

Proline User Guide

Release 2.3.0

Proline Software Suite

Proline is a production grade software suite, which provides an environment for large-scale MS data management, visualization, analysis and curation with the main objective of promoting the production and sharing of high quality proteomic datasets. Proline can be used (i) to produce reliable identification and quantification results through robust automated processes, (ii) for data curation, (iii) to systematically save and keep track of metadata from processing steps, parameters and generated data, and (iv) to submit highly qualified datasets to public repositories.



A workflow in Proline is implemented as a collection of tasks (see Figure) that can be performed by the user through the graphical user interface. Users can import multiple identification results corresponding, for example, to fractions and replicates of a biological sample and combine them before or after validation. The resulting datasets can then be compared or quantified using spectral counting or DDA label-free quantification, before exporting the results in different file formats.

The software suite is based on two main components: a **server** handling processing tasks and based on **relational database management system** storing the data generated and two different graphical user interfaces, both allowing users to start tasks and visualize the data: **Proline Studio** which is a rich client interface and **Proline Web** the web client interface. An additional component called **ProlineAdmin** is used by system administrators to set up and manage Proline. **Proline Zero** is an all-in-one, "zero installation" solution containing the **server** and **Proline Studio**.

Document organization

This document is organized in two main sections:

- The <u>Concepts & Principles</u> section presents the main concepts and algorithms implemented in Proline as well as the different parameters of these algorithms.
- The <u>How to</u> section gives more details on how to proceed with the Proline Studio graphical interface.



You could also find a description of <u>available converters</u> to create files in **mzDB format** (Bouyssié et al., 2015) from spectrometers raw files.

Proline Concepts & Principles

- Dataset types:
 - o <u>Result File</u>
 - o <u>Search Results</u>
 - o <u>Decoy Searches</u>
 - o <u>Identification Summary</u>
 - o <u>Modification Dataset</u>
- Data Processing:
 - o <u>Protein Inference</u>
 - o Protein and Proteins Sets scoring
 - o Validation Algorithm
 - o FDR Estimation
 - o <u>Protein Sets Filtering</u>
 - o <u>Combining datasets</u>
 - o Identify Modification Sites or Clusters
 - o <u>Compare with Spectral Count</u>
 - o Label Free LC-MS quantitation workflow
 - o Isobaric labelling quantitation
 - o <u>Post-processing of quantitative results</u>
 - o <u>Aggregation of quantitative results</u>
- Data Import/Export:
 - o Identification Summary Export
- <u>Advanced Features</u>:
 - o <u>Allow multiple imports in parallel</u>

Identification results

Proline considers different types of identification data: Result Files, Search Results and Identification Summaries which will be defined in the following sections.

Result File

A **result file** produced by a search engine can be imported into Proline in their native format. OMSSA (.omx files), Mascot (.dat files) and X!Tandem (.xml files) search engines are currently supported. In addition, the mzIdentML format is supported to allow the output from any other search engine compatible with this standard to be imported (e.g. MS-GF+). A first version for MaxQuant support has been implemented. It is possible to import only search results or to import search results as well as quantitation (beta version) values from MaxQuant files.

Search engines may provide different types of searches for MS and MS/MS data. It is important to highlight that Proline only supports MS/MS ions searches at this point.



Search Result

A **search result** is the raw interpretation of a given set of MS/MS spectra given by a search engine. It contains one or many peptides matching the submitted MS/MS spectra (**PSM**, i.e. Peptide Spectrum Match), and the protein sequences these peptides belong to. The **search result** also contains additional information such as search parameters, used protein sequence databank, etc.

A **search result** is created when a result file is imported in Proline. During this step, no filtering or thresholding is applied: along with the search parameters, all submitted spectra, peptide spectrum matches (PSMs) and protein hits suggested by the search engine are retained in the Proline database to allow subsequent validation of putative identifications. In the case of a target-decoy search, two **search results** are created: one for the target PSMs, one for decoy PSMs.

Content of a search result

Importing a result file creates a new search result in the database which contains the following information:

- Search Settings: software name and version, parameters values
- Peaklist and Spectrum information: file name, MS level, precursor m/z, ...
- Search result data:
 - o Protein sequences
 - o Peptide sequences
 - o Spectra
 - o Two kinds of Matches:
 - Peptide Spectrum Matches (PSM), i.e. the matching between a peptide and a spectrum, with some related data such as the score, fragment matches...
 - Protein Matches, i.e. the proteins in the databank corresponding to the PSMs identified by the search engine

Search engine specificities

- Mascot
 - \circ $\;$ The PSM score corresponds to the Mascot ion score.
- OMSSA
 - The PSM score corresponds to the negative common logarithm of the E-value:
 Score = -log10(E-value). Note that Proline only supports OMSSA Result Files generated with the 2.1.9 release.
- X!Tandem
 - The X!Tandem standard hyperscore is used as a PSM score. Note that Proline supports X!Tandem Result Files generated with the Sledgehammer release (or later).

Decoy Searches

Proline handles decoy searches performed from two different strategies:

- Concatenated searches:
 - A protein databank is created by concatenating target protein sequence to decoy protein sequence.
 Decoy could be created using reverse or random strategy. A unique search is done using that databank.



- o When importing a Search Result from a decoy concatenated databank, decoy data are extracted from the Result File and stored in Proline databases as a decoy Search Result independent of the target Search result. Nevertheless both searches are linked to each other.
- Separated searches:
 - o Two searches are done using the same peaklist, one on a target protein databank and one on a decoy protein databank. These searches are then combined to retrieve useful information such as FDR. Mascot allows the user to check a decoy option and automatically creates a decoy databank.
 - o The two performed searches are stored in Proline databases and are linked together.

Identification Summary

An **Identification Summary** is a set of identified proteins inferred from a subset of the PSM contained in the <u>search</u> <u>result</u> that have been declared valid. The subset of PSM taken into account are the PSM that have been validated by a filtering process (example: PSM fulfilling some specified criteria such as score greater than a threshold value).

Content of an identification summary

- Peptide Set
 - $\circ~$ A set of peptides identifying one or more proteins.
- Protein Set
 - Typical Protein or representative protein. Protein "chosen" to represent the ProteinSet.
 - Sameset : all proteins identified by the same set of peptides than the typical one.
 - Subset: Proteins identified by a subset of peptides. All these peptides should also identify typical protein.

Protein Inference

All peptides identifying a protein are grouped in a Peptides Set. A same Peptides Set can identify many proteins, represented by one Proteins Set. In this case, one protein of this Protein Set is chosen to represent the set, it is the Typical Protein. If only a subset of peptides identify a (or some) protein(s), a new Peptide Set is created. This Peptide Set is a subset of the first one, and identified Proteins are Subset Proteins.



- In the first example, P2 and P5 are identified by the same peptide set {pe1, pe4, pe5, pe8}. P2 was chosen as typical protein. One subset composed of {pe4, pe5, pe8} identifies subset protein P4.
- In the second example, another protein set represented by P3 shares some peptides with the protein set represented by P2. Both protein sets have specific peptides.
- Sharing could involve many protein sets as shown in example 3.





All peptides sets and associated protein sets are represented, even if there are no specific peptides. In both cases above, no choice is done on which protein set / peptide set to keep. These protein sets could be filtered after inference (see <u>Protein sets filtering</u>).

Modification Dataset

From an Identification Summary, users can ask to Identify Modification Sites and Clusters, thus creating a Modification Dataset containing the result. This dataset contains

- Modification Sites: a modification type at a given location on a given protein. They are only extracted for the
 proteins that are representative of a validated protein set. Only modifications of interest are used in this
 process.
- Modification Clusters: co-localized modification sites are grouped into clusters. Identify Modification Sites and Clusters describe this grouping process.

Proteins and Protein sets scoring

There are multiple algorithms that could be used to calculate the Proteins and Protein Sets scores. Proteins scores are computed during the importation phase while Protein Sets scores are computed during the validation phase.

Protein

Each individual protein match is scored according to all peptide matches associated with this protein, independently of any validation of these peptide matches. The sum of the peptide matches scores is used as protein score (called standard scoring for Mascot result files).

Protein Set

Each individual protein set is scored according to the validated peptide matches belonging to this protein set (see <u>inference</u>).

Scoring schemes

Mascot Standard Scoring

The score associated with each identified protein (or protein set) is the sum **of the score of all peptide matches** identifying this protein (or protein set). In case of duplicate peptide matches (peptide matched by multiple queries) only the match with the best score is considered.

Mascot MudPIT Scoring

This scoring scheme is also based on the sum of all non-duplicate peptide matches score. However the score for each peptide match is not its absolute value, but the amount that it is above the threshold: the score offset. Therefore, peptide matches with a score below the threshold do not contribute to the protein score. Finally, the average of the thresholds used is added to the score. For each peptide match, the "threshold" is the homology



threshold if it exists, otherwise it is the identity threshold. The algorithm below illustrates the MudPIT score computation procedure:

```
Protein score = 0
For each peptide match {
    If there is a homology threshold and ions score > homology threshold {
        Protein score += peptide score - homology threshold
    } else if ions score += peptide score - identity threshold
    }
}
Protein score += 1 * average of all the subtracted thresholds
```

- if there are no significant peptide matches, the protein score will be 0.
- homology and identity threshold values depend on a given p-value. By default Mascot and Proline compute these thresholds with a p-value of 5%.
- In the case of separated target-decoy searches we obtain two values for each threshold: one for the target search and another one for the decoy search. In order to obtain a single value we apply the following procedure:
 - o the homology threshold is the decoy value if it exists else the target value
 - o the identity threshold is the mean of target and decoy values.

The benefit of the MudPIT score over the standard score is that it removes many of the junk protein sets, which have a high standard score but no high scoring peptide matches. Indeed, protein sets with a large number of weak peptide matches do not have a good MudPIT score.

Mascot Modified MudPIT Scoring

This scoring scheme, introduced by Proline, is a modified version of the Mascot MudPIT one. The difference with the latter is that it does not take into account the average of the subtracted thresholds:

```
Protein score = 0
For each peptide match {
    If there is a homology threshold and ions score > homology threshold {
        Protein score += peptide score - homology threshold
    }
    else if ions score += peptide score - identity threshold
    }
}
```

This score has the same benefits as the MudPIT one. The main difference is that the minimum value of this modified version will be always close to zero while the genuine MudPIT score defines a minimum value which is not constant between the datasets and the proteins (i.e. the average of all the subtracted thresholds).

Fisher

This scoring scheme relies on the Fisher's test to define protein scores and p-value from the scores of the best subset of peptides. The scoring is extensively described in (<u>https://doi.org/10.1021/acs.analchem.0c00328</u>).

Search result validation

Once a result file has been imported and a search result created, the validation is performed in four main steps:

- 1. <u>Peptide Matches filtering and Validation</u>
- 2. <u>Protein Inference</u> (peptides and proteins grouping)
- 3. <u>Protein and Proteins Sets scoring</u>
- 4. Protein Sets Filtering and Validation



Finally, the <u>identification summary</u> issued from these steps is stored in the identification database. Different validation of a Search Result can be performed and a new Identification Summary of this Search Result is created for each validation.

When validating a merged Search Result, it is possible to propagate the same validation parameters to all childs Search Results. In this case <u>Peptide Matches filtering and validation</u> will be applied on childs as well as Protein Sets filtering. <u>Note:</u> actually, Protein Sets validation is not propagated to childs Search Results.

Peptide matches filtering

Peptide Matches identified in search results can be filtered using one or multiple predefined filters (described hereafter). Only validated peptide matches will be considered for further steps.

Score filter

All PSMs with a score lower than a given threshold are discarded. For some search engines, Proline computes the PSM score value itself by applying a mathematical transformation to another PSM property. For instance, the score values for PSMs from X!Tandem search results correspond to the log10 transformation of the PSMs expectation values.

Pretty Rank filter

This filter is applied after having temporarily joined target and decoy PSMs corresponding to the same query. For each query, target/decoy PSMs are then sorted by score. As in Mascot, a pretty rank is computed for each PSM depending on their ranking: PSM with almost equal score (difference < 0.1) are assigned the same rank. All PSMs with a pretty rank greater than the cut-off specified are discarded.

Minimum sequence Length filter (Length)

PSMs corresponding to peptide sequences shorter than the cut-off stipulated will be discarded when this parameter is applied.

Mascot e-Value filter (e-Value)

This filter is used to select PSMs based on the Mascot expectation value (e-value) which reflects the difference between the PSM's score and the Mascot identity threshold (p=0.05). PSMs with an e-value greater than the threshold specified are discarded.

Mascot adjusted e-Value filter (Adjusted e-Value)

Proline can compute an adjusted e-value. It first selects the lowest threshold between the identity and homology e-values (p=0.05). Then, it computes the e-value using this selected threshold. PSMs for which the adjusted e-value is greater than the specified cut-off are discarded.

Mascot p-Value based on identity filter (Identity p-Value)

Given a specific p-value, the Mascot identity threshold is calculated for each query and all peptide matches associated with the query for which the score is lower than the identity threshold calculated are discarded.

Mascot p-Value based on homology filter (Homology p-Value)

Given a specific p-value, the Mascot homology threshold is inferred for each query and all peptide matches associated with the query which have a score lower than the calculated homology threshold are discarded.

Single PSM per MS Query filter

This filter validates only one PSM per Query. To select a PSM, following rules are applied:

For each query:



- Select PSM with higher score.
- If several PSM with same score:
 - o Choose PSM which identify the protein which have the max number of valid PSM
 - o If still equality choose the PSM with the lower delta moz
 - o If still equality, select the first PSM using alphabetic order
 - o If still equality, select the first one. Warning : may be different in different versions of Prolline...

Single PSM per Rank filter

This filter selects only **one PSM per pretty rank**, which is already the case when a given pretty rank is associated with a single PSM. When multiple PSMs have the same pretty rank, the same selection than "Single PSM per Query" is used

- Select PSM that identifies the protein which has the max number of valid PSM.
- If several PSM are equals
 - o Choose the PSM with the lower delta moz.
 - o If still equality, select the first PSM using alphabetic order.
 - o If still equality, select the first one. Warning : may be different in different versions of Proline...

Thus, if this filter is combined with the "Pretty rank" filter, the result obtained should be identical to the result of the "Single PSM per MS query" filter.

Single Sequence per Rank filter

This filter selects the best **PSM per pretty rank** and all PSMs that have the **same sequence**, without considering Post translational modification. The best PSM is selected using the same rules as the Single PSM per Rank filter described above.

Isotope Offset filter

This filter validates PSMs which have been validated using an isotope offset less or equal than specified one. This corresponds to identification search engine parameters such as Mascot isotope error and could be used to invalidate PSM identified using one or more 13C.

Peptide matches validation

In addition to these filters a PSM level validation step may be specified using:

- A target-decoy approach (Elias and Gygi, 2007), which can be performed by adjusting a user-specified validation criterion until it reaches a user-specified false discovery rate (FDR). The search engine score can be used as a generic validation criterion for any of the search engines supported by Proline. For results obtained with the Mascot search engine, other criteria can be used to control the FDR: Mascot e-Value, Mascot adjusted e-Value, Mascot p-Value based on identity threshold, or Mascot p-Value based on homology threshold.
- A method based on the Benjamini-Hochberg procedure to control the FDR proposed in (<u>https://doi.org/10.1021/acs.analchem.0c00328</u>)



FDR estimation for Target Decoy validation

There are several ways to calculate FDR depending on the database search type. In Proline the FDR is calculated at PSM and protein levels using the following rules:

• if the Search has been done on a concatenated Target/Decoy bank or if rank filter has been used during validation :

 $FDR = 2 \frac{nbr DecoyPSM}{nbr TargetPSM + nbr DecoyPSM}$

<u>Note</u>: when computing PSM FDR, peptide sequences matching a Target Protein and a Decoy Protein are taken into account in both cases.

• if the Search has been done on a separated Target/Decoy bank :

 $FDR = \frac{nbr \, DecoyPSM}{nbr \, TargetPSM}$

Protein sets filtering

Any Identification Summary, generated by a validation process or by merging datasets could be filtered.

Filtering consists in invalidating Protein Sets which don't follow specified criteria. Invalidated Protein Sets are not taken into account for further algorithms or display.

Available filtering criteria are defined below.

Specific peptides filter

This filter invalidates protein sets that don't have at least x peptides identifying only that protein set. The specificity is considered at the DataSet level.

This filtering goes through all Protein Sets from worse score to best score. For each, if the protein set is invalidated, associated peptides properties are updated before going to the next protein set. Peptide property is the number of identified protein sets.

Peptides count filter

This filter invalidates protein sets that don't have at least x peptides identifying that protein set, independently of the number of protein sets identified by the same peptide.

This filtering goes through all Protein Sets. For each, if the protein set is invalidated, associated peptides properties are updated before going to the next protein set. Peptide property is the number of identified protein sets.

Peptide sequence count filter

This filter invalidates protein sets that don't have at least x different peptide sequences (independently of PTMs) identifying that protein set.

This filtering goes through all Protein Sets from worse score to best score. For each, if the protein set is invalidated, associated peptides properties are updated before going to the next protein set. Peptide property is the number of identified protein sets.

Protein set score filter

This filter invalidates protein sets which score is below a given value.



Protein sets validation

Once prefilters (see above) have been applied, a validation algorithm can be run to control the FDR. In the same way as for PSM, FDR may be controlled using :

- the Benjamini-Hochberg procedure, see (<u>https://doi.org/10.1021/acs.analchem.0c00328</u>)
- a target decoy strategy.

Target-Decoy Strategy:

See how_FDR is calculated.

At the moment, it is only possible to control the FDR by changing the Protein Set Score threshold. Three different protein set scoring functions are available.

Given an expected FDR, the system tries to estimate the best score threshold to reach this FDR. Two validation rules (R1 and R2) corresponding to two different groups of protein sets (see below the detailed procedure) are optimized by the algorithm. Each rule defines the optimum score threshold allowing to obtain the closest FDR to the expected one for the corresponding group of protein sets.

Here is the procedure used for FDR optimization:

- protein sets are segregated in two groups, the ones identified by a single validated peptide (G1) and the ones identified by multiple validated peptides (G2), with potentially multiple identified PSMs per peptide.
- for each of the validation rules, the FDR computation is performed by merging target and decoy protein sets
 and by sorting them by descending score. The score threshold is then modulated by using successively the
 score of each protein set of this sorted list. For each new threshold, a new FDR is computed by counting the
 number of target/decoy protein sets having a score above or equivalent to this value. The procedure stops
 when there are no more protein sets in the list or when a maximum FDR of 50% is reached. It has to be
 noted that the two validation rules are optimized separately:
 - o G2 FDR is first optimized leading to the R2 score threshold. The validation status of G2 protein sets is then fixed.
 - o final FDR (G1+G2) is then optimized leading to the R1 score threshold. Only the G1 protein sets are here used for the score threshold modulation procedure. However the FDR is computed by taking into account the G2 validated target/decoy protein sets.

