, and the associated variation.

Next, at each step, a branch structure enables the user to view reanalyses of each experiment. The reanalyses can be performed by the user offline with any combination of software tools and settings. MassIVE.quant stores the intermediate files and allows the user to check for the presence of script files, accuracy of parameters and completeness of documentation.

In order to scale the submission procedure and to ensure the reproducibility of a quantitative workflow, MassIVE.quant maintains datasets with four levels of curation (Bronze, Silver, Gold, and Platinum), reflecting the documentation and the reproducibility of the quantitative workflow. During the submission, the infrastructure checks whether the submission of the dataset or reanalysis meets the minimal requirements for the entry-level of curation. The submitter can then request the advanced review to level up.

MassIVE.quant further automates the statistical analysis of quantified proteins with an online MSstats workflow accessible with a user-friendly interface. This workflow can be used to reproduce the statistical analysis steps in MassIVE.quant reanalyses, as well as to analyze new private or public datasets. The MSstats Comparison workflow in MassIVE.quant automatically compares MSstats outputs across alternative reanalyses, and produces figures such as **Figure 2(o)**. This enables the user to evaluate the implications of alternative reanalyses on conclusions regarding differential protein abundance.

The online user documentation clearly describes the structure and the vocabulary used by MassIVE.quant, and provides detailed instructions for contributing data, reanalyses and comparisons (**Supplementary Note 1**). **Supplementary Figure 1 and 2, Supplementary Table 1** describe the infrastructure of MassIVE.quant and gives an example of the reanalysis submission workflow.

The impact of the choice between various analysis options is best understood in the presence of some notion of ground truth. Therefore, we populated MassIVE.quant with a collection of ten datasets with controlled mixtures with known changes in protein abundance. These include three datasets collected using label-free DDA, four datasets collected using DIA, one dataset collected using SRM with heavy labeled isotope peptides, and two DDA dataset collected using chemical labeling (TMT) (**Supplementary Table 2**). These datasets vary in background proteomes, number of conditions and replicates, and type and number of differentially abundant proteins.

We also populated MassIVE.quant with 95 reanalyses of these ten controlled datasets using multiple software tools, performed by the developers of the tools or by expert users. All the DDA experiments in **Figure 1** were processed with up to six tools for identification and four tools for quantification. For example, data from Choi *et al.*¹⁶ (DDA:Choi2017) were processed with eight different combinations of parameter settings in Skyline. The combinations of algorithms, tools, and settings generated 10 distinct quantification reports. Finally, up to five different types of downstream statistical analysis per dataset using MSstats¹⁷ generated 22 distinct tests for differential protein abundance for DDA:Choi2017.

To demonstrate the utility of documentation, reanalysis, and curation in basic biology investigations, we further populated MassIVE.quant with a collection of biological datasets, at the time of publication, including 8 DIA/SWATH, 7 SRM, 12 DDA, and 6 experiments collected DDA-TMT acquisition, analyzed with multiple tools; 25 datasets with platinum level of curation, 18 datasets with gold (**Supplementary Table 3**). For example, the DIA experiment by Selevsek *et al.*¹⁸ (DIA:Selevsek2015) was reanalyzed four times using different analysis strategies and different processing tools and parameter settings. **Figure 2, Supplementary Table 4, Supplementary Figure 3 and 4** illustrate how changes in data processing propagated themselves into discrepancies in the number of quantified proteins, frequency of missing values, and lists of differentially abundant proteins. **Figure 2(a-d)** illustrate these discrepancies in the special case of one protein. The analysis strategies and processing tools affected protein-level summaries in terms of scale, variation, and patterns of missing values. This in turn impacted the estimates of fold changes (**Figure 2(e-h)**) and tests for differential abundance (**Figure 2(i-l)**). Analysis with filtering in Skyline, applied to limit the DIA features to those known to be informative *a priori*

(Skyline:lowCV, RMSV000000251.1) detected a smoother, and therefore more biologically plausible, pattern of differential abundance in time (**Figure 2(m)**). While the true differential abundance is unknown, changes identified by the majority of tools are more likely to be real (**Figure 2(n-o)**). Such comparisons help curate the results of biological investigations.

To summarize, MassIVE.quant provides an opportunity for large-scale deposition of heterogeneous experimental datasets and facilitates a community-wide conversation about the benefits of its use. We hope that the community will find the resource useful and welcome user-driven submissions of both new datasets and documented reanalyses of the existing datasets.

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Author contributions

M.C., J.C., N.B., O.V. designed the research. M.C., T.H. collected datasets and performed statistical analysis. J.C., B.P. implemented MassIVE.quant. T.T. performed statistical analysis. C.C., E.S., M.T. experimented with new controlled mixtures. C.C., M.T., R.H., G.C.T., Y.P-R., J. M., M. M., S.G., M.P., E.V., B.W., O.M.B., A.I.N., L.R., T.D., E.S. analyzed data up to quantification. M.C., N.B., O.V. wrote the manuscript, with input from all authors.