The separation of protein sets in two groups allows to increase the power of discrimination between target and decoy hits. Indeed, the score threshold of the G1 group is often much higher than the G2 one. If we were using a single average threshold, this would reduce the number of G2 validated proteins, leading to a decrease in sensitivity for a same value of FDR.

Combining datasets

Identification results can be combined to construct a parent dataset, and create a non-redundant list of identified peptides and proteins. This combination can be performed either before validation (on search results) or after validation (on identification summaries). Since this operation could be recursively performed, it leads to hierarchical structuring of search results and/or identification summaries. On the one hand, combination before validation (taking into account all PSMs identified by the search engine) may, for example, be relevant when analyzing results obtained after peptide fractionation: in that case, several peptides belonging to the same protein may be spread across different result sets; these sets should be merged before protein validation. On the other hand, merging



identification summaries is appropriate when seeking to group the validated results from a series of individual samples to be compared or when combining data from different search engines.

When datasets are combined, their PSMs are collected to generate a non-redundant set of peptides before recomputing protein inference. Additionally, the mappings between peptides and FASTA entries observed across the different datasets are also collected and merged into a single final mapping list. This list reflects thus the whole set of peptide and protein matches that were observed in the individual datasets.

Users can combine search results or identification summaries. The main difference is the set of spectra and peptides (and thus PSMs) considered. When combining search results, all spectra, peptides and PSMs in the dataset are considered, whereas when combining identification summaries, only validated PSMs are taken into account. In addition, Proline can be used to control how PSMs are collected in the parent dataset: in union mode, PSMs originating from combined datasets are added, while in aggregation mode, all PSMs identifying the same peptide are aggregated into a single representative PSM.



Combining datasets in Proline. Datasets are represented as a tripartite graph composed of spectra, peptides and proteins; edges between spectrum and peptides represent PSM. When the blue and orange datasets are combined, PSMs from both datasets are collected together, generating a non-redundant set of peptides. The combination can be performed in 2 modes: union or aggregation mode.

Identifying Modification sites and clusters

A list of Post translational modification (PTM) sites identified among the peptides of an identification summary can be extracted by Proline. A modification site is characterized by a modification type, at a given location on a given protein. The list of modification sites extracted by the software is restricted to the proteins that are representative of a validated protein set and to the modifications of interest specified by the user. This means that a peptide



identified with two different modifications of equal interest to the user will appear twice in the list, one for each modification location.

In a second phase, co-localized modification sites are grouped into clusters as soon as evidence of their co-existence exists. The required evidence is a peptide sequence identified in the dataset with all the clusterized modification sites.

The figure below represents different peptides (blue rectangles), co-localized on a protein sequence (in red). In this example, modifications of interest (Phosphorylation (Ph)) are shown in orange. Peptide 1 proves that Phosphorylation at positions m, x and y occurs simultaneously. Peptides 2 and 3 are considered as isomorphic since the oxidation of peptide 2 is ignored (only Phosphorylations have been declared of interest by the user in this example). Peptides 4 and 5 are partially isomorphic: they confirm Phosphorylation respectively at position (m, x) and (x, y). Conversely peptides 6 and 7 are not in accordance, suggesting that there are two other proteoforms, one with Phosphorylations at position (m,y) but no modification at x and one with a Phosphorylation at position m but not at x. These two peptides could be grouped into another cluster.



The user can choose between two different modification clustering methods:

- Partially Isomorphic Matching (aka Exact Position matching): Two sequence matches are clusterized if they are Isomorphic or Partially Isomorphic. Partially Isomorphic matches could belong to multiple clusters.
- Only Isomorphic Matching: two sequence matches are clusterized if they are Isomorphic.

In Proline, clusters co-localisation concept is used. Co-localized clusters are clusters that have overlapping on protein sequence. These clusters may share one or more sites, but they could also cover the same protein sequence without any common site. See co-localized clusters example below.





Spectral Counting

Principles

Proline can be used to compare protein sets based on spectral counts through a previously presented algorithm (Hesse et al., 2016). This algorithm notably computes a weighted spectral count metric (called adjusted spectral count in the original publication). Basically, the algorithm takes both unique and shared peptides into account, and for each shared peptide, the proportion of MS/MS spectra that should be attributed to the different protein sets is determined. This proportion (also called weight) is based on the spectral counting of proteotypic (or specific) peptides identifying the different protein sets sharing the peptide to be attributed.

Spectral counting is calculated from a hierarchy of identification summaries. The parent identification summary (at the top of the hierarchy) is where the list of protein sets to compare and the list of specific peptides are created. The list of specific peptides is then used to compute the protein sets' respective weights but users can choose any "child" dataset where the weights must be calculated.

First, Proline compute the peptide spectral count at each level of the dataset hierarchy using the following rules:

- If the dataset is a "leaf" identification summary of the hierarchy (not issued from a merge, no child dataset), the peptide spectral count is the number of MS/MS spectra matching a peptide (equal to the number of peptide spectrum matches).
- If the dataset is a merged identification summary, the peptide spectral count is the sum of its child peptide spectral count.
- If the dataset is a merged of search results, the peptide spectral count is the sum of validated children peptide spectral count. Validated children are PSMs that meet validation criteria applied to parent Identification Summaries.



Once, peptide spectral count is calculated for each peptide, the protein spectral count is computed using the following rules:

- Protein basic spectral count (BSC) is the sum of the peptide spectral count of all peptides matching a protein.
- Protein specific spectral count (SSC) is the sum of the peptide spectral count of specific peptides. A specific peptide is a peptide which does not identify any other protein sets in the context of the identification summaries.
- Protein weighted spectral count (WSC) is the Protein specific spectral count (SSC) + weighted peptide spectral count of shared peptides.

Simple example



Protein sets weights

The protein set respective weights computation is based on the proteotypic peptides. The level in the dataset hierarchy where these weights are calculated can be chosen by the user, it could be the top level of the dataset hierarchy or at a lower level. In the following example, the weights (W_{P1} and W_{P2}) are calculated at the "parent dataset" level. At this level, P1 and P2 are respectively identified by the red and dark blue peptides/psm, each protein set weight is calculated using their specific spectral counting. These weights are thus used to calculate the weighted spectral count of P1 and P2 in the two "child" dataset "Condition 1" and "Condition 2".





FAQ

Why is the BSC less than Peptide Count ?

When running SC even on a simple hierarchy (1 parent, 2 childs) in some cases we obtain a BSC value smaller than the peptide count of the protein set. This occurs only for *invalid protein sets*. *Invalid protein sets* are the ones that are present at the parent level but are filtered at child level (if a *specific peptide* filter has been applied for example).

Indeed, the peptide count value is read in the child protein sets. On the other hand, the BSC is calculated by getting the spectral count information at child level for each peptide identified at parent level. If a protein set is invalid, its peptides are not taken into account during the merging so some of them could be missing at the parent level if they were not identified in the other child.

This case is illustrated in the following figure:



Label-free LC-MS quantitation workflow

Proline detects chromatographic peaks from raw data **converted to the mzDB format** (Bouyssié et al., 2015). Depending on the Proline version and the instrument used to acquire data, two <u>converters are available</u> to generate mzdbFile.

After a first **signal extraction** step, the algorithm associates the chromatographic peaks detected with validated PSMs, first by retrieving the corresponding MS/MS spectra acquired during the peptide elution, and then by **matching the precursor** m/z value of these spectra to the chromatographic peak m/z value. After the **deisotoping** step, the abundance of each ion is estimated from the apex of the chromatographic peak, which corresponds to the theoretically most abundant isotopologue (inferred from the peptide's atomic composition). The software then **aligns** the retention time of these annotated ions for all the LC-MS runs to be compared, and uses this information



to **cross-assign** MS/MS data to ions (i.e. chromatographic peaks) that were detected but not identified in other runs . The resulting ion abundances are finally stored in the Proline database, making them available for rapid data visualization and further post-processing.

Finally, peptide ion measurements can be **summarized as protein abundances** using different computational methods. The user can opt to perform additional operations such as excluding peptides or ions based on their characteristics (missed cleavages, variable modifications, sequence specificity, etc.) or normalizing peptide and protein abundances between runs. These post-processing steps can be executed on-demand using different parameters or methods; there is no need to repeat the whole quantification process when changes are made.

Signal extraction

During an LC-MS experiment, the m/z and intensity values for each peptide ion detected are recorded in MS1 scans acquired during the elution of this peptide from the chromatographic column. Most existing peak picking algorithms analyze these MS scans individually or sequentially. The Proline algorithm performs the signal detection in a different way. It first takes advantage of the mzDB format to detect chromatographic peaks in spectrum slices (5 m/z wide by default) across the whole chromatographic time. Then, in a given slice, m/z peaks are sorted in decreasing order of intensity. Thus, starting from the most intense m/z peak (apex), the algorithm searches for a peak with the same m/z value in the previous and subsequent MS1 scans, while applying a user-defined m/z tolerance. This lookup procedure stops when the ion signal is absent from more than a predefined number of consecutive scans. The [RT, m/z, intensity] peak list obtained, which is comparable to an extracted ion chromatogram (XIC), is then smoothed using a Savitzky-Golay filter (Savitzky and Golay, 1964). The resulting smoothed chromatogram is then split into the time dimension to form chromatographic peaks, by applying a peak picking procedure that will search for significant minima and maxima of signal intensity. When the signals of two ions overlap in the time dimension, a minimum is generally surrounded by two maxima. If the corresponding valley is deep enough, i.e. at least 66% of the lower surrounding maximum, this minimum will be considered significant (and thus will trigger the generation of two peaks). Once the smoothed chromatogram has been fully analyzed, the algorithm removes the corresponding detected peaks from the current spectrum slice, and performs another lookup using the next available apex. The result of this whole procedure is a list of chromatographic peaks defined by an m/z value, an apex elution time and an elution time range.

Parameters

These parameters are used by signal extraction algorithms.

- Extraction m/z tolerance: this corresponds to the error tolerance used when searching for a m/z peak in the previous and subsequent MS1 scans (see above).
- Use previous/last peakel detection: If a quantitation has already been done on a run, use the previously detected peakels file for this run. **Warning**: In this case, the extraction m/z tolerance parameter is ignored since the signal extraction is already done !

PSM assignment and deisotoping

In a single run, validated PSMs are MS/MS spectra assigned to a peptide sequence, and each spectrum is characterized by a precursor mass, a charge state and a retention time (RT). The algorithm assigns PSMs to detected chromatographic peaks by matching the spectrum precursor m/z ratio to the chromatographic peak m/z and verifies that the spectrum retention time falls within the peak time range. The PSM charge state assigned is then used to search for chromatographic peaks corresponding to the ion's isotopologues, considering the precursor mass-to-charge ratio of the spectrum as the monoisotope. The peptide ion intensity is summarized by retaining only



the apex of the peak corresponding to the theoretically most abundant isotopologue (inferred from the peptide's atomic composition). All peptide ion signals (a.k.a. LC-MS features) extracted from an mzDB file and assigned to a PSM are used to construct an LC-MS map. For the sake of simplicity no distinction is made between LC-MS runs and LC-MS maps in this manuscript.

Parameters

• PSM/Peakel matching m/z tolerance is the m/z error allowed to match a peakel to a PSM

Clustering is applied to group peakels that are matching the same identified ion.

- Cluster time computation: you have the choice between 2 computation methods: most intense or median. For "most intense" method, the cluster time corresponds to the time of the most intense feature composing the cluster. For the "median" method, cluster time is the median of the feature times forming the cluster.
- Cluster intensity computation: you have the choice between 2 computation methods: most intense or sum. For "most intense" method, the cluster intensity corresponds to the intensity of the most intense feature of features forming the cluster. For the "sum" method, cluster intensity is the sum of the intensities of features composing the cluster.

Retention time alignment

As soon as the PSM are matched to peakels, the software then aligns the retention time of the annotated ions for all the LC-MS runs to be compared, and uses this information to cross-assign MS/MS data to ions (i.e. chromatographic peaks) that were detected but not identified in other runs. Because chromatographic separation is not completely reproducible, LC-MS runs must be aligned. The retention time alignment procedure is a critical step in MS1 label-free quantification. Proline's alignment algorithm selects a **reference run** and generates a set of functions that will be used to predict the RT (retention time) for missing features from another run. These functions are obtained by performing pairwise alignments between the different runs to be compared (Bylund et al., 2002; Jaitly et al., 2006; Sadygov et al., 2006). The first step consists in computing a scatter plot (see Figure below) of the observed time difference between the two runs as a function of the reference run's time-scale. This mapping can be based on the **peptide identity** (same sequence and same post-translational modifications) of identified features, or by mapping the detected features of the pair of runs, taking user-defined time and mass error ranges into account (the default feature mapping time and m/z tolerance values are set to 600 seconds and 5 ppm, respectively).





RT prediction using computed alignments. The two scatter plots on the left correspond to computed run alignments between the reference run and two other runs (A and B). The red curves on these plots correspond to the median RT prediction for each alignment, obtained by applying a moving median calculation. The graphs on the right illustrate the case of a peptide ion that is present in runs A and B, but has only been fragmented by MS/MS in run A. Knowing the retention time in run A (T_A), we can predict T_B by two consecutive time conversions. T_A is first converted to the reference run scale ($T_{REF} = T_A + D_A$) using the first run alignment, then to $T_B = T_{REF} + D_B$ using the alignment for the second run.

RT prediction functions are then obtained by **smoothing** these scatter plots using a moving median calculation or a local regression. To decrease the number of alignment combinations (i.e., pairs of maps), the reference run is determined by an iterative method. The algorithms begin by selecting a random run as a reference and compute all alignments against this map. The algorithm then determines a new reference run by selecting the run with the smallest sum of RT differences in the resulting run alignments. The iteration stops after a user-specified maximum number of iterations or when the reference run remains unchanged between two iterations. The software can also be configured to compute all possible RT alignment combinations (all possible pairs of maps, "exhaustive" option), but this can be very computationally expensive when there is a high number of maps to be compared. The alignments computed can then be used to predict the retention times for peptide ions in a specific sample where they were not identified.

Parameters

Alignment

- Map Alignment: alignment can be disabled. In this case the cross assignment step could be executed but without any retention time prediction
- Method : is the name of the method used to determine the reference run
 - Exhaustive: the exhaustive algorithm computes the distance between maps for each possible couple of maps and selects the map with the lowest sum of distances to be the reference map.



- Iterative: the algorithm iteratively selects the best reference run as described above.
 - Maximum number of iterations: this option is available only for the iterative method. This is
 a stop condition of the iterative algorithm, when the algorithm has reached its maximum
 number of iterations, it stops.
- Ignore alignment error : allow or not to continue quantitation even if there are some errors in alignment.

Feature mapping

- Feature mapping is needed to build the alignment scatter plot of the observed time difference between two runs. The method used could be:
 - Feature coordinates : the mapping is based on features RT and m/z coordinates taking into account a tolerance for each of these values.
 - m/z tolerance : m/z window used to match features between two compared maps.
 - Peptide Identity : the mapping is based on peptides identification (same sequence and same post-translational modifications)
- Time tolerance in seconds: time window used to match features/peptides between two compared maps.

Alignment smoothing

When features of two runs are matched, a trend can be extracted from the scatter plot by using a smoothing method.

- Smoothing method: you have the choice between three smoothing methods: LOESS, time window or landmark range:
 - LOESS : no specific parameter
 - Landmark range
 - Number of landmarks/window size: time of aligned map is corrected using median computed on windows containing a specified number of landmarks. The run is divided into windows of size the specified number of landmarks. You have to provide the number of landmarks by window. The smoothing method is applied considering the number of landmarks present in the window, and computes the median point for this window.
 - Sliding window overlap: overlap is used to compute the step to move the smoothing window forward to calculate a smoothing point for this new smoothing window. Overlap gives the percentage of overlapping between two consecutive windows. For example, if window size is 200 (seconds or landmarks depending on which smoothing method is selected) and overlap is 20%, the step forward = 200*((100-20)/100) = 160 seconds or landmarks, i.e. the smoothing window is moved forward by a step of 160, so two successive windows overlaps each other by a step of 40 seconds or landmarks corresponding to 20% of 200
 - Time window : time of aligned map is corrected using median in a time window.
 - Window size/time interval: You have to provide the time interval. This time interval corresponds to the window size in which time median will be computed.
 - Minimum number of landmarks: This allows you to specify the minimum number of landmarks a window must contain to compute a median on it, it is not significant to compute a median on less landmarks.



Sliding window overlap: overlap is used to compute the step to move the smoothing window forward to calculate a smoothing point for this new smoothing window. Overlap gives the percentage of overlapping between two consecutive windows. For example, if window size is 200 (seconds or landmarks depending on which smoothing method is selected) and overlap is 20%, the step forward = 200*((100-20)/100) = 160 seconds or landmarks, i.e. the smoothing window is moved forward by a step of 160, so two successive windows overlap each other by a step of 40 seconds or landmarks corresponding to 20% of 200.

MoZ calibration

Delta moz (between experimental and theoretical moz) may change during a run. By specifying a moz calibration method, you can specify to use the recalibrated theoretical moz to cross assign instead of using the best feature moz. In both cases, a moz tolerance should be specified. see below

Cross Assignment

Proline uses a hybrid approach to retrieve intensity values for ions that were not identified. As indicated above, identified and quantified features are obtained by detecting chromatographic peaks in raw files without a-priori, using an identification-based deisotoping method. During the PSM assignment step, the identification data provides the monoisotopic mass and the charge state for the ion, guiding the deisotoping procedure to group together the detected chromatographic peaks. These grouped peaks are then removed from the list of peaks to be assigned, thereby reducing the data density when annotating subsequent chromatographic peaks during the cross-assignment step. Ions that were not identified in a run can then be sought out in this restricted list of detected peaks using their m/z and RT coordinates. The m/z value is the theoretical m/z value obtained following identification of this ion in another run. The RT value is predicted from the apex RT of the feature detected in the run providing the highest identification score. This RT prediction computation may involve the use of one or two alignment functions. Using these two coordinates, associated with user-defined m/z and RT tolerances, the algorithm seeks a corresponding signal among the chromatographic peaks that have not already been assigned to an identified ion. To avoid the propagation of erroneous cross-assignments between runs, an additional but optional control (named "use only confident features") is applied to ensure that this peak is the monoisotope of a peptide ion with a charge state identical to the master feature one. This is done by fitting its observed isotope pattern to a theoretical one.

Parameters

- allow cross assignment between all runs or only between runs of the same groups: the user can completely disable the cross assignment or can control runs between which cross assignment is applied:
 - Between all runs
 - Within groups only: cross assignment is applied only between runs belonging to the same group.

Feature mapping

- m/z tolerance (ppm or dalton): when mapping features from two different maps of the map set, the delta m/z between features must be lower than the m/z tolerance to be considered as the same feature seen on two different maps.
- Time/RT tolerance (seconds): when mapping features from two different maps of the map set, delta time between features must be lower than the time tolerance to be considered as the same feature seen on two different maps.