Competing financial interests

O.M.B., J.M. and L.R. are employees of Biognosys AG. Spectronaut is a trademark of Biognosys AG. M.T. and T.D. are employees of Hoffmann-La Roche Ltd. All other authors declare no competing financial interests.

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Figure Legends

Figure 1 : Outline of MassIVE.quant repository structure, and reanalysis of three DDA-based experiments. Each step can be performed with multiple algorithms and software tools, generating tool-specific files in diverse formats. For the experiments in the figure, MassIVE.quant stores the intermediate outputs from combinations of algorithms and tools for peptide ion identification and quantification. For example, DDA:Choi2017 was processed with eight combinations of parameter settings in Skyline. Each reanalysis is saved with a unique reanalysis ID, prefixed by RMSV, under the experiment repository prefixed by MSV in MassIVE.quant.

Figure 2 : Re-analyses of DIA:Selevsek2015, profiling changes in proteome abundance of *S. cerevisiae* over 6 time points: T0(0 min), T1(15 min), T2(30 min), T3(60 min), T4(90 min), T5(120 min), n=3 biologically independent samples per each time point, in response to osmotic stress (RMSV000000251) (a)-(d) Discrepancies of quantification of protein YKL096W across data processing tools. Gray lines: fragments reported by each tool. Red lines: protein quantification summarized by MSstats. (a) Skyline:lowCV used Skyline to quantify a subset of the fragments with low coefficient of variation; (b) Skyline:All used Skyline to quantify all detectable peptides,

with a maximum of 6 fragments each; (c) data processed by Spectronaut; (d) data processed by DIA-Umpire. (e)-(h) Discrepancies in detecting differential abundance for protein YKL096W across data processing tools, with statistical analysis by MSstats. Dark red dot, center for error bars: model-based estimates of $\log_2$ fold change of protein abundance, as determined by MSstats. Error bars: 95% confidence intervals for the $\log_2$ fold changes, as determined by MSstats. Stars(*) correspond to adjusted p-values < 0.05 . (i)-(l) Volcano plots, summarizing differential abundance between T5 and T0. Dashed line: FDR=0.05; blue dots: significantly down-regulated proteins; red dots: significantly up-regulated proteins (counts are shown at the top left corner; other time points are shown in **Supplementary Figure 3**). (m) Number of differentially abundant proteins across all time points and all tools, FDR=0.05. (n) Venn diagram of differentially abundant proteins between two processing approaches by Skyline, comparing T5 versus T0. (o) Venn diagram of differentially abundant proteins across all tools, comparing T5 versus T0 (other time points are shown in **Supplementary Figure 4**).

Online Methods

Manual and tutorials

MassIVE.quant user manuals and video tutorials explain how to 1) create an account in MassIVE.quant, 2) upload files to MassIVE account via FTP, 3) submit quantification reanalysis to MassIVE.quant, 4) access reanalyses, 5) run MSstats workflow in MassIVE.quant, and 6) compare the results of statistical analysis by MSstats. User manuals are available at MassIVE.quant section in <https://ccms-ucsd.github.io/MassIVEDocumentation/>. The video tutorial is available in <https://www.youtube.com/channel/UCPeNhYFItiabsoOITPZBc5Q>

Data analysis for DIA:Selevsek2015

Skyline:All – All files for ProteomeXchange ID PXD001010 were downloaded, along with the forward and reversed FASTA and mzXML files ($n = 46$) used in the library peptide search (requested of the authors). Using Skyline (daily version 3.7.1.11571) first a spectral library was built using the iProphet score cut-off 0.0242 suggested in the paper as achieving 1% FDR at the PSM level, with 1,031 ambiguously matched peptides excluded, resulting in 82,439 unique peptides (104,993 entries). Because the Biognosys iRT standard peptides used were not included in the search, an iRT library was created from these files by adding all detected peptides with the iRT standards added as targets in Skyline and importing the mzXML files for MS1 extraction. The iRT values were then calculated using both the extracted peaks for the iRT standards and target peptide MS1 peaks where the peak contained a matching MS/MS ID, because the runs used fractionated samples and all peptides were not expected in all runs. For DIA, allowing unique peptides of length 7 to 45 resulting from semi-tryptic cleavage with up to 2 missed cleavages, with Carbamidomethyl (C) and optionally Oxidation (M), precursors of charge states 2, 3, or 4, from 400-1,200 m/z (the range covered by the DIA method), with 6 product transitions found in the library, of y or b type and 1 or 2 charge state (excluding y1, y2, b1, and b2). Chromatogram extraction was set to use TOF extraction at 18,000 resolving power with high-selectivity extraction applying to all MS/MS spectra within 10 minutes of predicted retention times using the iRT library. Importing the FASTA file and then removing duplicate peptides and empty proteins resulted in targets for 4,603 proteins, 68,910 peptides, 87,042 precursors, and 522,252 fragment ions, at 32% protein, 2.7% unique peptide FDR by reversed sequence decoy counting (decoys/targets). The protein FDR is likely overstated because the FASTA file contains only 6,717 protein sequences, which means as many as 2/3 of false peptides can be expected to occur in a true protein, while the

same is not true for detections of reversed peptides. Even at 10% protein FDR, however, this target set seemed to contain a higher error rate than we felt desirable. For these experiments, we decided to rebuild the library using the iProphet score cut-off 0.9, with 361 ambiguously matched peptides excluded, resulting in 64,501 unique peptides (84,245 entries). For our most inclusive method, we chose to include only fully-tryptic peptides and no variable modifications (dropping Oxidation (M)), which resulted in targets for 4,152 proteins, 36,889 peptides, 48,082 precursors, and 288,492 fragment ions, at 2.6% protein, 0.29% unique peptide FDR by reversed sequence decoy counting. An equal number of shuffled sequence decoys were generated for mProphet model generation. The 18 runs were then imported into the template an mProphet model trained and applied. The MSstats report was exported for further analysis.

Skyline:lowCV – Using Skyline (daily version 3.7.1.11571) and starting from the settings for the broadly inclusive test, we restored semi-tryptic cleavage and the variable modification Oxidation (M), expecting unstable peptides to be filtered by our first experiment. Importing the forward and reversed FASTA file resulted in targets for 4,246 proteins, 58,168 peptides, 74,314 precursors, and 445,884 fragment ions, at 4.5% protein, 0.34% unique peptide FDR by reversed sequence decoy counting. Reversed sequences, accounting for 193 proteins and 200 peptides, were left in the targets list to be carried through the subsequent “Reproducibility” experiment. An equal number of shuffled sequence decoys were generated for mProphet model generation. The runs ($n = 8$ – reported as $n = 4$ in the paper) acquired for the “Reproducibility” experiment were then imported into the template, an mProphet model trained and applied. Next, the targets were filtered for peptides detected at q value less than 0.01 in at least 4 (of 8) runs, and where the CV of the detected peak areas, median normalized, was less than 20%, resulting in targets for 2,212 proteins, 13,744 peptides, 18,910 precursors, and 113,460 fragment ions, at 1% protein, 0.18% unique peptide FDR

by reversed sequence decoy counting. The remaining peptides comprised 249 of 2,367 –10.5% – Oxidation (M), 679 of 18,479 – 3.7% – unmodified semi-tryptic, and 12,816 of 37,322 – 34.3% – unmodified tryptic peptides. As expected, Oxidation (M) and semi-tryptic peptides made it through this filter at much lower rates than unmodified fully tryptic peptides. An equal number of shuffled sequence decoys of matching types were generated for mProphet model generation. The 18 runs were then imported into the template an mProphet model trained and applied. The MSstats report was exported for further analysis.

Spectronaut – A spectral library was generated using all available raw files from the original publication using the Pulsar search engine integrated in Spectronaut 11(11.0.15038) with default settings. The uniprot yeast reference proteome was used for the spectrum centric data analysis for library generation. DIA data was then analyzed using default settings and exported using the built in MSstats Report (v3.7.3) export schema.

DIA-Umpire – The raw files were converted to mzXML files with centroiding. The resulting mzXML files were processed by the signal extraction (SE) module of DIA-Umpire to generate pseudo-MSMS spectra. The generated pseudo-MS/MS spectra were searched using X! Tandem, Comet and MSGF+ search engines. The output files from the search engines were further analyzed by PeptideProphet and combined by iProphet. FDR filtering was done with PeptideProphet and ProteinProphet. DIA-Umpire's Quant module was for the quantification analysis. The outputs for all-level quantification (FragSummary, PeptideSummary, ProtSummary) were used in further analysis.

Statistical analysis for DIA:Selevsek2015

R package MSstats v3.10.6 was used to pre-process the output from Skyline, Spectronaut, and DIA-Umpire before statistical analysis, to have protein quantification, and to perform differential abundance analysis. MSstats estimated $\log_2$ fold change and the standard error by linear mixed effect model for each protein. To test two-sided null hypothesis of no changes in abundance, the model-based test statistics were compared to the Student T distribution with the degrees of freedom appropriate for each protein and each dataset. The resulting p-values were adjusted to control the False Discovery Rate with the method by Benjamini-Hochberg. Parameter settings as well as the R code used to analyze DIA:Selevsek2015 are available in reanalysis container, RMSV000000251 in MassIVE.quant.

Data Availability

All the datasets that support this study are publicly available in MassIVE.quant (<https://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp>) with MassIVE and ProteomeXchange identifiers. Additionally, identifiers for all the datasets are listed in Supplementary Table 2, 3, and 5.