Filtering/Correction

- Use only confident features: is applied to ensure that the cross-assigned peak is the monoisotope of a peptide ion with a charge state identical to the master feature one. This is done by fitting its observed isotope pattern to a theoretical one.
- Master feature intensity filter (optional): a filter can be applied to the map features to keep the best features (above threshold) to build the master map.
 - Two methods are available to filter features: the filter can be applied directly on intensity values (*Intensity method*) or it can be a proportion of the map median intensity (*Relative intensity method*).
 - intensity threshold/value: this provides the threshold for the filtering method. Only features above or below (depending on the *operator*) this threshold are considered for the master map building process.

If you choose Relative intensity for master feature filter type, the only possibility you have is percent, so features which intensities are beyond the relative intensity threshold in percentage of the median intensity are removed. If you choose Intensity for master feature filter type, you also have only one possibility at the moment of the intensity method: basic. Features which intensities are beyond the intensity threshold are removed and not considered for the master map building process.

Normalizing LC-MS maps

The comparison of LC-MS maps is confronted to another problem which is the variability of the MS signals measured by the instrument. This variability can be technical or biological. Technical variations between MS signals in two analyses can depend on the injected quantity of material, the reproducibility of the instrument configuration and also the software used for the signal processing. The observed systematic biases on the intensity measurements between two successive and similar analysis are mainly due to errors in the total amount of injected material in each case, or the LC-MS system instabilities that can cause variable performances during a series of analysis and thus a different response in MS signal for peptides having the same abundance. Data may not be used if the difference is too important. It is always recommended to do a quality control of the acquisition before considering any computational analysis. However, there are always biases in any analytic measurement but they can usually be fixed by normalizing the signals. Numerous normalization methods have been developed, each of them using a different mathematical approach (Christin, Bischoff et al. 2011). Methods are usually split in two categories, linear and non-linear calculation methods, and it has been demonstrated that linear methods can fix most of the biases (Callister, Barry et al. 2006). Three different linear methods have been implemented in Proline by calculating normalization factors as the ratio of the sum of the intensities, as the ratio of the median of the intensities, or as the ratio of the median of the intensities.

Sum of the intensities

How to calculate this factor:

- 1. For each map, sum the intensities of the features
- 2. The reference map is the median map
- The normalization factor of a map = sum of the intensities of the reference map / sum of the intensities of the map

Median of the intensities

How to calculate this factor:



- 1. For each map, calculate the median of the intensities in the map
- 2. The reference map is the median map
- 3. The normalization factor of a map = median of the intensities of the reference map / median of the intensities of the map

Median of ratios

This last strategy was published in 2006 (Dieterle, Ross et al. 2006) and gives the best results. It consists in calculating the intensity ratios between two maps to be compared then set the normalization factor as the inverse value of the median of these ratios (cf. figure below). The procedure is the following:

- 1. For each map in a "map set", sum the intensities of the features
- 2. The reference map is the median map
- 3. For each feature of the master map, ratio = intensity of the feature in the reference map / intensity of the feature for this map
- 4. Normalization factor = median of these ratios



Distribution of the ratios transformed in log2 and calculated with the intensities of features observed in two LC-MS maps. The red line representing the median is slightly off-centered. The normalization factor is equal to the inverse of this median value. The normalization process will refocus the ratio distribution on 0 which is represented by the black arrow

Proline makes this normalization process for each match with the reference map and has a normalization factor for each map, independently of the choice of the algorithm. The normalization factor for the reference map is equal to 1.

Isobaric labeling quantitation workflow

Common isobaric labeling quantitation methods, such as TMT 6 to 18plex or iTRAQ 4 or 8plex, are supported in Proline. Adding a missing xPlex method can be easily done. This technique may be used to compare multiple samples in a single run. This is done by reading all reporters ion peak intensity each corresponding to a different sample.

Data is red from the MS2 spectrum stored in mzDB file (see <u>available converters</u> paragraph). These quantitation results are stored in the Proline database and aggregated to define peptide ion abundances which are also stored in the Proline database, making them available for rapid data visualization and further post-processing.

Finally, in the same way as for label free quantitation, peptide ion measurements can be **summarized as peptides and then protein abundances** using different computational methods.



The user can opt to perform additional operations such as excluding peptides or ions based on their characteristics (precursor intensity fraction, missed cleavages, variable modifications, sequence specificity, etc.). A purity correction matrix can also be provided to correct the reporter ion abundances. Indeed some manufacturers provide such matrices indicating the percentages of each reporter ion that have masses differing by +/- n Da from the nominal reporter ion mass due to isotopic variants.

Post-processing of quantitative results

This procedure is used to compute peptide and protein abundances. It is available for LC-MS and isobaric labeling quantitative results. Several filters can also be set to increase the quality of quantitative results.

Here is the description of the parameters that can be modified by the user.

PSM filters

For isobaric labeling only

 Discard PSM on Precursor Ion Fraction value. "The PIF is defined as the fraction of ion current in the isolation window that is due to the targeted precursor ion and therefore ranges between 0 and 1. The PIF is determined for each targeted precursor ion and each tandem mass spectrum based on the closest full scan, which could be the previous or the consecutive one" (J. Proteome Res. 2011, 10, 4, 1785–1793).

Purity correction matrix

For isobaric labelling only

In isobaric labelling quantitation, isobaric tags are used in samples to compare their abundances. Due to isotopic contamination in tags, reporter ion peaks will contribute to those of neighboring reporter ions. A purity correction matrix is provided with the labelling kit by the manufacturer. This matrix can be used to correct the abundance of the reporter ions.

Peptide filters

- **Peptides selection method:** define which peptides are to be considered for quantitation.
 - All peptides: all peptides are taken into account at this step. Further filters may invalidate some peptides.
 - **Specific peptides**: peptides shared between different protein sets are discarded for protein set abundance calculation and statistical analysis.
 - **Razor and specific:** In addition to specific peptides, quantitation of shared peptides are considered for the "best" ProteinsSet. The best is actually based on the score.
- **Discard missed cleaved peptides**: if checked, peptides containing missed cleavages are discarded from the statistical analysis. It has to be noted that perfect tryptic peptides whose sequence is included in an observed missed cleaved peptide are also discarded if this option is enabled.
- **Discard modified peptides**: if checked, peptides containing specific modifications are discarded for protein set abundance calculation and the statistical analysis.
 - only those modified peptides or also non-modified peptides whose sequence is the same as these peptides may be discarded depending on the chosen filtering method.



Peptide and protein common parameters

• Normalization: the normalization factors are computed as the median of the ratio distributions between each run and a run of reference. A similar procedure is used for the <u>normalization of LC-MS features</u>.

Summarize PSM (reporter ions) into peptide ions abundance

For isobaric labelling only.

To calculate peptide ions abundance, associated reporter ions abundances can be **summed** or the **median** can be computed.

Summarize peptides ions into peptide abundance

To calculate peptide abundance, associated peptide ion abundances can be summed or the best ion is used. To choose the best peptide ion following rules are defined :

- Select the peptide ions identified and quantified in the maximum of quant channels(runs)
- If more than one, select peptide ions with the maximum of PSMs count
- If more than one, choose the peptide ion with the higher abundance.

Summarize peptides abundances into protein abundance

Peptide abundances can be summarized into protein abundances using several mathematical methods:

- sum: for each quantitative channel (raw file) the sum of observed peptides abundances is computed
- **mean**: for each quantitative channel (raw file) the mean of observed peptides abundances is computed
- **mean of TOP3** peptides: same procedure but applied on the 3 most abundant peptides. Peptides are sorted by descending median abundances (computed across all compared samples for peptide). Then the 3 first peptides are kept.
- median: for each quantitative channel, the median of observed peptides abundances is computed
- **median profile**: a matrix of peptide abundance ratios is first computed (rows correspond to peptides and columns to quantitative channels). The median of these ratios is then computed for each column. The relative values are then converted back into absolute values using a scaling factor. This factor is computed as the maximum value from the **means of TOP3 peptides** abundances.
- **normalized median profile**: matrix of peptide abundance ratios is first computed (rows correspond to peptides and columns to quantitative channels). This matrix is then normalized and then summarized using the median method described above. The obtained median abundances are then adjusted by using a scaling factor. This factor is computed as the maximum value from the **means of TOP3 peptides** abundances.
- Median Ratio Fitting: MRF computes a matrix of abundance ratios calculated between any two runs from ion abundances for each protein. For each pair-wise ratio, the median of the ion ratios is then calculated and used to represent the protein ratio between these two runs. A least-squares regression is then performed to approximate the relative abundance of the protein in each run in the dataset. This abundance is finally rescaled to the sum of the ion abundances across runs.

Aggregation of quantitative results

Two or more label free quantitations can be combined such that an ion quantified in multiple aggregated quantitations is represented only once in the aggregation result. The abundance of this ion is a combination of its



abundance measured in the different aggregated quantitations. This could be useful to combine for example quantitation of fractions into a single quantitation result.

For Isobaric labelling quantitations, the same process is used but combining reporter ions abundances. These combined PSMs will then be summarized to get peptide ions abundances.

The experimental design of the aggregation is based on the experimental design of the aggregated quantitation: the number of group/condition and the number of replicates per condition remains the same. However, the user can modify the correspondence between the groups and replicates if needed. In the following example the abundance of ion1 in the aggregated quantitation (in green) is based on the quantitation of this same ion in "Fraction 1" and "Fraction 2". Since ion2 is quantified only in "Fraction 1", its abundance values in the aggregated quantitation are the same as the abundances measured in "Fraction 1".



In this simple example, the correspondence between experimental designs is such that the abundance of ion1 in the replicate Repl1 is based on the measured abundance of ion1 in Repl1' in "Fraction 1" and Repl1'' in "Fraction 2". This could be modified by the user to take into account differences in the replicates order in aggregated quantitations or to account for the absence of a replicate (see for example replicate 3 in "Fraction 1").

In the current version, the abundance at the aggregation level is the sum of the abundances in aggregated quantitations.

Identification Summary Export

When exporting a whole Identification Summary in an excel file, the following sheets may be generated:

- Search settings and info : Contains information on project and search settings parameters
- Import and filters : Summary of used parameters during import, filtering and validation process
- *Protein sets* : List of all Protein Sets, valid or invalidated (configurable in custom option) during <u>Protein Sets</u> <u>Filtering</u>. Some columns description :
 - o *#sequences (#specific sequences)* : number of different peptide sequences identifying the Protein Set (specific : which does not identify any other valid Protein Set)



- o *#peptides* (*#peptides*) : number of different peptide (sequence + PTM) identifying the Protein Set (specific : which does not identify any other valid Protein Set)
- o *#spectral_count* (*specific_spectral_count*) : number of different peptide spectrum matches identifying the Protein Set (specific : which does not identify any other valid Protein Set)
- *Protein matches in protein set* : list of Protein Matches in each Protein Set. A same Protein Match could thus appear a few times if it belongs to different Protein Sets. (same column as protein set)
- *Best PSM from protein sets* : List of best peptide spectrum matches (a single PSM per peptide is listed) for each Protein Set. Some columns description :
 - o *#psm_prot_sets* : number of Valid Protein Sets identified by this PSM.
 - o *#psm_prot_matches* : number of Protein Match, which belong to at least 1 valid Protein Set, identified by this PSM.
 - o #psm_db_prot_matches : number of Protein Match, validated or not, identified by this PSM. This is equivalent to the number of proteins in fasta files containing the PSM.
- All PSMs from protein sets : List of all peptide spectrum matches for each Protein Set. (same column as best PSM from protein sets)
- PTM Cluster (filled only if "identify Modifications sites" has been run): All clusters (protein-set of localised ptms) identified in this identification summary.
- *Dataset statistics* : Some statistical values for the exported Identification Summary : number of Protein Set, modified peptides ...

Advanced features

Allowing multiple imports

By default, Proline does not allow importing multiple results files at the same time. This was due to the obsolete Peptide database which has been removed in Proline version 2.0. Nevertheless, if multiple results files could now be imported at the same time there is still a restriction at project level. Indeed, result files should be inserted sequentially in the same project. In addition, depending on Proline Server configuration, there could not be as many import threads as wanted or as the number of projects.

This is why a configuration allows the user (administrator) to specify groups of projects which will belong to the same "import thread". For instance, if Proline Server enables 3 threads for import services, you can specify 2 groups of projects:

- Import Thread 1 : project 1, project 3, project 5
- Import Thread 2 : project 6, project 7
- Import Thread 3 : all other project (and new projects)

This means that for projects 1, 3 and 5 imports will be sequential but in parallel of import to project 6 or 7...

If you specify more groups than allowed thread, some project groups will be grouped into a single one.

To define a project group, you should add the following string to *project.serialized_properties* in uds_db database. Currently this could be done only with PGAdmin (or any database server administration tool) :

{"import_group":"1"}

In the following example, the user has defined 3 specific groups, and the default group is always defined for all other projects.



9	uds_d	b 📑 l	Localhost 🛛 🚍 project 🔀				
■	Propr	iétés 🔣 Do	onnées 📅 ER Diagram				🍕 uds_db 🏾 🍔
	proje	ct 💈 Entr	ez une expression SQL pour filtrer les résu	ltats (utilisez Ctrl+Espace)			
ile		12 <mark>3</mark> id 📲	ABC name	Tt ABC description	🥝 creation_timestamp 🏾 Ҭ	nsc serialized_properties ↓↓↑	123 owner_id 🏹
Ğ	1	26	20220211_XPLnoFAIMS_import parallèl	e 20220211_XPLnoFAIMS_import	2022-02-11 16:32:17	{"import_group":1}	2 🗹
⊞	2	27	20220211_XPLFAIMS_import parallèle	20220211_XPLFAIMS_import par	2022-02-11 16:33:14	{"import_group":2}	2 🗹
e	3	28	20220211_Batch4_import parallèle	20220211_Batch4_import parallè	2022-02-11 16:33:36	{"import_group":3}	2 🗹
ť							



Available converters to mzDB

Raw2mzdb

Raw file conversion to mzDB

This <u>converter</u>, named <u>raw2mzdb</u>, is based on ProteoWizard, ensuring compatibility with a wide range of instrument vendors.

Installation

- 1. get the zip archive on Proline download section
- 2. install of MSFileReader from Thermo (<u>here</u>, will install all necessary c++ redistribuables)
- 3. ensure your regional settings parameters are '.' for the decimal symbol and ',' for the list separator

Use case procedure

Open a command line window in the directory containing raw2mzdb.exe

Enter:

raw2mzdb.exe -i <rawfilename> -o <outputfilename>

By default, the raw file will be converted in the "fitted" mode for the MS1 (MS2 is often in centroid mode and can not be converted in fitted mode). If the MS2 (or superior) are acquired in high resolution (i.e in profile mode), you could specify that you want to convert several MSs in the required mode: **raw2mzdb.exe -i <rawfilename> -o <outputfilename> -f 1-2** will try to convert MS1 to MS2 in fitted mode.

There are two other available conversion modes:

- 1. "profile", the command line is then: raw2mzdb.exe -i <rawfilename> -o <outputfilename> -p 1 (means you want profile mode for MS1, others MS will be stored as they were stored in the raw file)
- 2. "*centroid*" : **raw2mzdb.exe -i <rawfilename> -o <outputfilename> -c 1** (means you want centroid mode for MS1, others MS will be stored as they were stored in the raw file)

Warning: for Thermo raw file, -n option is mandatory. Anyway, it is recommended to use mzDBConverter. See below

mzDBConverter

New converter based on manufacturer libraries. It currently supports Thermo raw files and Bruker TimsTof files.

This converter is distributed with the automatic installer or can be downloaded from <u>proline download page</u>. To display a complete list of option type :

- mzdbConverter thermo --help
- mzdbConverter brucker --help

mzdbConverter.bat thermo -i <InputFilename> -o <outputFilename> -c 2,3 -f 1

This will convert Thermo <inputFilename> raw file info <outputFilename> mzdb file, using centroid mode for MS2 and MS3 and fitted mode for MS1 spectra.



Proline Studio

How to

Note: Read the Concepts & Principles documentation to understand main concepts and algorithms used in Proline.

• List of Abbreviations

Creation/Deletion

- Open a session and access to my projects
- <u>Create a new project</u>
- <u>Create a Dataset</u>
- Import a Search Result
- <u>Delete Data</u>
- <u>Connection Management</u>

Display

- Display MSQueries, Peptides/PSM or Proteins of a Search Result
- <u>Display MSQueries</u>, PSM, Peptides, Protein Sets, PTM Protein Sites or Adjacency Matrices of an Identification Summary
- Display Modification Sites or Clusters of a Modification Dataset
- Display Search Result & Identification Summary Properties
- Display Spectral Counts
- <u>Display Quantitation results</u>
- <u>Create and Save a User Window</u>
- Frame Toolbars Functionalities
- Filter tables
- <u>Search tables</u>
- <u>Graphics : Scatter Plot / Histogram</u>
- <u>Statistical Reports (MSDiag)</u>
- <u>MS Files (MzScope)</u>
- General Settings

Save, import and export

- Import Search Result file (Mascot/OMSSA/X!Tandem/MzIdent/MaxQuant)
- Export data
- Generate Spectrum Matches

Algorithm and other operation

- Validate a Search Result
- Filter Protein Sets
- <u>Change Typical Protein of a Protein Set</u>
- <u>Combine datasets</u>



- Data Analyzer
- <u>Calculator</u>
- Update Spectrum using Peaklist software
- Identify Modification Sites/Cluster
- Annotate and Edit Modification Clusters
- <u>Administration</u>

Quantitation

- Spectral Count
- Label Free Quantitation
- Isobaric Labeling Quantitation
- Quantitations Aggregation
- <u>Compute Post Processing on abundances</u>



List of Abbreviations

Calc. Mass: Calculated Mass Delta MoZ: Delta Mass to Charge Ratio Exp. MoZ: Experimental Mass to Charge Ratio Ion Parent Int.: Ion Parent Intensity Missed Cl.: Missed Cleavage Modification D. Mass: Modification Delta Mass Modification Loc.: Modification Location Next AA: Next Amino-Acid Prev. AA: Previous Amino-Acid Protein Loc.: Protein Location of the Modification Protein S. Matches: Protein Set Matches PSM: Peptide Spectrum Match **PTM:** Post Translational Modification PTM D. Mass: PTM Delta Mass **RT**: Retention Time SC: Spectral Counting

Server Connection

When you start Proline Studio for the first time, the Server Connection Dialog is automatically displayed.

Server Connection				
Server Parameter				
Server host : Host				
User Parameters				
User : Username				
Password :				
Remember Password				
Default V OK X Cancel				

You must fill the following fields:

- Server Host: this information must be asked to your IT Administrator. It corresponds to the Proline server name
- User: your username (an account must have been previously created by the IT Administrator).
- Password: password corresponding to your account (username).



If the field "Remember Password" is checked, the password is saved for future use. Server connection dialog continues to open with Proline Studio, the user though does not need to fill in his password, unless the last one is changed after his last login.

Create a New Project

Projects %	=
< Select a Project >	-
	7



To create a Project, click on "+" button at the right of the Project Combobox. The Add Project Dialog opens. Fill the following fields:

- Name: name of your project
- Description: description of your project

You can specify other people to share this new project with them. Then click on OK Button

Creation of a Project can take a few seconds. During its creation, the Project is displayed grayed with a small hourglass over it.



Create a Dataset

In the Identification tree, you can create a Dataset to group your data

To create a Dataset:

- right click on Identifications or on a Dataset to display the popup.
- click on the menu "Add Dataset..."



Projects × Ms	Files	
Proline_Project	ct	
	s	
🕂 📄 All Im	Display Search Result	>
🛄 Trash	Display Identification Summary	>
	Add Dataset	
	Add Identification Folder	
	Copy Search Result	
	Paste Search Result	
	Rename	>
	Clear	
	Delete	
	Import Search Result	
	Import MaxQuant Result	
	Validate Search Result	

On the dialog opened:

- fill the name of the Dataset
- choose the type of the Dataset
- optional: click on "Create Multiple Datasets" and select the number of datasets you want to create

Add	l Dataset	:		23	
ſ	Dataset Parameters				
	Name:	Replicate			
	Type:	Biological Sample		•	
			Create Multiple Datasets	3 🜩	
	🕑 Defa	ult	V OK Cancel		

Let's see the result of the creation of 3 datasets named "Replicate":

Projects %	•
ProjectTest	
Identifications Identifications Imported Replicate 1 Replicate 2 Replicate 3 Trash	



Create a Folder

In both Identification and Quantitation tree, you can create Folders to organize your data

To create a Folder :

- right click on Identifications, Quantitations or on a Folder to display the popup.
- click on the menu "Add Identification Folder..." or "Add Quantitation Folder..."



Import a Search Result

See Concept & Principle section

Import Mascot/X!Tandem/OMSSA/MzIdentML

There are two possibilities to import Search Results:

- import multiple Search Results in "All Imported" and put them later in different datasets.
- import directly a Search Result in a dataset.



Import in "All Imported"

To import in "All Imported":

- right click on "All Imported" to show the popup
- click on the menu "Import Search Result..."



Import directly in a Dataset

It is possible to import Search Results directly in a Dataset. Even in this case, Search Results are available in "All Imported".

To import a Search Result in a Dataset, right click on a dataset and then click on "Import Search Result..." menu. Same dialog and parameters as in "Import in "All Imported"" above will be displayed.





Import Search Result Parameters

In the Import Search Results Dialog:

- select the file(s) you want to import thanks to the file button (the Parser will be automatically selected according to the type of file selected)

- select the different parameters (see description below)

- click on OK button

Note 1: You can only browse the files accessible from the server according to the configuration done by your IT Administrator. Ask him if your files are not reachable. (Look for *"Setting up Mount-points"* paragraph in <u>Installation & Setup</u> page).

Note 2: Proline is able to import OMSSA files compressed with BZip2.

Parameters description:

- Software Engine: the software which generated your Result File (this parameter will be automatically set when files are selected or you can select it)
- Instrument: mass-spectrometer (with specific configuration) used for sample analysis
- Fragmentation Rule Set: The fragmentation rules specified in software. by clicking on in you could visualize all rules for a specific rule set. This is necessary to generate spectrum matches.
- Peaklist Software: the software used for the peaklist creation (mandatory for LCMS XIC quantitation)
- Decoy Strategy: The type of decoy search which was performed.
 - o "No Decoy": if the search was performed against a target database only.
 - o "Concatenated Decoy": if target and decoy sequences were merged into a single database.
 - o "Software Engine Decoy": if the decoy sequences were generated on-the-fly by your search engine.
 - o Decoy Accession Regex: for concatenated searches only. Select the rule to apply for the discrimination of target and decoy protein matches.
- Parser Parameters: according to your Software Engine, some extra-parameters are displayed:
 - o Mascot:
 - Subset Threshold: the percentage of score between a given protein match and the master protein match (superset). Protein matches with a relative score lower than
 Master_protein_score * (1-subset threshold)
 won't be imported.

Omssa:

0

- Usermods file path: an XML file containing the definitions for each user defined PTM used in the OMSSA search.
- PTM Composition file path: a text file containing the chemical composition for each user defined PTM. This is required for PTMs not already imported in another Search Result. The format is the following:

PTM name=<PTM composition> (one per line).

Example: Acetyl peptide N-term=H(-6) C(-7) O(-1)

• X!Tandem:


■ Protein parsing rule: Specify the rule (regular expression) to be used to retrieve protein accession from protein info. As an example, to use the Uniprot *Entry Name* as protein accession, use the following rule: ...\|[^|]*\|([^]*)

nport Search Results	Carrier and Protection A.	×
Files Selection		
mascot_data/Proline_Tests	/SmallRuns/F071232.dat	
mascot_data/Proline_Tests	/SmallRuns/F071233.dat	
mascot_data/Proline_rests	Jomalikuns/F071234.uat	
Parameters		
Software Engine :	Mascot	•
Instrument :	LTQ-ORBITRAP XL (A1=FTMS F=CID A2=TRAP)	•
Fragmentation Rule Set :	ESI-TRAP (A1=TRAP F=CID A2=TRAP)	
Peaklist Software :	Mascot Distiller	•
Decoy Parameters		
Decoy :	Concatenated Decoy	-
Decoy Accession Regex :	###REV###\\$+	
Parser Parameters		
Subset Threshold : 1.0)	
Save Door	d OK X Cancel	

Importing a Search Result can take some time. While the import is not finished, the "All Imported" or "selected dataset" is shown grayed with an hourglass and you can follow the imports in the Tasks Log Window (Menu Window > Tasks Log to show it).



To show all the Search Results imported, double click on "All Imported", or right click to popup the contextual menu and select "Display List"



If needed, from the All Imported window, you can drag and drop one or multiple Search Result to an existing dataset.

Projects %	-	Та	asks Log 🛛 ProjectTest : All Imported 🛛	
ProjectTest	- 🗈 🖉 🔹		id Search Result Name Peaklist Path MSISearch F	MSIS
		1	2 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\\Data\\Clair F067897.dat	/mnt/
Se Identifications		2	4 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\\Data\\Clair F067898.dat	/mnt/
- All Imported		3	10 K12 DH5 QEx T12 QEx2_002086.raw (DH5_10) D:\\MSData\\D F075556.dat	/mnt/
- Replicate 1	/	4	15 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\\Data\\Clair F067897.dat	/mnt/
- Replicate 2		E.	17 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\\Data\\Clair F067898.dat	/mnt/
- Replicate3	•	6	19 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\\Data\\Clair F067899.dat	/mnt/
_	Drag & D	o	р	

Import MaxQuant result

To import a MaxQuant Search Result, right click on a dataset and then select "Import MaxQuant"



Note 1: MaxQuant import will generate a dataset hierarchy with the result from the different acquisition.

The following dialog will be displayed



• • • • • • • •		Load Identification Da
Import MaxQuant Results	×	Load Data for Datase
Files Selection		Load Data for Datase
		Load Quantitation Da
		Load Identification Da
		Load Projects for Use
	14	Check User dupierris
	Sec.	Load Projects for Use
		Check User hesse
		Get Server File Syste
		Load Data for Datase
		Load Data for Datase
	🗫 Ouvrir	×
	Rechercher dans	: 📙 🗸 🤌 🥬 🖽 -
		50-5
		MQ_YO
	Documento r	
	Documents 1	
	Bureau	
Parameters		
Testement of Colority		
Instrument: < Select >		
Accession receyn :	Documents	
Import quantitation values		
	~ 50	
📊 Save 🕕 Load 🖌 🖌 🛠	CEPC	
	S	Nom du dossier : D:\DEV\Proline_Datz
	Déceau	
	Reseau	Type de fichier : Tous les fichiers \checkmark

- select the directory containing the files generated by MaxQuant. This folder should look like:

<root_folder>\mqpar.xml <root_folder>\combined\txt\summary.txt <root_folder>\combined\txt\proteinGroups.txt <root_folder>\combined\txt\parameters.txt <root_folder>\combined\txt\msmsScans.txt <root_folder>\combined\txt\msms.txt

- select the Instrument: mass-spectrometer used for sample analysis different parameters
- specify, if needed, the regular expression to extract protein accessions from MaxQuant protein ids.
- you can choose to import also quantitative data
- click on OK button

Delete Data

You can delete Search Results, Identification Summaries and Datasets in the data tree. You can also delete XIC or Spectral Counts in the quantitation tree.

Delete the Datasets (identification or quantitation...) **from the tree view** (Search Result always accessible from "All Imported" view...).

There are two ways to delete data: use the contextual popup or drag and drop data to the Trash.

Delete Data from the contextual popup

Select the data you want to delete, right-click to open the contextual menu and click on delete menu.



Projects 2 MzDB Fil	es
Project Document	ation
 Identifications All Imported dataset <lidatas< td=""><td>Display Search Result Display Identification Summary</td></lidatas<>	Display Search Result Display Identification Summary
	Add Dataset Rename Clear Delete
	Import Search Result Validate Search Result Merge Datasets Filter ProteinSets Change Typical Protein Generate Spectrum Matches Compare With SC
	Export Properties

The selected data is put in the Trash. So it is possible to restore it while the Trash has not been emptied.

Delete Data by Drag and Drop

Select the data you want to delete and drag it to the Trash. It is possible to restore data while the Trash has not been emptied



Empty the Trash

To empty the Trash, you have to Right click on it and select the "Empty Trash" menu.





A confirmation dialog is displayed and if accepted Dataset will be removed from the Trash.

Search Results are not completely removed, you can retrieve them from the "All Imported" window.

Delete a Project

It is not possible to delete a Project by yourself. If you need to do it, ask your IT Administrator.

Connection Management

Once user is connected (see Server Connection), it is possible to:

• Reconnect with a different login

Server Connect	tion
Server Param	neter
Server Host :	GRE046180
User Parame	ters
User :	newUserName
Password :	•••••
	Remember Password
👌 Default	V OK X Cancel

Change password

Change Password	×
Old password :	••••
New password :	•••••
Confirm new password :	•••••
	V OK Cancel



Display MS Queries, Peptides/PSM or Proteins of a Search Result

All information, validated or not, can be accessible from this menu. Indeed, Search Result contains all data imported from a result file without any validation consideration.

Functionality Access

To display data of a Search Result:

- right click on a Search Result
- click on the menu "Display Search Result >" and on the sub-menu "MSQueries" or "PSM" or "Proteins"



MSQueries Window

If you click on MSQueries sub-menu, you obtain this window:

	Initial Id	Charge	m/z	#PSMs	First Scan	Last Scan	First Time	Last Time	Spectrum Title	
41	20 813	3	763.3928	2	25212	25212	105.8300	105.8300	22101: Scan 25212	(rt=105.83) [D:/Data/M
42	20 812	3	763.3925	2	25001	25001	104.8920	104.8920	21908: Scan 25001	(rt=104.892) [D:/Data/
43	20 811	3	763.3915	2	24916	24916	104.5090	104.5090	21838: Scan 24916	(rt=104.509) [D:/Data/
44	20 8 10	2	1144.5831	1	24929	24929	104.5670	104.5670	21849: Scan 24929	(rt=104.567) [D:/Data/
45	20 809	3	763.3789	0	22071	22071	91.7158	91.7158	19135: Scan 22071	(rt=91.7158) [D:/Data/
46	20 808	3	763.3782	0	13836	13836	55.5833	55.5833	11432: Scan 13836	(rt=55.5833) [D:/Data/
47	20 807	3	763.3781	0	22155	22155	92.0904	92.0904	19208: Scan 22155	(rt=92.0904) [D:/Data/
48	20 806	3	763.3739	0	18214	18214	74.5564	74.5564	15506: Scan 18214	(rt=74.5564) [D:/Data/
49	20 805	3	763.3493	0	26964	26964	113.6810	113.6810	23827: Scan 26964	(rt=113.681) [D:/Data/
50	20 804	3	763.3462	0	17478	17478	71.3421	71.3421	14808: Scan 17478	(rt=71.3421) [D:/Data/
51	20 803	3	763.0883	2	28919	28919	122.3630	122.3630	25749: Scan 28919	(rt=122.363) [D:/Data/
52	20 802	2	1144.1276	1	28910	28910	122.3260	122.3260	25742: Scan 28910	(rt=122.326) [D:/Data/
53	20 80 1	2	1144.0883	1	19772	19772	81.4564	81.4564	16985: Scan 19772	(rt=81.4564) [D:/Data/
54	20 800	3	763.0597	1	28202	28202	119.2060	119.2060	25069: Scan 28202	(rt=119.206) [D:/Data/
55	20 799	2	1144.0857	1	28210	28210	119.2400	119.2400	25076: Scan 28210	(rt=119.24) [D:/Data/M
56	20 798	3	763.0583	0	23012	23012	95.9305	95.9305	20060: Scan 23012	(rt=95.9305) [D:/Data/I
57	20 797	3	763.0579	1	21384	21384	88.6501	88.6501	18482: Scan 21384	(rt=88.6501) [D:/Data/
58	20 796	3	763.0460	1	8310	8310	32.6783	32.6783	6254: Scan 8310 (rt	t=32.6783) [D:/Data/MS
59	20 795	4	572.5167	1	3993	3993	16.5747	16.5747	2221: Scan 3993 (rt	t=16.5747) [D:/Data/MS
60	20 794	3	763.0040	1	13152	13152	52.6821	52.6821	10784: Scan 13152	(rt=52.6821) [D:/Data/
61	20 /93	2	1144.0024	1	13235	13235	53.0366	53.0366	10864: Scan 13235	(rt=53.0366) [D:/Data/
62	20 792	3	63.0038	1	13240	13240	53.0584	53.0584	10869: Scan 13240	(rt=53.0584) [D:/Data/
63	20 /91	2	1144.0020	1	13150	13150	52.6/31	52.6/31	10782: Scan 13150	(rt=52.6731) [D:/Data/
04	20 790	3	/62.7336	1	11987	11987	47.7521	47.7521	9685: Scan 11987 (rt=47.7521) [D:/Data/M
00	20 769		E 1143.3933	1	12/00	12/00	31.1014	31.1014	10440; Scart 12/60	(r l=51, 1014) [D:/Dala/i
00	20 788	4	372.2874	0	6157	8157	32.0894	32,0694	0112; Scan 8157 (n	1=32.0094) [D:/Data/MS
Don	tide Length	PTMe	Score Cal	Mass	Evo MoZ Pom	Charge	Missed Cl Rank	PT Ion Pare (Decov MsOu	Spectrum Title



Upper View: list of MSQueries. Some columns may not be (correctly) filled if the Peaklist software were not correctly specified during import. It is possible to change this information using '<u>Update Spectrum ..</u>'

Bottom Window: list of all Peptides linked to the current selected MSQuery.

Note: Abbreviations used are listed here

PSMs (Peptides) Window

If you click on PSMs sub-menu, you obtain this window:

\mathbb{D}		Peptide	Length	PTMs	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Rank	RT	Ion Par	MsQuery	Spectrum Title	r,
ñ	7756	VTFLAWQYR		9	2.35	1182.6185	592.3171	0.82	2	C	3	28.0916		4595	5178: Scan 714	4 🔺
$\overline{\mathbf{v}}$	7757	RTGSLALFYR	1	10	2.74	1182.6509	592.3338	1.81	2	1	. 1	42.9726		4596	8636: Scan 108	5
ã	7758	IIPTVDRTLR	1	10	0.26	1182.7085	592.3578	-6.13	2	1	. 6	48.3877		4597	9823: Scan 121	.3
<u>~</u>	7759	LIKIQEGNLR	1	10	0.26	1182.7084	592.3578	-6.10	2	1	. 6	48.3877		4597	9823: Scan 121	3
	7760	LLDSITVPVAR	1	11	62.26	1182.6973	592.3578	3.35	2	C	1	48.3877		4597	9823: Scan 121	.3
	7761	QRGSITPRIR	1	10	16.95	1182.6945	592.3578	5.63	2	2	2 2	48.3877		4597	9823: Scan 121	.3
	7762	EVCFACVDGK	1	10 Carbamidometh	32.08	1183.5002	592.7587	2.17	2	C	1	22.3758		4599	3740: Scan 560	11
	7763	EVCFACVDGK	1	10 Carbamidometh	27.59	1183.5002	592.7593	3.32	2	C	1	22.0342		4600	3657: Scan 550	19
0	7764	AIEIYTDMGR	1	0 Oxidation (M8)	30.56	1183.5543	592,7853	1.47	2	C	1	20.4658		4604	3236: Scan 507	10
	7765	AYSMLTITER	1	10	46.98	1183.5907	592.8040	2.26	2	0	1	39.9774		4606	7942: Scan 101	2
	7766	GLTVMFEIMK	1	LO Oxidation (M9)	0.45	1183.5981	592.8040	-3.99	2	C	2	39.9774		4606	7942: Scan 101	2
	7767	EPSQADIALLK	1	11	52.48	1183.6448	592.8315	3.11	2	C	1	34.3582		4608	6664: Scan 873	2
	7768	HLGTLNFGGIR	1	1	2.83	1183.6462	592.8315	1.95	2	C	3	34.3582		4608	6664: Scan 873	2
	7769	XAAAPDDLALLK	1	12	19.63	1183.6448	592.8315	3.11	2	C	2	34.3582		4608	6664: Scan 873	2
	7770	AIEAVAISPWK	1	11	52.44	1183.6600	592.8387	2.44	2	C	1	51.6265		4609	10550: Scan 12	9
	7771	MMPTPVILLK	1	0 Acetyl (Protein	11.63	1183.6709	592.8431	0.59	2	C	1	104.5440		4610	21845: Scan 24	1 9: Y
		<													>	>



Upper View: list of all Peptide Spectrum Matches

Middle View: Spectrum, Spectrum Error, Spectrum Values and Fragmentation Table of the selected PSM. If no annotation is displayed, you can generate Spectrum Matches by clicking on the according button

Bottom Window: list of all Proteins identified by the currently selected Peptide.