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Supplementary Information

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Supplementary Note 1 : Links to the documentation and the video tutorials for MassIVE.quant

- MassIVE.quant website :
<http://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp>
MassIVE.quant website contains a summary of the datasets in MassIVE.quant and links to the next steps of data exploration and reanalysis.
- MassIVE.quant user manuals : MassIVE.quant section in
<https://ccms-ucsd.github.io/MassIVEDocumentation/>
- MassIVE.quant video tutorials :
<https://www.youtube.com/channel/UCPeNhYFItiabsoOITPZBc5Q>

User manuals and video tutorials explain how to:

1. Create an account in MassIVE.quant
2. Upload files to MassIVE account via FTP
3. Submit quantification reanalysis to MassIVE.quant : ‘Add analysis’ workflow
4. Access reanalyses
5. Run MSstats workflow in MassIVE.quant : ‘MSstats’ workflow
 - a. For a new quantification, using offline data processing tools
 - b. For a published quantification, using MassIVE.quant
6. Compare the results of statistical analysis by MSstats : ‘MSstats comparison’ workflow

Supplementary Figure 1 : Repository structure for submitting reanalyses in MassIVE.quant

DDA:Choi2017 was used as an example to show how reanalyses are organized in MassIVE.quant. 'Dataset' is set up for each experiment or publication. For example, DDA:Choi2017, including all RAW files, metadata, and identifications, is stored as a 'Dataset' with an unique identifier, prefixed by MSV, which in this case is MSV000079843. MSV000079843 is the MassIVE dataset identifier for the DDA:Choi2017 experiment. Users can set up a 'Reanalyses Container' under each dataset (i.e., MassIVE dataset) to aggregate groups of related reanalyses, such as the MassIVE.quant sets of reanalyses for each dataset reported here. Each container has a unique reanalysis identifier (for example, RMSV000000249 and RMSV000000259) to capture container-specific metadata (e.g., related experiment) and to facilitate aggregating single-identifier references to related sets of reanalysis. Any number of reanalyses can be stored under each 'Reanalyses container'. Finally, each reanalysis also has a unique reanalysis identifier (such as RMSV000000249.1 and RMSV000000249.2) to enable direct references to individual reanalyses, regardless of the context of datasets to which each reanalysis is related.

Supplementary Figure 2 : Example of data analysis steps and corresponding input or output files.

DDA:Cox2014, quantified by MaxQuant, was used for demonstration.

Data processing tool	File type	Which category in MassIVE.quant this file should be stored
All	Input.csv	Quantification results
	Annotation.csv	Metadata
	testResult_byMSstats.csv	Statistical analysis of quantified analytes
	msstats.log	Methods and protocols
	Rscript.R	Methods and protocols
	Description.pdf	Methods and protocols
MaxQuant	evidence.txt	Quantification results
	proteinGroups.txt	Quantification results
	parameters.txt	Quantification results
	mqpar.xml	Methods and protocols
	fasta	Sequence database
Spectronaut	input.xls	Quantification results
	SpectronautAnalysis.sne	Supplementary files
DIAUmpire	FragSummary.xls	Quantification results
	PeptideSummary.xls	Quantification results
	ProtSummary.xls	Quantification results
OpenSWATH	input.tsv or input.txt	Quantification results
Skyline	.sky.zip	Supplementary files

Supplementary Table 1 : Categories for intermediate files for data processing tools.

All examples are quantification files and the output of MSstats for statistical analysis.

Quantification results : quantification result files from data processing tools/quantification tools. These should be used for downstream analysis (for example, PSM-level or transition-level or protein-level quantified peak intensities data).

Metadata : An annotation file should include the information about design of experiments and is submitted in the Metadata section. For example, it should contain information regarding biological replicate, technical replicate, fraction, condition or group for the corresponding MS runs, and corresponding channels.

Supplementary files : If the processing software provides software-specific files, it can be shared. For example, sky, skyd, sky.zip, blib, irtdb, protodb, optdb, and midas for Skyline; .pdresult for Proteome Discoverer; .sne for Spectronaut.

Methods and protocols : Any file types for any methods and protocols related to the corresponding reanalysis. For example, a text file to record parameter settings or extra steps, parameter files themselves, script file containing command lines for other statistical analysis tools or any downstream analysis. For example, R or Python script.

Statistical analysis of quantified analytes : Result files for statistical analysis. It could be a table or figure of results for statistical analysis. For example, table (.txt, .csv), r data format or pdf for figures.