Note: Abbreviations used are listed here

Proteins Window

If you click on Proteins sub-menu, you obtain this window:



	Proteir	1		3	Score				Peptides			Mass				
	1 🗟 PY	1_YEAST						3235.37			77				245040.77	7
:	2 🚺 KP1	K1_YEAST						2937.61			47	'			54544.63	3
:	3 🚺 EF:	YEAST						2860.99			57	'			93289.18	3
·	4 🚺 G3	3_YEAST						2760.79			35	i			35746.67	7
	5 🚺 EN	D2_YEAST						2651.52			37	'			46914.17	7
1	6 🚺 PD	1_YEAST						2510.19			38	1			61495.39	,
	7 🚺 HS	71_YEAST						2405.36			37	'			69657.25	5
:	8 🚺 HSI	72_YEAST						2325.76			40				69470.01	L
- ·	9 🚺 EN	01_YEAST						2261.95			33				46816.14	ł
1	о 🚺 но	082_YEAST						2260.61			43	1			80899.75	5
1	1 🔓 PG	(_YEAST						2073.21			34	ł			44738.43	3
1	2 🚺 HSI	82_YEAST						2068.16			39				81406.40)
1	3 🚺 HSI	75_YEAST						2056.18			35	i			66601.59)
1.	4 🔂 HSI	76_YEAST						1989.48			34	ł			66594.57	7
1	5 🚺 FA	32_YEAST						1913.23			48				206946.94	ł
1	6 🚺 FA	1_YEAST						1903.58			43				228691.28	3
1	7 🚺 G3	2_YEAST						1890.95			28				35846.85	5
	Prev. AA	Peptide	Next AA	Score	Start	Stop	McOuery				-	-				
		HILSIK		20.40	1.012	1.017	maquery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Mis	Ion P	PTM	
2 IN			M	30.48	1 912	1917 2 202	274	Rank	Calc. Mass 709.45	Exp. MoZ 355.73	Ppm 0.13	Charge 2	Mis	Ion P	РТМ	
2 1	•	YGLEVR	M	30.48 18.41	1 912 2 197	1 917 2 202	274 647	Rank 2	Calc. Mass 709.45 753.42	Exp. MoZ 355.73 377.72	Ppm 0.13 -0.94	Charge 2 2	Mis 0 0	Ion P	PTM	
3 K		YGLFVR QVIAEAK	M T	30.48 18.41 15.38	1 912 2 197 1 628	1917 2 202 1 634	274 647 687	Rank 2	Calc. Mass 709.45 753.42 757.43 777.38	Exp. MoZ 355.73 377.72 379.72 389.70	Ppm 0.13 -0.94 1.62	Charge 2 2 2	Mis 0 0	Ion P	PTM	
3 K 4 R 5 K		YGLFVR QVIAEAK WSPFNK VSINEPK	M T D	30.48 18.41 15.38 15.63 25.70	1912 2 197 1 628 1 796	1917 2 202 1 634 1 801 205	274 647 687 886 976	Rank 2	Calc. Mass 709.45 753.42 757.43 777.38 785.43	Exp. MoZ 355.73 377.72 379.72 389.70 393.72	Ppm 0.13 -0.94 1.62 -0.54 -2.60	Charge 2 2 2 2 2 2 2	Mis 0 0 0	Ion P	PTM	
3 K 4 R 5 K	· · ·	YGLFVR QVIAEAK WSPFNK VSINEPK	M T D L	30.48 18.41 15.38 15.63 25.70 5.22	1 912 2 197 1 628 1 796 199 21	1 917 2 202 1 634 1 801 205 27	274 647 687 886 976	Rank	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31	Charge 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K 4 R 5 R 6 R	· · ·	YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK	M T D L D	30.48 18.41 15.38 15.63 25.70 5.22 8.74	1 912 2 197 1 628 1 796 199 21 1 734	1 917 2 202 1 634 1 801 205 27 1 741	274 647 687 886 976 1405	Rank	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K 4 R 5 R 7 K	· · · ·	YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGEK	M T D L D D	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02	1 912 2 197 1 628 1 796 199 21 1 734 1 350	1 917 2 202 1 634 1 801 205 27 1 741 1 357	274 647 687 886 976 1405 1434 1658	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P		
3 4 K K K K R K K R	· · · ·	YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLFAK	M T D D D L L	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287	1 917 2 202 1 634 1 801 205 27 1 741 1 357 294	274 647 687 886 976 1405 1434 1658 2192	Rank	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P		
3 K 4 R 5 R 7 K 9 R	· · · · · · · · · · · · · · · · · · ·	YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR	M T D L D L L K	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259	1917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265	274 647 687 886 976 1405 1434 1658 2192 2433	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K R K R K R K R K R K R K R K R K R K	· · · · · · · · · · · · · · · · · · ·	YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR EGVLDLMK	M T D L D L K K A G	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941	1917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948	274 647 687 886 976 1405 1434 1658 2192 2433 2650	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23 452.74	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 R K R K R K R K R K R K R K R K R K R		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR EGVLDLMK LLIEAISR	M T D L D L K K A G	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75 46.36	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941 1 480	1917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487	274 647 687 886 976 1405 1434 1658 2192 2433 2650 2823	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56	Exp. MoZ 355.73 377.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23 452.74 452.74	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 R K R K R K R K R K R K R K R K R K R		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR EGVLDLMK LLIEAISR DYPVVITK	M T D D D L K A G N Y	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75 46.36 2.96	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941 1 480 1 167	1917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487 1 174	274 647 687 886 976 1405 1434 1658 2192 2433 2650 2823 3152	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56 933.52	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23 452.74 457.79 467.77	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07 -0.24	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P 	PTM	
3 K 4 R 5 K 6 R 7 K 8 K 9 R 10 K 11 R 12 K 13 R 14 R		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR EGVLDLMK LLIEAISR DYPVVITK ILAIDVGMK	M T D D D D D L K K A G G N Y Y	30,48 18,41 15,38 15,63 25,70 5,22 8,74 12,02 36,64 17,33 17,75 46,36 2,96 22,82	1 912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941 1 480 1 167 229	1917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487 1 174 237	274 647 687 886 976 1405 1434 1658 2192 2433 2650 2823 3152 3599	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56 933.52 958.55	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23 452.74 457.79 467.77 480.28	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07 -0.24 -0.02	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K 4 R 5 K 6 R 7 K 8 K 9 R 10 K 11 R 112 K 113 R 114 R 115 R		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSNVLEAK VIECNVR EGVLDLMK LLIEAISR DYPVVITK ILAIDVGMK	M D D D L K A G G Y Y Y	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75 46.36 2.96 2.82 47.98	1912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941 1 480 1 167 229 8 15	1 917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487 1 174 237 822	274 647 687 886 976 1405 1434 1658 2192 2433 2650 2823 3152 3599 3670	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56 933.52 958.55 964.49	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 409.73 409.73 418.75 437.26 445.23 452.74 457.79 467.77 480.28 483.25	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07 -0.24 -0.04 5 -0.24 -0.54 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -2.60 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -1.62 -0.54 -0.04 -1.22 -0.72 -1.34 -0.07 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.72 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.55 -0.54 -0.55 -0.54 -0.55 -0.54 -0.55 -0.5	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K 4 R 5 K 6 R 7 K 8 K 9 R 8 K 10 K 11 R 112 K 113 R 114 R 115 R		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK SLLATGFK LISNVLEAK VIECNVR EGVLDLMK LLIEAISR DYPVVITK ILAIDVGMK TFEEAIQK DTYIVDNK	M D D D L K A G G Y Y Y A I	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75 46.36 2.96 22.82 47.98 8.88	1912 2 197 1 628 1 796 199 21 1 734 1 350 287 1 259 1 941 1 480 1 167 229 8 155 2 151	1 917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487 1 174 237 822 2 158	274 647 687 886 976 1405 1434 1658 2192 2433 2650 2823 3152 3599 3670 3706	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56 933.52 958.55 964.49 966.47	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 437.26 445.27 467.77 460.28 483.25 483.25	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07 -0.24 -0.24 -0.24 -0.24 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.54 -0.57 -0.54 -0.54 -0.57 -0.54 -0.54 -0.54 -0.57 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.54 -0.55	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	
3 K R K 6 R K 8 9 R K 10 1 R K		YGLFVR QVIAEAK WSPFNK VSINEPK LVTLELK VDVGMGIK SLLATGFK LSINUEAK UIECNVR EGVLDLMK LLIEAISR DYPVVITK LLAIDQMK TFEEAIQK DTYIVDNK	M T D D D L L K A G G V Y Y Y L L	30.48 18.41 15.38 15.63 25.70 5.22 8.74 12.02 36.64 17.33 17.75 46.36 2.96 22.82 47.98 8.37,56	1912 2 197 1628 1796 199 21 1734 1 350 287 1 259 1 941 1 480 1 167 229 815 2 151 1 311	1 917 2 202 1 634 1 801 205 27 1 741 1 357 294 1 265 1 948 1 487 1 174 237 822 2 158 1 318	274 6477 687 886 976 1405 1434 1658 2192 2433 2650 2823 3152 3599 3670 3706	Rank 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Calc. Mass 709.45 753.42 757.43 777.38 785.43 814.52 817.44 835.48 872.50 888.45 903.47 913.56 933.52 958.55 964.49 966.47 976.51	Exp. MoZ 355.73 377.72 379.72 389.70 393.72 408.27 409.73 418.75 437.26 445.23 452.74 457.79 467.77 480.28 483.25 483.25 483.25	Ppm 0.13 -0.94 1.62 -0.54 -2.60 3.31 -0.04 -1.22 -0.09 -0.72 -1.34 -0.07 -0.24 -0.02 -0.45 3.54 0.56	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Mis 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ion P	PTM	

Upper View: list of all Proteins

Bottom View: list of all Peptides identifying the selected Protein.

Note: Abbreviations used are listed here

Display MS Queries, PSM, Peptides, Protein Sets or Adjacency Matrices of an Identification Summary

From this menu, all displayed information is Identification Summary data, which has been validated according to user specified rules. To view the raw information as defined at import, use the Search Result sub menu.

Functionality Access

To display data of an Identification Summary:

- right click on an Identification Summary
- click on the menu "Display Identification Summary >" and on the sub-menu "MSQueries", "PSM", "Peptides", "Protein Sets", "PTM Protein Sites" or "Adjacency Matrix"



Didentification → Identification → All Import → op root → op rt →	ions ported 210	>	
🗄 💮 t 👘	Display Identification Summary	,	MSQueries
- 🛍 T	Display Identification Summary		PSMs
	Add Dataset		Pentides
	Add Identification Folder		Protein Sets
	Copy Search Result		Adiacency Matrix
	Paste Search Result		
	P		Modification Sites
	Rename	1	Modification Clusters
	Clear		New User Window
	Delete	_	Manage User Windows
	Import Search Result		
	Import MaxQuant Result		
	Malidate Caralle Daniela		

MSQueries Window

If you click on MSQueries sub-menu, you obtain this window:

	,	-																
	Charge	m/z		#Peptide Ma	tches	First Scan	Last Scan		First Time	Last	Time S	pectrum Ti	tle					
1		3 1004	2088		3		0	0	100.7630		24	955: Scan	37188 (rt=1	00.763) [D	:/Data/Claire/Ol	EMMA 121101	L_43b.raw]	
2		2 710	8333		1		0	0	35.8804		76	65: Scan 1	1718 (rt=35	.8804) [D:/	'Data/Claire/OEI	MMA121101_	43b.raw]	
3		2 433	2238		6		0	0	31.9367		66	30: Scan 1	l0122 (rt=31	.9367) [D:/	/Data/Claire/OEl	MMA121101_	43b.raw]	
4		3 393	2524		1		0	0	69.5741		16	646: Scan	25427 (rt=6	9.5741) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
5		4 550	5493		0		0	0	58.1151		13	611: Scan	20740 (rt=5	3.1151) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
6		2 374	7152		0		0	0	48.7761		11	077: Scan	16949 (rt=4	3.7761) [D	:/Data/Claire/Ol	EMMA 121101	L_43b.raw]	
7		2 703	.8414		3		0	0	37.0394		79	56: Sum o	f 2 scans in r	ange 12189	9 (rt=37.0394)	to 12217 (rt=	=37.1083) [[):/Da
8		2 905	9039		0		0	0	88.8100		22	001: Scan	33032 (rt=8	3.81) [D:/D	ata/Claire/OEM	MA121101_4	i3b.raw]	
9		4 487	7362		2		0	0	72.0339		17	306: Scan	26438 (rt=7	2.0339) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
10		2 771	8703		1		0	0	50.9328		11	626: Scan	17831 (rt=5	0.9328) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
11		2 594	8139		2		0	0	21.4588		39	43: Scan 5	5960 (rt=21.4	1588) [D:/D	ata/Claire/OEM	MA121101_4	i3b.raw]	
12		3 884	1173		4		0	0	109.4620		26	582: Scan	39515 (rt=1	09.462) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
13		4 848	9335		1		0	0	136.2080		30	209: Scan	45171 (rt=1	36.208) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
14		3 585	6480		2		0	0	63.5996		15	092: Scan	22981 (rt=6	3.5996) [D	:/Data/Claire/Ol	EMMA121101	L_43b.raw]	
15		4 411	2476		0		0	0	62.9898		14	928: Scan	22727 (rt=6	2.9898) [D	:/Data/Claire/OI	EMMA121101	[_43b.raw]	
16		2 768	9158		7		0	0	30.9361		63	56: Sum o	f 2 scans in r	ange 9734	(rt=30.9361) to	o 9756 (rt=30).9912) [D:/	Data
17		2 386	7380		10		0	0	10.7135		10	91: Scan 1	1850 (rt=10.)	7135) [D:/D	ata/Claire/OEM	MA121101_4	i3b.raw]	
18		1 568	3451		0		0	0	23.7393		45	05: Scan 6	6833 (rt=23.)	7393) [D:/D	ata/Claire/OEM	MA121101_4	i3b.raw]	
19		2 633	3152		3		0	0	12.7999		16	15: Scan 2	2609 (rt=12.)	7999) [D:/D	ata/Claire/OEM	MA121101_4	i3b.raw]	
20		3 756	0681		7		0	0	113.8170		27	309: Scan	40618 (rt=1	13.817) [D	:/Data/Claire/Oi	EMMA121101	[_43b.raw]	
21		2 763	8879		6		0	0	37.8659		81	61: Scan 1	l2525 (rt=37	.8659) [D:/	/Data/Claire/OEI	MMA121101_	43b.raw]	
22		3 663	3690		0		0	0	112.0440		27	101: Scan	40277 (rt=1	12.044) [D	:/Data/Claire/Oi	EMMA121101	[_43b.raw]	
23		3 748	7043		0		n	0	47 4860		03	80. Scan 1	4390 (++-47	4960) [D+	Data/Claire/OE	MMA121101	43h rawl	_
Pep	tide		РТМ	Score	Calc. Mas	s Ex	p. MoZ		Ppm Cha	arge	Missed Cl.	Rank	RT	Protei	Protein Sets	Ion Par	Decoy	Valir
TFSV	GFNEVVASO	QLNAIVVV		0.00	300	9.5903	1004.	2088	4.03	3		1	2 100.763	0	0			
IGLQ	IVQFINEPS/	AALLAHA		50.22	300	9.5962	1004.	2088	2.73	3		0	1 100.763	0	1 SSZ1_YEAST			
VKDN	PLOPYIKOM	YSFISSII		0.05	300	9.6128	1004.	2088	-3.02	3		2	2 100.763	0	0		× .	

Upper View: list of MSQueries.

Bottom Window: list of all Peptides linked to the current selected MSQuery.

Note: Abbreviations used are listed here

This view contains all MSQueries even if it doesn't bring an identification.

PSMs Window

If you click on PSM sub-menu, you obtain this window:



s Log	🛛 🗶 F06	7897 PSM 🛛 🗱										4		•
	Peptide	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion P	PTM	Protein Sets	E.	Π
1	ILFQK	21.38	113	2	647.40	648.41	-0.42	1	0			PUT1_YEAST		
2	LIFAGK	28.56	113	1	647.40	648.41	-0.42	1	0			RL402_YEAST		
3	LLFQK	21.38	113	3	647.40	648.41	-0.42	1	0			ARGR2_YEAST		
4	LVDLIK	25.99	182	1	699.45	350.73	-0.28	2	0			PHSG_YEAST		
5	LVDLLK	25.99	182	2	699.45	350.73	-0.28	2	0			BRR2_YEAST		
6	LVDLIK	24.51	185	1	699.45	350.73	0.63	2	0			PHSG_YEAST		
7	LVDLLK	24.51	185	2	699.45	350.73	0.63	2	0			BRR2_YEAST		L
8	LVDLIK	22.23	187	1	699.45	350.73	1.17	2	0			PHSG_YEAST		L
9	LVDLLK	22.23	187	2	699.45	350.73	1.17	2	0			BRR2_YEAST		L
10	VPFGGVK	31.28	200	1	702.41	352.21	0.24	2	0			ALDH6_YEAST		L
11	AAFIER	28.59	223	1	705.38	353.70	-0.37	2	0			RIR4_YEAST		L
12	AAFIER	23.53	225	1	705.38	353.70	1.47	2	0			RIR4_YEAST		L
13	LADFLK	24.19	227	1	705.41	353.71	-0.91	2	0			SYYC_YEAST		L
14	AIDLFK	25.18	230	1	705.41	353.71	2.06	2	0			SYRC_YEAST] /	l
15	AIDLFK	25.12	232	1	705.41	353.71	2.65	2	0			SYRC_YEAST		l
16	LADFLK	20.84	232	2	705.41	353.71	2.65	2	0			SYYC_YEAST		l
17	AGAFITK	34.03	239	1	706.40	354.21	-0.79	2	0			PYRD_YEAST		l
18	AGAFITK	41.53	240	1	706.40	354.21	0.20	2	0			PYRD_YEAST		l
19	KAMITK	27.74	240	2	706.40	354.21	-4.54	2	1		Oxidation (M3)	FMC1_YEAST	7 /	l
20	FGMDLK	34.44	256	1	709.35	355.68	-0.34	2	0			RIR4_YEAST] /	l
21	FGMDLK	23.01	258	1	709.35	355.68	0.61	2	0			RIR4_YEAST		l
22	FGMDLK	20.96	259	1	709.35	355.68	0.70	2	0			RIR4_YEAST		l
23	DHLLGR	24. 1 <mark>8</mark>	264	1	709.39	355.70	-0.91	2	0			RL16B_YEAST		ĺ
24	HILSIK	22.97	268	1	709.45	355.73	-0.69	2	0			PYR1_YEAST		l
25	HLISIK	22.97	268	2	709.45	355.73	-0.69	2	0			OTC_YEAST	1	ĺ
26	HILSIK	23.16	269	1	709.45	355.73	-0.58	2	0			PYR1_YEAST		l
27	HLISIK	23.16	269	2	709.45	355.73	-0.58	2	0			OTC_YEAST		l
28	HLSLLK	21.12	269	3	709.45	355.73	-0.58	2	0			SKP2_YEAST		l
29	HILSIK	24.81	272	2	709.45	355.73	-0.18	2	0			PYR1_YEAST	1 /	l
30	HLISIK	24.81	272	3	709.45	355.73	-0.18	2	0			OTC_YEAST		ĺ
31	HLSLLK	28.22	272	1	709.45	355.73	-0.18	2	0			SKP2_YEAST		ĺ
32	HILSIK	30.48	274	2	709.45	355.73	0.13	2	0			PYR1_YEAST		ĺ
33	HLISIK	30.48	274	3	709.45	355.73	0.13	2	0			OTC_YEAST		ĺ
34	HLSLLK	32.31	274	1	709.45	355.73	0.13	2	0			SKP2_YEAST		l
35	HILSIK	25.34	275	2	709.45	355.73	0.60	2	0			PYR1_YEAST	1	ĺ
36	HLISIK	25.34	275	3	709.45	355.73	0.60	2	0			OTC_YEAST	1	ĺ
37	HLSLLK	26.93	275	1	709.45	355.73	0.60	2	0			SKP2_YEAST	1	l
38	HILSIK	28.43	276	1	709.45	355.73	0.80	2	0			PYR1 YEAST	-	L

Note: Abbreviations used are listed here

Peptides Window

If you click on Peptides sub-menu, you obtain this window:

	DF136916	6 Peptides 🗙														
Peptide	Length	PTMs	Sco	ore	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Rank	RT	Protein Se	Protein Sets	PSM Count	MsQuery	Spectrum Title
1 LVQDVANNTNEEAGDGTTTAT	2	25		216.83	2559.2412	1280.6292	1.01	2		1	1 28.488		1 CH60_MOUSE	1	73704	4 17395: Scan 1923
2 SCSGVEFSTSGSSNTDTGKVSGTL	.6 2	27 Carbamidomet.		216.10	2722.2239	1362.1228	2.59	2			1 19.910		2 G3UX26_MOUSE,	3	78783	3 9695: Scan 12125
3 METYCNSGSTDTSSVINAVTHALT	Г/ З	31 Carbamidomet.		205.80	3318.5132	1107.1788	0.39	3	0	1	1 103.887		1 BDH_MOUSE	4	93848	8 83798: Scan 7668
4 NMGGPYGGGNYGPGGSGGSGGY	G 2	25 Oxidation (M2)		204.69	2204.8931	1103.4567	2.63	2	: 0	1	1 15.569		1 ROA2_MOUSE	1	. 58750	0 5154: Scan 8165 (
5 METYCNSGSTDTSSVINAVTHALT	r/ 3	31 Oxidation (M1)		202.35	3334.5083	1112.5104	0.35	3	(1	1 101.495		1 BDH_MOUSE	1	94179	9 81821: Scan 7496
8 LLLAGYDDFNCNVWDALKADR	2	21 Carbamidomet.		196.45	2468.1794	1235.0977	0.49	2	! :	L	1 93.214		1 GBB1_MOUSE	6	70303	3 74683: Scan 6898
7 GGGGSFGYSYGGGSGGGFSAS	. 3	32		196.04	2704.1538	1353.0887	3.37	2	2 (1	1 59.366		1 #C#P35527	2	78238	8 44642: Scan 4357
8 LCYVALDFEQEMATAASSSSLEK	2	23 Carbamidomet.		192.61	2549.1665	1275.5916	0.83	2	: 0		1 96.830	ł	4 ACTG_MOUSE, #	. 53	73330	77800: Scan 7159
9 EQGSSGLGSGSSGGGGSSSGL	2	28		192.56	2401.0742	1201.5471	2.26	2	! (1	1 22.515	1	1 AAK1_MOUSE	1	67623	3 11996: Scan 1429
	-			101.01	0101 0017	1001 5015	4.00				1 10 550		ADDING HOUSE		5000	
Protein Set Descri	iption	Score			Proteins	Peptides		Obse	rvable Peptides	Sp	ectral Count	Specific	Spectral Count	Sequence Count	Mass	s
/DAC2_MOUSE sp Q60	0930 VDAC	2_MOU		2115.78	4 (1	■,3🔺)		28		17		73	12		26	31
GUX26_MOUSE tr G3U	X26 G3UX2	26_MO		1851.23	1 (1	, 0 ()		24		16		63	2		24	30 -
vical Protein: Js musculus OX=100	090 GN=Vd	ac2 PE=1 SV=2	Ŧ	Prev. AA	Pr	entide	Next AA		Length		PTMs	Score		itart	Ston	Cal
ical Protein: us musculus OX=100	090 GN=Vd	ac2 PE=1 SV=2	T	Prev. AA	Pe	eptide	Next AA		Length		PTMs	Score	e S	itart	Stop	Ca
ical Protein: us musculus OX = 10(090 GN=Vd	ac2 PE=1 SV=2	₹ ≫	Prev. AA	Pe SC	eptide SGVEFSTSGSSNTDT	Next AA GKY		Length		PTMs 27 Carbamidometh	Scon	216.10	itart	Stop 47 47	Ca 73 65
ical Protein: Js musculus OX=100 Protein Descriptio VDAC2 MOUSE sp106093	090 GN=Vd	Same:	₹ ≫	Prev. AA	Pe SC SC	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT FDTTESPNTGK	Next AA GKY G V		Length		PTMs 27 Carbamidometh 19 Carbamidometh	Scon /I (C2) /I (C2)	e S 216.10 138.60 108.67	itart	Stop 47 47	Ca 73 65
ical Protein: _us musculus OX=100 Protein Description vDAC2_MOUSE sp [Q60931 a A0A286YCR8 M	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A	lac2 PE=1 SV=2	₹	Prev. AA	Pe SC SC LTT	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT FDTTFSPNTGK FDTTFSPNTGK	Next AA GK Y G V K S		Length	1	PTMs 27 Carbamidometh 19 Carbamidometh 13	Score (I (C2) (I (C2)	2 S 216.10 138.60 108.67 92.23	itart	Stop 47 47 109	Ca 73 65 121 122
ical Protein: Js musculus OX=10 Protein Descriptic 3 A0A226YCR8 M tr JA0A286 4 D3Y275 MOUSE tr JA0X28	090 GN=Vd on 0 VDAC2_M 5YCR8 A0A	lac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 5 K	Pe SC LTT LTT	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT FDTTFSPNTGK FDTTFSPNTGKK SCSGVEFSTSGSSNT	Next AA GKY GV K S DV		Length	1	PTMs 27 Carbamidometh 19 Carbamidometh 13 14 21 Carbamidometh	Scon ((C2) ((C2) ((C4)	2 S 216.10 138.60 108.67 92.23 82.73	itart	Stop 47 47 109 109 45	Ca 73 65 121 122 65
Ical Protein: Js musculus OX=10 Protein Descriptic VDAC2_MOUSE splQ6093 AD4286YCR8_M trIA04286 D3Y2T5_MOUSE trID3Y2T5 D3YUT5_MOUSE trID3Y2T5	090 GN=Vd on 60 [VDAC2_M 57 CR8 [A0A 5 [D3YZT5_M 8 [D3YUN8	lac2 PE=1 SV=2		Prev. AA	L Pe SC LTT TK	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT PDTTFSPNTGK FDTTFSPNTGKK SCSGVEFSTSGSSNT CIPPPYADI GK	Next AA GKY GV K S DV A		Length	1	PTMs 27 Carbamidometh 19 Carbamidometh 13 14 21 Carbamidometh 13 Oxidation (M2):	Scon (I (C2) (I (C2) (I (C4) Carbus	216.10 138.60 108.67 92.23 82.73 82.31	itart	Stop 47 47 109 109 45 12	Ca 73 65 121 122 65 24
ical Protein: s musculus OX=10 Protein Descriptic 3 VDAC2_MOUSE sp Q6093 3 A0A266/CR8_M tr IA0A286 3 D3YZT5_MOUSE tr ID3YZNS 3 D3YUN8_MOUSE tr ID3YUN8	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A 5 D3YZT5_N 8 D3YUN8_	Same:	¥ >> => •	Prev. AA 1 K 2 K 3 K 4 K 6 K 0 R 7 K	Pe SC LTT LTT TKC PM	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT PDTTFSPNTGK FDTTFSPNTGKK SCSGVEFSTSGSSNT UTDNTLGTEIAIEDOI VTDNTLGTEIAIEDOI	Next AA G V G V K S D V A C L		Length	1	PTMs 27 Carbamidometh 19 Carbamidometh 13 14 14 12 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh	A (C2) A (C2) A (C4) Carb	e S 216.10 138.60 108.67 92.23 82.73 82.31 80.75	itart	Stop 47 47 109 109 45 12 87	Ca 73 65 121 122 65 24 108
ical Protein: is musculus OX=10 Protein Descriptic § VDAC2_MOUSE sp[06093 § ADA286YCR8_M tr IADA286 § DSYZT5_MOUSE tr ID3YZT5 § D3YUN8_MOUSE tr ID3YUN8	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A 5 D3YZT5_M 8 D3YUN8_	Same:	₹ ****	Prev. AA 1 K 2 K 3 K 4 K 5 K 6 R 7 K 8 M	Pe SC SC LTT LTT TK PM W	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT FDTTFSPNTGK FDTTFSPNTGK SCSGVEFSTSGSSNT CIPPPYADLGK VTDNTLGTEIAIEDQI CVPVCPRPMCIPPF	Next AA GKY GV K S DV A C L YA		Length		PTMs 27 Carbamidometh 19 Carbamidometh 13 14 14 13 Oxidation (M2); 22 Carbamidometh 13 Acetvi (Protein I	A (C2) A (C2) A (C2) A (C4) Carb A (C18) I-ter	e S 216.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14	itart	Stop 47 47 109 109 45 12 87 2	Ca 73 65 121 122 65 24 108 24
Cal Protein: Is musculus OX=10 Protein Descriptic 3/ VAC2_MOUSE tsp(26033) 3/ A04286/CR8_M tr A04286 6/ 037275_MOUSE ts/100432 3/ D37UN8_MOUSE ts/1037UN8	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A 5 D3YZT5_M 8 D3YUN8_	Same:		Prev. AA 1 K 2 K 3 K 4 K 6 K 6 R 7 K 8 M 8 K	Pr SC SC LTT LTT TK PM WT AE GF	SGVEESISGSSNIDT SGVEESISGSSNIDT POTTESPNTGK POTTESPNTGK SCSGVEESISGSSNI CIPPPYADLGK VTDNTLGTEIAIEDQI CCVPVCPRPMCIPAIEDQI CCVPVCPRPMCIPAIEDQI CCVPVCPRPMCIPAIEDQI	Next AA GKY G V K S D V A C L Y A T		Length		PTMs 27 Carbamidometh 19 Carbamidometh 13 14 21 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 23 Acetyl (Protein I	Score Id (C2) Id (C1) Id (C18) Id-ter	e S 216.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14 61.21	itart	Stop 47 47 109 109 45 12 87 2 33	Ca 73 65 121 122 65 24 108 24 44
cal Protein: Js musculus OX=00 Protein Descriptic © VDAC2_MOUSE sp(2603) © ADA286YCR8_M tr[ADA286 © D372TS_MOUSE tr[D37UN8 © D37UN8_MOUSE tr[D37UN8	090 GN=Vd on 10 VDAC2_N 5YCR8 A0A 5 D3YZT5_N 8 D3YUN8_	Same: 4		Prev. AA 1 K 2 K 3 K 4 K 6 K 6 R 7 K 8 M 9 K 1 1 K 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Per SC LTT LTT TK PM WM AE GF	eptide SGVEFSTSGSSNTDT FDTTFSPNTGK FDTTFSPNTGK SCSGVEFSTSGSSNT (IPPPYADLGK VTDNTLGTEIAIEDQI CCVPVCPRPMCIPPF GFGLVKDVK	Next AA G V G V K S D V A C L Y A T W		Length		PTMs 27 Carbanidometh 19 Carbanidometh 13 14 14 12 Carbanidometh 13 Oxidation (M2); 22 Carbanidometh 23 Acetyl (Protein 1 12 11 Carbanidometh	Score A (C2) A (C2) Carb A (C18) I-ter A (C2)	2 5 216.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14 61.21 57.73	tart	Stop 47 47 109 109 45 12 87 2 33 76	Cr 73 65 121 122 65 24 108 24 408 24 44 86
ical Protein: Js musculus OX=10 Protein Descripti Jacob C2_MOUSE sp(2603 Jacob Anology MoUSE (b) 100711 Jacob Dorarts_MOUSE (b) 100711 Jacob Dorarts_MOUSE (b) 1007110	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A 5 D3YZT5_N 8 D3YUN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 6 R 7 K 8 M 9 K 10 K 11 M	Pr SC SC LTT LTT TC PM WW AE GF WW AE	eptide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT FDTTFSPNTGK FDTTFSPNTGKK SCSGVEFSTSGSSNT CIPPPYADLGK VTDNTLGTEIALEDQI GFGLVKLDVK EFGLVRLDVK EFGLVFTFEK CVPVCPR	Next AA G V G V K S D V A C L Y A T W W		Length		PTMs 27 Carbamidometh 19 Carbamidometh 13 21 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 23 Acetyl (Protein 1 12 21 Carbamidometh 10 Acetvl (Protein 1 0 Acetvl (Protein 1	Scon A (C2) A (C2) A (C4) Carb A (C4) I-ter A (C2) I-ter	e S 216.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14 61.21 57.73 55.98	tart	Stop 47 47 109 109 45 12 87 2 33 76 2	Ca 73 65 121 122 65 24 108 24 44 86 11
cal Protein: Js musculus OX=00 Protein Descriptic Jobac2_MOUSE sp(26093 JA023687CR8_M tr1A0288 JO37715_MOUSE tr1D37UN8	090 GN=Vd on 10 VDAC2_M 5YCR8 A0A 5 D3YZT5_V 8 D3YUN8_	Aac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 R 7 K 8 M 9 K 10 K 11 M 12 M	Per So SC LTT LTT TK PM WI AE GF GF	aptide SQUEFSTSGSSNTDT SQUEFSTSGSSNTDT PDTTFSPNTGK PDTTFSPNTGK SSOUFETSGSSNT CIPPPYADLGK UTDVTLGTEATEOQI CCVPV_PRMCIPPF GFGLWLDW EYGLTFTEK CCVPV_PRM. IPPF CVPV_PRM. IPPF	Next AA Gr Y G V K S D V A C C L Y A T W P P Y A		Length		PTMs 27(Carbamidometh 12) 24) 24) 24) 24) 24) 24) 24) 25) 24) 25) 24) 24) 25) 24) 24) 24) 24) 24) 24) 24) 24) 24) 24	Score A (C2) A (C2) Carb A (C4) Carb A (C18) I-ter A (C2) I-ter I-ter	2 S 215.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14 61.21 57.73 55.98 54.31	tart	Stop 47 47 109 109 45 12 87 2 33 76 2 2 2	C 73 65 121 122 65 24 108 24 44 86 11 24
kcel Protein: Js musculus OX=10 Protein Descripti ≩ VDAC2_MOUSE sp(2603 ≩ ADA2367/084 hr HADA324 ≩ D372/15_MOUSE tr[D372/16 ≩ D372/16_MOUSE tr[D371/16	090 GN=Vd on 10[VDAC2_IV 5YCR8[A0A 5]D3YZI75_V 8]D3YUN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 K 6 R 7 K 8 M 9 K 10 K 11 M 12 M 13 K	Pe S⊂ LTT LTT TK P M M M GF GF GF	Epide SGVEFSTSGSSNTDT SGVEFSTSGSSNTDT PDTTFSPNTGK SC SGVEFSTSGSSNT CIPPPYADLGK TICPPYADLGK TICPTFADLGK CCVPV-PRPMCIPPF GFQ.UKUDW EFQCTFTEK CCVPV-PR CCVPV-PR CCVPV-PR-	Next AA GVY G V K S D V A C L T W Y A T W P P Y A L		Length		PTMs 27 Carbamidometh 13 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 23 Acetyl (Protein 1 12 Carbamidometh 10 Acetyl (Protein 1 23 Acetyl (Protein 1 8	Score A (C2) A (C2) Carb A (C4) Carb A (C18) I-ter A (C2) I-ter I-ter	2 S 216.10 138.60 108.67 92.23 82.31 80.75 64.14 61.21 57.73 56.98 54.31 53.99	tart	Stop 47 47 109 109 12 87 2 33 76 2 33	C2 73 65 121 122 65 24 108 24 44 46 11 24 40
ical Protein: Js musculus OX=00 Protein Descripti J VDAC2_MOUSE sp[Q6093 J A023867CR8_M tr1A0A284 J D37/TF_MOUSE tr1D37/LINK	090 GN=Vd on 10[VDAC2_V 5YCR8[A0A 5]D5YCIT5_Y 8]D3YUN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 K 6 R 7 K 8 M 9 K 10 K 11 M 12 M 13 K 14 K	Per SC LTT LTT LTT TC PM WW AE GF GF GF GF GF YK	epide SovEFSTSGSSTDT SovEFSTSGSSTDT PDTTFSPNTGK PDTTFSPNTGK SCSOVEFSTSGSSTDT CVPPVADLGK VTDNTLGTEIAIEDQI CVPV-PRPM_UPPF GFQ.WLDW EYGLTFTEK CVPV-PRPM_UPPF GFQ.WL	Next AA GY G V K S D V A C C L W P Y Y A L W		Length		PTMs 27 Carbamidometh 19 Carbamidometh 13 14 12 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 13 Acetyl (Protein 1 23 Acetyl (Protein 1 8 13 Carbamidometh 13 Carbamidometh	Score Score A (C2) A (C2) A (C2) A (C2) A (C18) I-ter I-ter I-ter I-ter I-ter	2 S 216.10 138.60 108.67 92.23 82.73 82.31 80.75 64.14 61.121 57.73 56.98 54.31 53.99 46.69	itart	Stop 47 47 109 109 2 33 76 2 2 33 76 2 33 76 2 2 3 74	C2 73 65 121 122 65 24 108 24 44 86 11 24 40 86
Ical Protein: Js musculus OX=10 Protein Descripti VDAC2_MOUSE Bol(2603 JADA2567084, HIADA26 JADA2567084, HIADA26 JOSTATS_MOUSE Ir(D3704 JD37045, MOUSE Ir(D3704)	090 GN=Vd on 10[VDAC2_V 5YCR8[A0A 5[D3YZT5_N 8[D3YUN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 R 7 K 8 M 9 K 10 K 11 M 12 M 13 K 14 K 15 R	Pet SC LTT LTT TK PM AE GF WW AE GF GF KK FM	Epide SOVEPSTSGSSNTDT SGVEPSTSGSSNTDT PDTTPSPNTGK PDTTPSPNTGK SCSOVEPSTSGSSNT (PPPPYADLGK TCPPPYADLGK TCPPPYADLGK CVPV-PR CCVPV-PR GFGLWLDW GFGLWL W-EYGLTFTEK W-EYGLTFTEK	Next AA GY GV K S DV A C C C C C C C C C C C C C C C C C C		Length		PTMs PTMs 27 Carbamidometh 19 Carbamidometh 13 21 Carbamidometh 23 Acetyl (Protein 1 20 Acetyl (Protein 1 23 Acetyl (Protein 1 23 Acetyl (Protein 1 36 Carbamidometh 13 Carbamidometh	Score A (C2) A (C2) A (C4) A (C2)	e S 216.10 138.60 108.67 92.23 82.73 82.73 64.14 61.21 57.73 56.99 54.31 53.39 94.69 44.99	itart	Stop 47 47 109 109 12 87 2 33 76 2 33 76 2 33 74	24 23 65 121 122 65 24 108 24 44 44 46 40 86 24
ical Protein: Js musculus OX=10 Protein Descripti Ja DAG2_MOUSE spi26093 Ja DAG2_MOUSE spi26093 Ja Darots_MoUSE tri JD3rtUNA D3rtunka_MOUSE tri JD3rtUNA	090 GN=Vd on 10[VDAC2_N 5YCR8[A0A 5]03YZT5_N 8]D3YUN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 R 7 K 8 M 7 K 8 M 10 K 11 M 12 M 13 K 14 K 15 R 16 R	P4 SS SS LT LT LT TK PM ME GF WW AE GF GF WW AE GF YK GF SN	spide SovEFSTSGSSITDT SovEFSTSGSSITDT SovEFSTSGSSITDT PDTTFSPNTGK FDTTFSPNTGK SC SovEFSTSGSSITU COPPPYADLGK VTDNTLGTEATEOP GFGLVKLUW EYGLTFTEK CCVPU-PR GFGLVK WETVGLTFTEK CCVPU-PR GFGLVK WETVGLTFTEK CUPPYADLGK	Next AA GY G V K S D V A C L C L T W Y A L W W A T		Length Length		PTMs PTMs PTMs PCarbamidometh 13 14 14 Carbamidometh 13 14 Carbamidometh 13 Oxidation (M2); 24 Carbamidometh 12 Carbamidometh 12 Carbamidometh 13 Carbamidometh 13 Carbamidometh 13 Carbamidometh 14 Carbamidometh 15 Carbamidometh 16 Carbamidometh	Score A (C2) A (C2) A (C2) A (C2) A (C18) I-ter I-ter I-ter I-ter A (C2) I-ter I-ter <td>2 S 216.10 133.60 108.67 92.23 82.73 82.73 80.75 64.14 61.21 57.73 56.98 54.31 53.99 46.69 44.99 43.18</td> <td>itart</td> <td>Stop 47 47 109 109 45 12 87 2 33 76 2 33 74 129</td> <td>Ca 65 121 122 65 24 108 24 44 86 11 24 40 86 24 186</td>	2 S 216.10 133.60 108.67 92.23 82.73 82.73 80.75 64.14 61.21 57.73 56.98 54.31 53.99 46.69 44.99 43.18	itart	Stop 47 47 109 109 45 12 87 2 33 76 2 33 74 129	Ca 65 121 122 65 24 108 24 44 86 11 24 40 86 24 186
kcal Protein: s musculus OX=10 Protein Descripti i VDAC2_MOUSE so(2603 i ADA256YCRA to FIADA254 i D3YLTR8_MOUSE triD3YLTR8 i D3YLTR8_MOUSE triD3YLTR8	090 GN=Vd on 010 [VDAC2_V 57CR8]A0A 57CR8]A0A 8]D37UN8_	ac2 PE=1 SV=2		Prev. AA 1 K 2 K 3 K 4 K 6 K 6 K 6 K 6 K 6 K 10 K 10 K 10 K 11 M 11 M 12 M 13 K 16 R 10 R 10 R 10 R 17 R 10 17 R 10 10 17 18 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	A P4 SS SC LTT TTC PM AE GF GF GF YKC AE GF DT DT	ppide SovEFSTSGSSVIDT SovEFSTSGSSVIDT SOVEFSTSGSSVIDT PDTTFSPNTGK PDTTFSPNTGK CPPPYADLGK CPPVPRACLEPQ GFQ.WLDW CVPV.PR CCVPV.PR CVPV.PR GFQ.WLDW CVPV.PR GFQ.WLDW CVPV.PR GFQ.WL W.EYGLTFTEK CVPV.PR GFQ.W W.EYGLTFTEK SovEFSTGVALGK FAVGYR	Next AA GY G V K S D V A C L Y A T T W W P Y A L W W A T T		Length Length		PTMs PTMs 27 Carbamidometh 19 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 23 Acetyl (Protein 1 10 Acetyl (Protein 1 8 13 Carbamidometh 8 3 Carbamidometh 8 13 Carbamidometh 8 17	Score A (C2) A (C2) Carb A (C4) A (C5) I-ter A (C2) I-ter A (C3)	s S 216.10 138.60 108.67 92.23 82.73 82.73 80.75 64.14 57.73 56.99 54.31 53.99 46.69 44.99 43.18 34.39	tart	Stop 47 47 109 45 12 87 2 33 76 2 33 74 12 13 76 2 33 74 12 13 74 12 13 2 33 74 12 13 73 74 12 13 73 73	Ca 73 65 121 122 65 24 108 24 44 486 11 1 24 40 86 24 1386 24 1386 24
akal Protein: s musculus OK=10 Protein Descripti. la VDAC2_MOUSE mo(2005 a ADA256YCRE mo(2005 a D37CH3_MOUSE trip37CH4 b D37CH3_MOUSE trip37CH4	090 GN=Vd on 10 [VDAC2_V SYCR8]A0A 5)CR8]A0A 8]D3YUN8_	ac2 PE=1 SV=2		Prev. AA 2 K 2 K 4 K 6 K 6 K 7 K 8 M 9 K 10 K 11 M 12 M 13 K 14 K 15 R 17 R	P P S SC LTT LTT TTT PM AE GF GF GF YTC AE GF OID	ppide SovEFSTSGSSVIDT SovEFSTSGSSVIDT SovFSTSGSSVIDT DITTFSPNTGK DDTTFSPNTGK DDTTFSPNTGK SCSVEFSTSGSSVIT (DPPYADLGK UTDNTLGTEALEDQU GFGLVALDW CVVV.PR CCVVV.PR GFGLVK CVVV.PR GFGLVK UV.EYGLTFTEK CVPV.PR GFGLVK FAVGYR FAVGYR FAVGYR	Next AA GY G K S D X A C L W P Y A T W P Y A T T T T		Length		PTMs 27 Carbamidometh 19 Carbamidometh 13 21 Carbamidometh 13 Oxidation (M2); 22 Carbamidometh 13 Oxidation (M2); 23 Acetyl (Protein 1 10 Acetyl (Protein 1 10 Carbamidometh 13 Carbamidometh 8 3 Carbamidometh 8	Score A (C2) A (C2) A (C2) A (C4) A (C18) I-ter A (C2) I-ter A (C2) A (C2) A (C2) A (C2) I-ter I-ter A (C2)	2 5 216.10 138.60 108.67 92.23 82.31 80.75 64.14 61.21 57.73 56.98 54.31 53.99 46.69 44.99 43.18 34.39	itart	Stop 47 47 109 109 12 87 33 76 2 23 33 76 2 12 33 76 2 23 33 74 12 12 2 28 28	Ca 73 65 121 122 65 24 108 24 44 86 11 11 24 40 86 24 186 24 186 44