Dataset name	Publication	Type of acquisition	(Background) Organism	No. of tech. replicates/condition	No. of conditions	Dataset ID in repository	Database search	Data processing tool	Reanalysis ID in MassIVE	
DDA:ControlledMix	Chiva et al., 2014	DDA	<i>E.coli</i>	3	5	PXD005642 MSV000084181	Andromeda	Skyline	RMSV000000261.1	
								MaxQuant	RMSV000000261.2	
							Proteome Discoverer, Mascot	Progenesis	RMSV000000261.3	
								Proteome Discoverer	RMSV000000261.4	
										+ 16 reanalyses in RMSV000000263
DDA:Choi2017	Choi et al., 2017	DDA	<i>E.coli</i>	3	4	PXD015300 MSV000079843	Comet, OMSSA, MS-GH+	Skyline	8 reanalyses in RMSV000000249	
							Andromeda	MaxQuant	RMSV000000249.2	
							Comet	Progenesis	RMSV000000249.3	
										+ 12 reanalyses in RMSV000000259
DDA:Cox2014	Cox et al., 2014	DDA	<i>E.coli</i>	4	2	PXD000279 MSV000081831	Andromeda	Skyline	RMSV000000255.7	
							Andromeda	MaxQuant	RMSV000000255.1	
							Comet	Progenesis	RMSV000000255.8	
DIA:Navarro2016	Navarro et al., 2016	DIA	Human / Yeast / <i>E.coli</i>	3	2	PXD002952 MSV000081024	Comet, Mascot	Skyline	RMSV000000250.1	
								Spectronaut	RMSV000000250.2	
								DIA-Umpire	RMSV000000250.3	
								OpenSWATH	RMSV000000250.4	
										+ 16 reanalyses in RMSV000000257
DIA:Bruderer2015	Bruderer et al., 2015	DIA	Human	3	8	PASS00589 MSV000081828	Pulsar	Skyline	RMSV000000252.1	
							Pulsar	Spectronaut	RMSV000000252.2	
							XITandem, Comet, MS-GH+	DIA-Umpire	RMSV000000252.3	
										+ 12 reanalyses in RMSV000000258
DIA:Röst2014	Röst et al., 2014	DIA	Human	3	8	PASS00289 MSV000081829	-	Skyline	RMSV000000253.1	
								OpenSWATH	RMSV000000253.2	
DIA:Huang2020	Huang et al., 2020	DIA	Mouse	5	5	PXD016647 MSV000084864	Andromeda In MaxQuant	Spectronaut	RMSV000000279.1	
		DIA	Human / <i>C.elegans</i> / <i>S. cerevisiae</i>	3	10				RMSV000000281.1	
		DIA	Human / <i>C.elegans</i> / <i>S.cerevisiae</i>	3	2	PXD005573 MSV000084874			RMSV000000282.1	
		DIA	<i>S.cerevisiae</i> / <i>E.coli</i>	3	2				RMSV000000283.1	
SRM:ControlledMix	New	SRM	Yeast	3	5	PXD015304 MSV000084220	-	MultiQuant	-	
TMT:ControlledMix-MS3	New	TMT	Human	10 tech rep samples/condition, 3 tech rep MS runs/mixture	4	PXD0015258 MSV000084264	Proteome Discoverer, Mascot	Proteome Discoverer	4 reanalyses in RMSV000000265	
TMT:ControlledMix-MS2	New	TM	Human	10 tech rep samples/condition, 3 tech rep MS runs/mixture	4	PXD015261 MSV000084266	Proteome Discoverer, Mascot	Proteome Discoverer	4 reanalyses In RMSV000000266	

Supplementary Table 2 : List of benchmark experiments with controlled mixtures, and resulting datasets, available in MassIVE.quant

MassIVE.quant stores the analysis results of six previously published dataset (DDA:Choi2017, DDA:Cox2014, DIA:Navarro2016, DIA:Bruderer2015, DIA:Röst, DIA:Huang2020) and four new experiments (DDA:ControlledMix, SRM:ControlledMix, TMT:ControlledMix-MS3, TMT:ControlledMix-MS2) obtained by up to 95 combinations of processing methods, software tools, and parameter settings. In all cases, statistical analyses of the identified and quantified proteins were performed with MSstats. Four experiments (DDA:ControlledMix, DDA:Choi2017, DIA:Navarro2016, DIA:Bruderer2015) were reanalyzed with up to five different MSstats options.