Upper View: list of all Peptides with best PSM information (charge, score ...)



Middle View: list of all Protein Sets identified by the selected peptide. Bottom Left View: list of all Proteins of the selected Protein Set Bottom Right View: list of all Peptides of the selected Protein Note: Abbreviations used are listed here

Protein Sets Window

If you click on Protein Sets sub-menu, you obtain this window:



View 1 (upper): list of all Protein Sets of the identification Summary

Note: In the column *Proteins*, 8 (2, 6) means that there are 8 proteins in the protein set : 2 in the sameset, 6 in the subset.

View 2: list of all Proteins of the selected Protein Set, sameset or subset.

View 3: list of all Peptides of the selected Protein. If a subset is selected only peptides matching that protein will be listed.

View 4a: Protein Sequence of the previously selected Protein and Spectrum of the selected Peptide. Other tabs display Spectrum, Spectrum Error and Fragmentation Table.

View 4b: Graphic representation of the Protein with matching peptide and associated modifications.

Note: Abbreviations used are listed here



Adjacency Matrix Window

If you click on Adjacency Matrix sub-menu, you obtain this window:



View 1: All the matrices. Each matrix corresponds to a cluster composed of linked Proteins/Peptides.

Note: use the Search tool to display an Adjacency Matrix for a particular Protein or Peptide

View 2: The currently selected matrix.

In the example, you can see two different protein sets which share only two peptides.

Thanks to the settings you can hide proteins with exactly the same peptides.



Display Modification Site or Cluster of a Modification Dataset

Functionality Access

To display modification data associated to an Identification Summary:

- right click on the Dataset
- click on the Display Modification > "Sites"; "Clusters"; "Annotated Clusters"

_	1 mm 3	2/ 1	NPCA_HUMA
	Display Search Result	>	TF3C2_HUM
			RGS12_HUM
	Display identification Summary		DD4D.2 HUM
	Display Modifications	>	Sites 4
	Add Dataset		Clusters 0
	Add Identification Folder		Annotated Clusters
	Conv Search Result		RN126_HUM
	copy search result		OTUD3_HUM
	Paste Search Result		DOCK6_HUM
			DOCK6_HUM
	Rename	~	DOCK6_HUM
	Clear Validation		DOCK6_HUM
	Clear All		00000 1100

If you click on one of "Display Modifications" sub-menu, you can obtain the following warning dialog.

This is due to the fact that you must run beforehand the "Identify Modification Sites" process. To do that, mouse right click on your Identification Summary and select the "Identify Modification Sites" menu.

PTM Cluster Loading Error	Х
To display Modification Sites or Modification Clusters, you must run "Identify Modification Sites" beforeha	ind.
ОК	

The Annotated Clusters menu may also cause the display of this warning. Indeed, Annotated Clusters display is only available after Clusters have been modified and saved.

All displays, Sites and Clusters, are structured in the same way. In Sites windows, the upper view will list all individual Sites while in Clusters windows, Sites will be clustered using rules specified by user (see <u>Identify Modification Sites</u>)

Clusters/Sites Windows



₽	Proline Stu	dio 2.2.0	Milestone	2022-04-2
File	Window	Heln		

	Id	d Status	Protein	Peptide	PTMs			PTMs Confid. (MD	Score		Peptide count	Site count	Sites Loc.	Sites C PT	M D.Mass	Spectrum title	2
	1	2 007 👛	B8YE68_HCMVA	LFGSSADEDDDDDDDDEKNIF I PIK	Phospho (54)			93.91	%	128.01		0	1 426	100.00	/9.9663	13780: Scan 1	l
	2	2 008 🖒	B8YE68_HCMVA	RLFGSSADEDDDDDDDDEKNIFTPIK	Phospho (S6)			95.85	%	160.41		5 1	1 427	100.00	79.9663	12158: Scan 1	l
	3	175 🖒	B8YE61_HCMVA	MEAGLSPYSVSSDAPSSFELVR	Oxidation (M1); Phospho (Sé	6)	99.97	%	126.19		4 1	1 59	99.97	95.9612	15373: Scan 1	L
	4	191 🖒	B8YE61_HCMVA	MEAGLSPYSVSSDAPSSFELVR	Oxidation (M1); Phospho (S	17)	90.74	%	66.71		4 1	1 70	91.80	95.9612	14624: Scan 1	l
	5	222 🖒	B8YE61_HCMVA	AFMEANGNHPEQICRSPPPPLPPR	Carbamidome	thyl (C14); Pho	ospho (100.00	%	74.87		1 :	1 625	100.00	136.9878	5850: Scan 76	j
	6	1 026 🖒	RRAS2_HUMAN	KFQEQECPPSPEPTR	Carbamidome	thyl (C7); Phos	spho (S	99.95	%	100.04		4 1	1 186	99.95	136.9878	1132: Scan 29)
	7	1 405 🖒	B8YEA6_HCMVA	RRDSPGGMDEPPSGWER	Phospho (S4)	Oxidation (M	8)	100.00	%	50.44		4 1	1 139	100.00	95.9612	1513: Scan 32	2
	8	1 484 🖒	B8YEA8_HCMVA	QDALPGPCIASTPK	Carbamidome	thyl (C8); Phos	spho (S	93.04	%	65.35		1 1	1 554	93.04	136.9878	5811: Scan 76	i
	9	1 485 🖒	B8YEA8_HCMVA	HRQDALPGPCIASTPKK	Carbamidome	thyl (C10); Pho	ospho (66.87	%	43.73		4 1	1 555	66.87	136.9878	1234: Scan 30)
1	10	1 546 🖒	PP71_HCMVA	SQASSSPGEGPSSEAAAISEAEAASGSFGR	Acetyl (Protei	n N-term); Pho	ospho (75.30	%	116.67		4 2	2 5;6	100.00;	201.9432	18865: Scan 2	2
1	11	1 549 🖒	PP71_HCMVA	SQASSSPGEGPSSEAAAISEAEAASGSFGR	Acetyl (Protei	n N-term); Pho	ospho (43.95	%	120.23		4 2	2 6;7	100.00;	201.9432	18942: Scan 2	2
1	12	1 552 🖒	PP71_HCMVA	SQASSSPGEGPSSEAAAISEAEAASGSFGR	Acetyl (Protei	n N-term); Pho	ospho (36.48	%	112.73		4 2	2 7;13	100.00;	201.9432	18572: Scan 2	2
1	13	1 554 🧀	PP71_HCMVA	SQASSSPGEGPSSEAAAISEAEAASGSFGR	Phospho (S12); Phospho (SI	13)	44.95	%	92.20		4 2	2 13;14	100.00;	159.9327	16807: Scan 1	
1	14	2 087 🖒	B8YE68_HCMVA	LSSPMTTTSTSQKPVLGK	Phospho (S2)	Oxidation (M	5)	50.00	%	98.02		4 1	1 764	50.00	95.9612	1262: Scan 31	-
																	l
1	15	2 088 🖒	B8YE68_HCMVA	LSSPMTTTSTSQKPVLGK	Phospho (S3)			81.32	%	84.74		4 1	1 765	81.32	79.9663	2196: Scan 41	l
1 1 Pł	15 18 hospho	2 088 🖒 2 130 🖒 no:643 Multiple S	B8YE68_HCMVA B8YE68_HCMVA ites: 165	LSSPMTTTSTSQKPVLGK RSTGTAAVGSPVKSTTGMK	Phospho (S3) Phospho (S10)		81.32 99.96	%	84.74 78.24		4 1 4 1	1 765 1 991	81.32 99.96	79.9663 79.9663	2196: Scan 41 980: Scan 280	1
Ph te P	15 16 hospho	2 088 👍 2 130 🖒 no:643 Multiple S es Site PSMs	B8YE68_HCMVA B8YE68_HCMVA ites: 165	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PVKSTTGMK	Phospho (S3) Phospho (S10)		81.32 99.96	%	84.74 78.24		4 1 4 J	1 765 1 991	81.32 99.96	79.9663 79.9663	2196: Scan 41 980: Scan 280	1
Př te P	nospha	2 088 🖒 2 130 no:643 Multiple S es Site PSMs	B8YE68_HCMVA B8YE68_HCMVA ites: 165	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PVKSTTGMK	Phospho (S3) Phospho (S10) Peptide	Score E	81.32 99.96 Exp Charge	% 8	84.74 78.24 PTMs F	PTMs C Spe	tt Acety	1 765 1 991 /(Protein N	81.32 99.96 Phospho(S):	79.9663 79.9663 Phospho(S	2196: Scan 41 980: Scan 280	1 1)
Př te P	16 18 rospho	2 088 2 130 10:643 Multiple S es Site PSMs MSQASSSP	BBYE68_HCMVA BBYE68_HCMVA ites: 165 GEGP SSEAAA	LS:PMTTTSTSQPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S10) Peptide 1 SQASSSP	Score E 92.20 S	81.32 99.96 Exp Charge 381.3 3	% % PTMs Phosp	84.74 78.24 PTMs F 159.9	PTMs C Spe 44.95 168	4 1 4 2 ct Acety	1 765 1 991	81.32 99.96 Phospho(S) 58	79.9663 79.9663 Phospho(S 3.71	2196: Scan 41 980: Scan 280	1 1) E
Pr te P	16 16 reptide	2 088 2 130 10	BBYE68_HCMVA BBYE68_HCMVA ites:165 GEGPSSEAAA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PVKSTTGMK	Phospho (S3) Phospho (S10	Peptide 1 SQASSSP 2 MSQASSS	Score E 92.20 9 86.01 1	81.32 99.96 Exp Charge 381.3 3 1030 3	% % PTMs Phosp Oxidat	84.74 78.24 PTMs F 159.9 175.9	PTMs C Spa 44.95 20.00 169	4 1 4 7 ct Acety 77:	1 765 1 991	81.32 99.96 Phospho(S) 58 40	79.9663 79.9663 Phospho(S 3.71 - 0.00 :	2196: Scan 41 980: Scan 280 5) Prob 44.95 20.00	1 1) E
Pr te P	reptide	2 088 2 130 to:643 Multiple S es Site PSMs MSQAS S S P	BBYE68_HCMVA BBYE68_HCMVA ites: 165	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S10	Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSS	Score E 92.20 5 86.01 1 74.78 1	81.32 99.96 Exp Charge 381.3 3 1030 3 1025 3	% % PTMs Phosp Oxidat Phosp	84.74 78.24 PTMs F 159.9 175.9 159.9	PTMs C Spr 44.95 20.00 19.99 167	4 1 4 1 7 7 7 7 7 7 7	1 765 1 991	81.32 99.96 Phospho(5) 58 40 39	79.9663 79.9663 Phospho(S 3.71 0.00	2196: Scan 41 980: Scan 280 ;) Prob 44.95 20.00 20.03	1 1) [
Př	reptide	2 088 1 10 10 10 10 10 10 10 10 10 10 10 10 1	BBYE68_HCMVA BBYE68_HCMVA ites:165 GEGPSSEAAA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK USEAEAASGSFGRLI	Phospho (S3) Phospho (S10	Peptide SQASSSP 2 MSQASSS 3 MSQASSS 4 SQASSSP	Score 5 92.20 5 86.01 1 74.78 1 100.78 5	81.32 99.96 5xp Charge 981.3 3 1030 3 395.4 3	% % PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs F 159.9 175.9 159.9 201.9	PTMs C Spa 44.95 168 20.00 169 19.99 167 31.21 170	4 1 4 1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(S) 56 40 39 0 62	79.9663 79.9663 Phospho(S 3.71 0.00 3.99 2.42	2196: Scan 41 980: Scan 280 ;) Prob 44.95 20.00 20.03 31.21	1 1)
Pr te P	reptide	2 088 6 2 130 6 no:643 Multiple S Site PSMs	B8YE68_HCMVA B8YE68_HCMVA ites: 165 GEGP S SEARA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PVKSTTGMK	Phospho (S3) Phospho (S10 TNVE	Peptide 1 <u>SQASSSP</u> 2 MSQASSS 3 MSQASSS 4 SQASSSPi	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 5xp Charge 981.3 3 1030 3 1025 3 395.4 3	% % PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs F 159.9 175.9 159.9 201.9	PTMs C Sp 44.95 168 20.00 169 19.99 167 31.21 170	t Acety	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(5) 58 40 39 0 62	79.9663 79.9663 Phospho(S 3.71 0.00 9.99 2.42	2196: Scan 41 980: Scan 280 5) Prob 44.95 20.00 20.03 31.21	
Př te P	reptide	2 088 6 2 130 6 no:643 Multiple S Site PSMs MSQASSSP	B8YE68_HCMVA B8YE68_HCMVA ites:165 GEGPSSEAAA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S10	Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSS 4 SQASSSP	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 381.5 Charge 381.5 3 1025 3 31025 3 3195.4 3	% % PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 159.9 175.9 199.9 201.9	PTMs C Spa 44.95 168 20.00 169 19.99 167 31.21 170	4 1 4 2 77 Acety 77 74 74	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(S) 58 40 39 0 62	79.9663 79.9663 79.9663 Phospho(S 3.71 0.00 2.42	2196: Scan 41 980: Scan 280 5) Prob 44.95 20.00 20.03 31.21	1
Př	reptide	2 088 6 2 130 6 130 7 130 6 130 7 130 7 100 7	B8YE68 HCMVA B8YE68 HCMVA Ites: 155 GEGPSSEAAA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK ISEAEAASGSFGRLI	Phospho (S3) Phospho (S10 TNVE	Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSS 4 SQASSSP	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 281.3 281.3 31030 3 395.4 3	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs F 159.9 175.9 159.9 201.9	PTMs C Spr 44,95 168 20.00 169 19.99 167 31.21 170	4 1 4 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(S) 58 40 39 0 62	79.9663 79.9663 Phospho(S 3.71 0.00 2.42	2196: Scan 41 980: Scan 280) Prob 44.95 20.00 20.03 31.21	
Pr te P	reptide	2 088 6 2 130 6 no:643 Multiple S es Site PSMs MSQASSSP	BYTES JCMVA BYTES JCMVA Https://doi.org/10.1000/100000000000000000000000000000	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S1C TNVE	Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSS 4 SQASSSP	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 381.3 Charge 381.3 3 1030 3 1025 3 395.4 3	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 159.9 175.9 175.9 201.9	PTMs C Spr 44.95 168 20.00 169 19.99 167 31.21 170	4 1 4 2 77 27 27 54	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(5) 58 40 39 0 62	79.9663 79.9663 79.9663 79.9663 8.71 9.99 2.42	2196: Scan 41 980: Scan 280) Prob 44.95 20.00 20.03 31.21	
Pr te P	rospho	2 088 6 2 130 6 130 7 130 6 130 7 130 7 100 7	BRYESS HCMVA BRYESS HCMVA Ites: 165 GEGP SE ARA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK ISE AE AASG SFGRLI	Phospho (S3) Phospho (S1C	Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSS 4 SQASSSP	Score E 92.20 86.01 100.78 9 100.78	81.32 99.96 361.3 Charge 361.3 3 1030 3 305.4 3 3	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs 1 159.9 199.9 201.9	2TMs C Sp 44.95 168 20.00 169 19.99 167 31.21 170	4 1 4 2 77 Acety 77 54 54	1 765 1 991	81.32 99.96 Phospho(S) 58 40 39 0 62	79.9663 79.9663 79.9663 Phospho(S 3.71 3.00 2.42 2.42	2196: Scan 41 980: Scan 280 5) Prob 44.95 20.00 20.03 31.21	
te P	eptide	2 088 6 2 130 6 no:643 Multiple S es Site PSMs	B8YE68_HCWVA B8YE68_HCWVA Ites: 155 GEGPS SEARA	LS:PMTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S10 TINVE) Peptide 1 SQASSEP 2 %SQASSS 4 SQASSSP	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 2011 - 2012	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs I 159.9 175.9 199.9 201.9	PTTMs C Spr 44,95168 20.00 169 19.99 167 31.21 170	4 1 4 2 2 2 2 7 2 7 2 7 2 7 2 7 2 7 	1 765 1 991 /(Protein N 100.0	81.32 99.96 Phospho(S) 55 40 39 0 62	79.9663 79.9663 79.9663 Phospho(S 3.71 0.00 3.99 2.42	2196: Scan 41 980: Scan 280 980: Scan 280 980: Scan 280 980: Scan 280 980: Scan 280 980: Scan 41 980: Scan 280 980: Scan	
te P	reptide	2 088 6 2 130 6 no:643 Multiple S es Site PSMs	BBYEGS JHCMVA BBYEGS JHCMVA Ittes: 165 GEGPSSEARA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK	Phospho (S3) Phospho (S10 TINVE) Peptide I SQASSSPI 2 VSQASSS 3 MSQASSS 4 SQASSSPI 4 SQASSSPI	Score E 92.20 86.01 74.78 100.78 9	81.32 99.96 381.3 3030 3 395.4 3	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs F 159.9 175.9 199.9 201.9	PTMs C Sp 44.95 163 20.00 169 19.99 167 31.21 170	t 1 t 2 t 4 t 2 t 4 t 2 t 4 t 2 t 4 t 2 t 4 t 2 t 4 t 4 t 2 t 4 t 4 t 4 t 4 t 4 t 4 t 4 t 4	1 765 1 991 4(Protein N 100.0	81.32 99.96 Phospho(5) 50 40 93 90 62	79.9663 79.9663 Phospho(5 3.72	2196: Scan 41 980: Scan 280 980: Scan 280 980: Scan 280 980: Scan 280 980: Scan 41 980: Scan 280 980: Scan 280	
te P	reptide	2 088 6 2 130 6 no:643 Multiple S es Site PSMs MSQAS SSP	B8YE68 HCMVA B8YE68 HCMVA Ites: 155 GEGPSSEARA	LS:PMTTTSTSQRPVLGK RSTGTAAVG:PWSTTGMK ISE AE AASG SFGRLI S4 amino acid (calculated <= protein length)	Phospho (S3) Phospho (S11 TINVE) Peptide 1 SQASSSP 2 MSQASSS 3 MSQASSSP 4 SQASSSP	Score E 92.20 9 86.01 1 74.78 1 100.78 9	81.32 99.96 2011 - 2012	PTMs Phosp Oxidat Phosp Acetyl	84.74 78.24 PTMs 1 159.9 175.9 201.9	2TMs C Sp 44,95 163 20.00 169 19.99 167 31.21 170	4 1 4 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	1 765 1 991 4(Protein N 100.0	81.32 99.96 Phospho(S) 65 40 39 0 62	79.9663 79.9663 Phospho(S 371 0 0.00 1 3.99 1 3.99 1 4.42 1	2196: Scan 41 980: Scan 280 980: Scan 280 20.00 20.03 31.21	