Dataset name	Publication	Type of acquisition	Organism	N of bio. replicates /condition	N of conditions	Dataset ID in repository	Database search	Data processing tool	Reanalysis ID in MassIVE	
DIA:Selevsek2015	Selevsek et al., 2015	SWATH	<i>S.cerevisiae</i>	3	6	PXD001010 MSV000081677	Sequest / Mascot	Skyline	RMSV000000251.1	
								Skyline	RMSV000000251.2	
							Pulsar	Spectronaut	RMSV000000251.3	
							XITandem / Comet / MS-GH+	DIA-Umpire	RMSV000000251.4	
							+18 reanalyses in RMSV000000262			
DIA:Varadarajan2020	Varadarajan et al., 2020	DIA	L.monocytogenes EGD-e	2 or 3	4	PXD014091 MXV000083881	SequestHT / Amanda / Percolator	Spectronaut	2 reanalyses in RMSV000000290	
			L.monocytogenes ScottA	3	4				2 reanalyses in RMSV000000291	
DIA:Huang2020	Huang et al., 2020	DIA	Human	4	3	PXD016647 MSV000084864	Andromeda in MaxQuant	Spectronaut	RMSV000000280.1	
DIA:Amon2019	Amon et al., 2019	DIA	Human	5	4	PXD009255 MSV000084982	Mascot / Comet	Spectronaut	RMSV000000303.1	
DIA:Muntel2019	Muntel et al., 2019	DIA	Human	3	2	PXD013658 MSV000084878	directDIA in Spectronaut	Spectronaut	3 reanalyses in RMSV000000299	
		DIA	Human	3	2				3 reanalyses in RMSV000000300	
		DIA	Human	3	2				3 reanalyses In RMSV000000301	
DIATMT:Muntel2019	Muntel et al., 2019	DIA	Mouse	2	5	PXD011691 MSV000084859	directDIA in Spectronaut	Spectronaut	RMSV000000268.1	
		DIA					Andromeda in MaxQuant		RMSV000000269.1	
		TMT	Mouse	2	5		Mascot	Proteome Discoverer	RMSV000000270.1	
		DIA	Mouse	2	5		directDIA in Spectronaut	Spectronaut	RMSV000000271.1	
		DIA					Andromeda in MaxQuant		RMSV000000272.1	
		TMT	Mouse	2	5		Mascot	Proteome Discoverer	RMSV000000273.1	
DIA:Andolina2018	Andolina et al., 2018	DIA	<i>P. aeruginosa</i>	3	2	PXD008577 MSV000084898	SequestHT	Spectronaut	RMSV000000278.1	
TMT:Shen2020	Shen et al., 2020	TMT	Human	14,17,19, or 20	4	PXD019221 MSV000085507	Proteome Discoverer	Proteome Discoverer	2 reanalyses In RMSV000000327	
TMT:Djomehri2020	Djomehri et al., 2020	TMT	Human	6 or 15	3	PXD014414 MSV000085343	MSFragger	Philosopher	3 reanalyses in RMSV000000323	
TMT:Yang2019	Yang et al., 2019	TMT	Human	9 or 18	2	PXD010175 MSV000084862	MS-GF+ / Perolator	OpenMS	RMSV000000289.1	
TMT:Plubell2017	Plubell et al., 2017	TMT	Mouse	5	4	PXD005953 MSV000082569	Proteome Discoverer / Mascot	Proteome Discoverer	4 reanalyses in RMSV000000264	
TMT:Paulo2016	Paulo et al., 2016	TMT	<i>S.cerevisiae</i>	3	3	PXD015257 MSV000084263	Proteome Discoverer / Mascot	Proteome Discoverer	4 reanalyses in RMSV000000267	
TMT:Specht2019	Specht et al, 2019	TMT	Human	6	7	MSV000084660	Andromeda In MaxQuant	MaxQuant	RMSV000000326.2	

Supplementary Table 3 : List of biological investigations in MassIVE.quant

The 33 published datasets with Gold or Platinum level of curation were currently available in MassIVE.quant. One experiment, DIA:Selevsek2015, was analyzed and quantified using four combinations of processing methods, software tools, and parameter settings. In all cases, differential abundance analysis of the quantified proteins were performed with MSstats or t-test in R. DIA:Selevsek2015 was analyzed using five different MSstats options. These information available for each dataset is sufficient to performing statistical analyses of differential abundance. Each dataset contains links to the publications or to metadata with additional descriptions provided by the original submitter.

Dataset name	Publication	Type of acquisition	Organism	N of bio. replicates /condition	N of conditions	Dataset ID in repository	Database search	Data processing tool	Reanalysis ID in MassIVE
SRM:Hüttenhain2019	Hüttenhain et al., 2019	SRM	Human	234	2	PXD015303 MSV000084048	-	Skyline	RMSV0000000305.1
SRM:Hüttenhain2019.2	Hüttenhain et al., 2019	DDA	Human/ Human immunodeficiency virus1	4	5	PXD009012 MSV000084855	Andromeda in MaxQuant	MaxQuant	RMSV0000000284.1
		DDA		2 or 4	10				RMSV0000000285.1
		DDA		2 or 4	10				RMSV0000000286.1
		SRM		4	5	MSV000084962	-	Skyline	-
		SRM		4	4	PXD017565 MSV000084963	-	Skyline	-
		SRM		4	4	PXD017566 MSV000084964	-	Skyline	-
		SRM		4 or 5	4	PXD017567 MSV000084965	-	Skyline	-
SRM:Lobingier2017	Lobingier et al., 2017	DDA	Human	3	12	PXD005758 MSV000084857	Andromeda in MaxQuant	MaxQuant	RMSV0000000287.1
		DDA		3	3				RMSV0000000288.1
		SRM		~ 5	13	PXD017570 MSV000084966	-	Skyline	-
		SRM		3	8	PXD017571 MSV000084967	-	Skyline	-
DDA:Luther2019	Luther et al., 2019	DDA	<i>E.coli</i>	3 or 4	2	PXD010174 MSV000084899	Comet / TPP(Trans-Proteomic-Pipeline)	Progenesis	RMSV0000000274.1
		DDA		3 or 4	2			Progenesis	RMSV0000000275.1
		DDA		3	2			Progenesis	RMSV0000000276.1
DDA:Vetterli2018	Vetterli et al., 2018	DDA	<i>E.coli</i>	3	2	PXD010988 MSV000084897	Comet / TPP(Trans-Proteomic-Pipeline)	Progenesis	RMSV0000000277.1
DDA:Jung2017	Jung et al., 2017	DDA	Mouse	3	34	PXD004263 MSV000079789	Andromeda	MaxQuant	RMSV0000000302.1
DDA:Malmström2016	Malmström et al., 2016	DDA	Mouse	1	13	PXD002896 MSV000081695	Andromeda	MaxQuant	RMSV0000000293.1
DDA:Meierhofer2016	Meierhofer et al., 2016	DDA	Mouse	6	2	PXD003155 MSV000084933	Andromeda	MaxQuant	RMSV0000000292.1
DDA:Rakus2016	Rakus et al., 2016	DDA	Mouse	5	3	PXD002152 MSV000084934	Andromeda	MaxQuant	RMSV0000000294.1
DDA:Deshmukh	Deshmukh et al., 2015	DDA	Mouse	3	2	PXD000288 MSV000084940	Andromeda	MaxQuant	RMSV0000000298.1
DDA:Guo2015	Guo et al., 2015	DDA	Mouse	3	2	PXD001293 MSV000084938	Andromeda	MaxQuant	2 reanalyses in RMSV0000000296
DDA:Azimifa2014	Azimifa et al., 2014	DDA	Mouse	~ 4	14	PXD000867 MSV000084937	Andromeda	MaxQuant	RMSV0000000295.1
DDA:Hornburg2014	Hornburg et al., 2014	DDA	Mouse	3	6	PXD000666 MSV000084939	Andromeda	MaxQuant	RMSV0000000297.1
DDA:Zhang2014	Zhang et al., 2014	DDA	Human	5	4	PXD015905 MSV000084238	Pepitome / MyriMatch / MS-GF+	IDPicker	RMSV0000000304.1

Supplementary Table 3, continued : List of benchmark biological investigations in MassIVE.quant

Software or tool for quantification	Skyline		Spectronaut	DIA-Umpire
Different processing steps	low CV	all		
No. of Proteins :				
- in report or output from tool	2,213	4,154	3,019	2,247
- after preprocessing by converter in MSstats	2,212	2,189	2,512	2,030
No. of Proteins possible to test	2,205	1,944	2,499	1,870
No. of Peptides per protein :				
- median	4	4	7	6
- range	1-145	1-157	1-227	1-202
No. of Fragments per peptide:				
- median	6	6	3	6
Proportion of missing :				
No. of NA + No. of Intensity ≤ 1 / total No. Intensity	31,688/ 2,041,110 = 0.0155	500,627/ 2,268,270 = 0.2207	291,629/ 2,173,896 = 0.1342	542,632/ 2,138,940 = 0.2537

Supplementary Table 4 : Summary for reanalyzed DIA:Selevsek2015 by three processing tools - Skyline, Spectronaut, DIA-Umpire.

Two different processing steps were performed in Skyline. Detailed description of processing steps for four reanalyses are available in MassIVE.quant RMSV000000251. ‘No. of Proteins: in report or output from tool’ is the number of protein ids reported from data processing tools. ‘No. of Proteins after processing by converter in MSstats’ is the number of proteins available the output of converter function in MSstats. ‘No. of Proteins possible to test’ is the number of proteins that can be fitted in a linear mixed model. It is less than ‘No. of Proteins’ because some proteins cannot be fitted and analyzed due to missingness issue. ‘Proportion of missing’ = the number of NA or normalized intensities less than or equal to 1 / total number of intensities. ‘Total No. Intensity’ = the number of unique features times the number of MS runs.

Supplementary Figure 3 : Volcano plots for pairwise comparisons between 0min and others in DIA:Selevsek2015

The plots summarize and show discrepancies of differential abundance between T5 and T0 across data processing tools, with identical statistical analysis using MSstats. To test two-sided null hypothesis of no changes in abundance, the model-based test statistics were compared to a Student T distribution. The p-values were adjusted to control the False Discovery Rate with the method by Benjamini-Hochberg. Each row is the time point comparison from starting point (0 min). Each column shows reanalysis by a different processing tool or step. Four reanalyses were performed by three spectral processing tools: Skyline:lowCV (first column) and Skyline:All (second column), Spectronaut (third column), and DIA-Umpire (fourth column). Detailed descriptions of processing steps for the four reanalyses are available in MassIVE.quant RMSV000000251. Dashed horizontal line : FDR=0.05, blue dots : significantly down-regulated proteins, red dots : significantly up-regulated proteins. The numbers of significantly up- or down-regulated proteins are shown top left corner of each volcano plot.

Supplementary Figure 4 : Venn diagram of differentially abundant proteins in DIA:Selevsek2015 (a, c, e, g) between two processing approaches by Skyline (Skyline:lowCV and Skyline:All), (b, d, f, h) across three processing tools (Skyline:lowCV, Spectronaut, DIA-Umpire). Each row is the time point comparison from starting point (0 min).

Name	Controlled mixtures	Type of acquisition	ID in repository
DDA:ControlledMix	Chiva et al., 2014 ¹	DDA	MSV000084181 (PXD005642)
DDA:Choi2017	Choi et al., 2017 ²	DDA	MSV000079843 (PXD015300)
DDA:Cox2014	Cox et al., 2014 ³	DDA	MSV000081831 (PXD000279)
DIA:Navarro2016	Navarro et al., 2016 ⁴	DIA	MSV000081024 (PXD002952)
DIA:Bruderer2015	Bruderer et al., 2015 ⁵	DIA	MSV000081828 (PASS00589)
DIA:Röst2014	Röst et al., 2014 ⁶	DIA	MSV000081829 (PASS00289)
SRM:ControlledMix	New	SRM	MSV000084220 (PXD015304)
TMT:ControlledMix-MS3	New	TMT	MSV000084264 (PXD015258)
TMT:ControlledMix-MS2	New	TMT	MSV000084266 (PXD015261)
DIA:Selevsek2015	Selevsek et al., 2015 ⁷	SWATH	MSV000081677 (PXD001010)
DIA:Varadarajan2020	Varadarajan et al., 2020 ⁸	DIA	MSV000083881 (PXD014091)
DIA:Huang2020	Huang et al., 2020 ⁹	DIA	MSV000084864 (PXD016647) MSV000008874 (PXD005573)
DIA:Amon2019	Amon et al., 2019 ¹⁰	DIA	MSV000084982 (PXD009255)
DIA:Muntel2019	Muntel et al., 2019 ¹¹	DIA	MSV000084878 (PXD013658)
DIATMT:Muntel2019	Muntel et al., 2019 ¹²	DIA/TMT	MSV000084859 (PXD011691)
DIA:Andolina2018	Andolina et al., 2018 ¹³	DIA	MSV000084898 (PXD008577)
TMT:Shen2020	Shen et al., 2020 ¹⁴	TMT	MSV000085507 (PXD019221)
TMT:Djomehri2020	Djomehri et al., 2020 ¹⁵	TMT	MSV000085343 (PXD014414)
TMT:Specht2019	Specht et al., 2019 ¹⁶	TMT	MSV000084660
TMT:Yang2019	Yang et al., 2019 ¹⁷	TMT	MSV000084862 (PXD010175)
TMT:Plubell2017	Plubell et al., 2017 ¹⁸	TMT	MSV000082569 (PXD005953)
TMT:Paulo2016	Paulo et al., 2016 ¹⁹	TMT	MSV000084263 (PXD015257)
SRM:Hüttenhain2019	Hüttenhain et al., 2019 ²⁰	SRM	MSV000084048 (PXD015303)
SRM:Hüttenhain2019_2	Hüttenhain et al., 2019 ²¹	SRM	MSV000084855 (PXD009012) MSV000084962 MSV000084963 (PXD017565) MSV000084964 (PXD017566) MSV000084965 (PXD017567)
SRM:Lobingier2017	Lobingier2017 et al., 2017 ²²	SRM	MSV000084857 (PXD005758) MSV000084966 (PXD018570) MSV000084967 (PXD017571)
DDA:Luther2019	Luther et al., 2019 ²³	DDA	MSV000084899 (PXD010174)
DDA:Vetterli2018	Vetterli et al., 2018 ²⁴	DDA	MSV000084897 (PXD010988)
DDA:Jung2017	Jung et al., 2017 ²⁵	DDA	MSV000079789 (PXD004263)
DDA:Malmström2016	Malmström et al., 2016 ²⁶	DDA	MSV000081695 (PXD002896)
DDA:Meierhofer2016	Meierhofer et al., 2016 ²⁷	DDA	MSV000084933 (PXD003155)
DDA:Rakus2016	Rakus et al., 2016 ²⁸	DDA	MSV000084934 (PXD002152)
DDA:Deshmukh	Deshmukh et al., 2015 ²⁹	DDA	MSV000084940 (PXD000288)
DDA:Guo2015	Guo et al., 2015 ³⁰	DDA	MSV000084938 (PXD001293)
DDA:Azimifa2014	Azimifa et al., 2014 ³¹	DDA	MSV000084937 (PXD000867)
DDA:Hornburg2014	Hornburg et al., 2014 ³²	DDA	MSV000084939 (PXD000666)
DDA:Zhang2014	Zhang et al., 2014 ³³	DDA	MSV000084238 (PXD015905)

Supplementary Table 5 : List of datasets for reference

Supplementary references for datasets

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