Upper View: This view lists all Modification Sites or Clusters. In this example, a Cluster view is shown. For each of them, many information is given : the number of Peptides belonging to the Cluster, how many sites have been clustered, The Confidence for the site combination, ...

Bottom Left view: Graphic illustration of the cluster/site peptides mapped to the protein sequence. All modification locations are represented above the protein sequence. An overview graphic is also given below.

Bottom Right view: Cluster/Site peptides list. The PSMs list is accessible in a hidden tab.

Display Additional Information Search on **Result/Identification Summary**

Functionality Access

To display properties of a Search Result or Identification Summary:

- right click on a Search Result/Identification Summary •
- click on the menu "Properties" •

Note: it is possible to select multiple Search Results/Identification Summaries to compare the values.

Proline_Project	~	
Identifications Identification		
	Display Search Result	>
··· () Repliac	Display Identification Summary	>
-	Add Dataset	
	Add Identification Folder	
	Copy Search Result	
	Paste Search Result	
	Rename	>
	Clear	
	Delete	
	Import Search Result	
	Import MaxQuant Result	
	Validate Search Result	
	Merge Datasets	>
	Filter ProteinSets	
	Change Typical Protein	
	Identify Modification Sites	
	Generate Spectrum Matches	
	Update Spectrum using Peaklist software	
	Retrieve Protein Sequences	
	Quantify	>
	Export	>
		-



Properties Window

Property window opened:

General Information: Various information on the analysis (instrument name, peaklist software...)
Search Properties: Information extracted from the Result File (date, software version, search settings...)
Search Result Information: Amount of Queries, PSM and Proteins in the Search Result.
Identification Summary Information: Information obtained after validation process
Validation xxx: Information on validation process : parameters used to validate and result
Sql Ids: Database ids related to this item

<u>Note:</u> Identification Summary Number may differ from Validation Results. Indeed, on one hand, peptide matches count in Validation Results takes into account all PSMs that have been validated. On the other hand, the PSM Number in the "Identification Summary Information" section considers only PSMs that identify a valid Protein Sets.



Pro Pro Pasta Term	Logs	;	× F083067 Properties ×		
Coord Information Coord Information Image: Coord Information International State International International State International Internatinterea International International International Inter	ň		Group	Туре	F083067
Book Market Program Description Description <thdescription< th=""> <thdescription< th=""> <th< td=""><td>T SD</td><td>1</td><td>General Information</td><td>Daw File Name</td><td></td></th<></thdescription<></thdescription<>	T SD	1	General Information	Daw File Name	
Bit Secon Resultises Game Levis UP 31 00P 412110, or Game Levis UP 31 00P 412100P 412110, or Game Levis UP 31 00P 412110, or Game Lev	~	2		Fasta Files	Accal/mascot-2 4//sequence/LIPS1LIPS2 D/current/LIPS1LIPS2 D
Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: constraint table Image: con		4		Search Result Name	Gamme Levure LIPS1 OEMMA121101 40
Image: start in the set in the s	0	5		Instrument Name	ESI-TRAP
Target Doxy Mede CORCHIVED Search Reperted Correct House Search Rese Result File time Search Rese Correct House Search Rese Search Rese Search Res Search Res Search Res Res Search Res Res Search Res Res Search Res Res	-	6		Fragmentation Rule Set	ESI-TRAP
Beach Properties Read File Name Extend Properties Sector Properties Read File Name File Name Sector Properties Sector Name File Name Sector Name Sector Name File Name Sector Name Sector Name Maximum Sector Name Maximum File Name Sector Name Maximum Sector Name Sector Name File Name Sector Name Sector Name Sector Name		7		Target Decoy Mode	CONCATENATED
Search Pagestes Result File Name Constraints 0 Sarch Table 22 septemice 215 1 Sarch Table 22 septemice 215 1 Sarch Table 23 septemice 215 1 Sarch Table 23 septemice 215 1 Sarch Table 23 septemice 215 1 Sarch Table 33 septemice 215 1 Sarch Table Call Mark Mass Charge 22 septemice 215 1 Sarch Table Sarce Sarch Table Sarce 33 septemice 215 2 Sarch Table Sarce 34 septemice 215 34 septemice 215 2 Sarch Table Sarce 35 septemice 215 34 septemice 215 2 Sarch Table Sarce Table Sarce 35 septemice 215 35 septemice 215 2 Sarce Table Sarce Table Sarce Sa		8		Peaklist Software	extract_msn.exe
Image: Second Tele Name Field and Celebrate Name Field and Celebrate Name Image: Second Name Name Software Name Name Image: Second Name Name Software Name Name Image: Second Name Name Software Name Image: Second Name Software Name Name Image: Second Name Software Name Software Name Image: Second Name Software Name		9	Search Properties		
Image: Search State 22 septimize 305 Image: Search State 36 Thorave Yearson 3.5.1 Image: Search State 3.5.1 3.5.1 Image: Search State 3.5.1 3.5.1 Image: Search State 3.5.1 3.5.1 Image: Search State 3.5.2 3.5.2		10		Result File Name	F083067.dat
Image: Software Name Material Image: Software Name Software Name Image: Software Name Software Name Image: Software Name Name Image: Software Name Software Name		11		Search Date	22 septembre 2015
Software version 2.5.1.1 Image: Software version 2.5.1.1 Image: Software version Trypending Image: Software version Carbonic Software version		12		Software Name	Mascot
Image: Transmit Transmit Transmit Image: Image: Transmit Transmit Image: Image: Charanistomer: Orbanistomer: Image: Image: Acetyl(Protein Netm), Constraint, Orbanistomer: Orbanistomer: Image: Image: Acetyl(Protein Netm), Constraint, Orbanistomer: Orbanistomer: Image: Image: Image: Orbanistomer: Orbanistomer: Image: Image: Image: Orbanistomer: Orbanistomer: <td></td> <td>13</td> <td></td> <td>Software Version</td> <td>2.5.1</td>		13		Software Version	2.5.1
Image: International Control of		14		Taxonomy	All entries
Image: Constraint of the		15		Enzyme	Irypsin/P
Indel Hostitation Control Image: Control of the statistical of the sta		16		Max Missed Cilvage	2 Contractification (C)
Image: Constraint Note Stress Activity Order Verling, C. 03, 01 Product Name Stress 2 + ext 3 + Product Name Stress <td< td=""><td></td><td>17</td><td></td><td>Variable Medifications</td><td>Carbandometry(C)</td></td<>		17		Variable Medifications	Carbandometry(C)
Image: Constraint of the second of		10		Fragment Mass Tolerance	Acetyl(Protein N-terni), Oxidation(N)
Paptide Mass Error Tolerance 2,50 pm Search Result Information Queries Market 2,2 and 3,6 Search Result Information Queries Market 3,80 pm Search Result Information Queries Market 3,80 pm Search Result Information Queries Market 3,80 pm PMI Namber 3,80 pm 3,80 pm <t< td=""><td></td><td>20</td><td></td><td>Pentide Charge States</td><td>2+ and 3+</td></t<>		20		Pentide Charge States	2+ and 3+
Image: Constraint of the set of		21		Pentide Mass Error Tolerance	5.0 ppm
A Fragment Mass Error Tolerance 0.0 ED Search Read Enformation Queries Number 7846 A PSM Number 7847 A PSM Number 7847 A PSM Number 6030 B PSM Number 6030 B PSM Number 6030 B PSM Decay Number 6030 B PSM Decay Number 5664 B PSM Number 9496 B PSM Number 9496 B PSM Number 9496 B Poten Sets Decay Number 0 PSM Number 9496 9496 B Peptode Decay Number 0 B		22		Fragment Charge States	2+ and 3+
Active Search Result Information Queries Number 378 /6 2 PSM Number 4221 2 Protein Number 6030 2 PSM Number 4221 2 Protein Number 6030 2 Protein Decoy Number 2506 3 Protein Sets Number 949 3 Protein Sets Decoy Number 2225 4 Protein Sets Decoy Number 2255 5 Protein Sets Decoy Number 0 6 Protein Sets Decoy Number 0 7 Protein Sets Decoy Number 0 8 Protein Sets Decoy Number 0 8 Protein Sets Decoy Number 0 9 Pagida Mumber 0 9 Peptide Sets Sets Sets Sets Sets Sets Sets Set		23		Fragment Mass Error Tolerance	0.8 Da
second 0 0 0 0 0 Second 0 0 <td< td=""><td></td><td>24</td><td>Search Result Information</td><td></td><td></td></td<>		24	Search Result Information		
Set Number -		25		Queries Number	37816
Poten Number 06030 1 PSM Ecory Number 25066 1 Protein Decory Number 5664 1 Protein Decory Number 9489 2 Protein Sets Number 9489 3 Protein Sets Number 9489 4 Protein Sets Number 9489 5 Protein Sets Number 9489 6 Protein Sets Decory Number 0 7 Protein Sets Decory Number 0 8 Protein Sets Decory Number 0 9 Protein Sets Protein 0 9 <t< td=""><td></td><td>26</td><td></td><td>PSM Number</td><td>42271</td></t<>		26		PSM Number	42271
B Member 25006 Ndentification Protein Secto Number 5664 Ndentification Protein Secto Number 988 A Protein Secto Number 988 B Protein Secto Number 988 B Protein Secto Number 0 B Protein Secto		27		Protein Number	6030
Image: Section Service of Control of Control Decory Number <		28		PSM Decoy Number	25006
Identification Summary Information Protein Sets Number 994 Image: Imag		29		Protein Decoy Number	5664
1 Protein Sets Number 949 2 Chech Sets Number 9493 3 Chech Sets Number 2225 3 Check Sets Number 2225 4 Check Sets Number 0 5 Check Sets Number 0 6 Check Sets Number 0 7 Check Sets Number 0 8 Check Sets Number 0 9 Check Sets Number 0 1 Peptide Number Namber Nam		30	Identification Summary Information		
3		31		Protein Sets Number	494
3		32		PSM Number	9489
a Check Protein Sets Decoy Number 0 a Check Number 0 b Peptide Decoy Number 0 c Peptide Decoy Number 0 c Check Number 0 c Peptide Ecoy Number 0 c Peptide Sets Decoy Decode Sets Decoy Number 0 c Peptide Sets Decoge Decode Sets Decode Sets Decoge Decode Sets Decoge Decode Sets Decode Decode Sets Decode Decode Sets Decode Decode Decode Decode Sets Decode Decode Decode Decode Dec		33		Peptide Number	2825
image:		34		Protein Sets Decoy Number	0
image: source of the section of th		35		PSM Decoy Number	0
Bit Control Bit Control Control Bit Control Control Control Bit Contrel Control Contrel <td></td> <td>36</td> <td></td> <td>Peptide Decoy Number</td> <td>U</td>		36		Peptide Decoy Number	U
Validation Parameters Deptide Validation Parameters peptide_filters#1 / description peptide math rank filter Peptide_filters#1 / properties / threshold_value PRETTY_RANK Peptide_filters#2 / properties / threshold_value 1 Peptide_filters#2 / properties / threshold_value 1 Peptide_filters#2 / properties / threshold_value 25.0 Protein_filters / properties / threshold_value 25.0 Protein_filters / properties / threshold_value 25.0 Peptide_filters#2 / properties / threshold_value 25.0 Protein_filters / properties / threshold_value 27 Valdation Results peptide_results / decov_matches_count 60 Peptide_results / target_matches_count 00 Protein_results / target_matches_count 00 Protein_results / target_matches_count 01 Protein_results / target_matches_count 11 Protein_results / target_matches_count 121 </td <td></td> <td>37</td> <td></td> <td>regult summary ptm dataset</td> <td>defined</td>		37		regult summary ptm dataset	defined
Induction autometer peptide_filters#1 / description peptide match rank filter 4		30	Validation Parameters	result_summary.pun_uataset	denned
4 peptide_filters#1 / parameter PPETTY_RAKK 4		40		peptide_filters#1/description	peptide match rank filter
42 peptide_filters#1 / properties / threshold_value 1 43		41		peptide_filters#1/parameter	PRETTY RANK
43		42		peptide filters#1/properties/threshold value	1
44 peptide_filters#2 / parameter SCORE 46		43		peptide filters#2 / description	peptide match score filter
46 peptide_filters#2 / properties / threshold_value 25.0 47 Control of threshold_value Peptide_filters / protein_filters / prometer Peptide_count 48 motion_filters / properties / threshold_value PEP_COUNT 49 Validation Results motion_filters / prometer Peptide_count 40 Validation Results motion_filters / prometer Peptide_count 40 Validation Results motion_filters / prometer motion_count 41 O Peptide_results / decoy_matches_count 00 42 O protein_results / farget_matches_count 00 43 option_results / farget_matches_count 00 44 option_results / target_matches_count 00 45 option_results / farget_matches_count 00 46 option_results / target_matches_count 00 47 option_results / target_matches_count 00 48 option_results / target_matches_count 00 494 Option_results / target_matches_cou		44		peptide_filters#2 / parameter	SCORE
40 protein_filters / description protein set filter on peptide count 41 protein_filters / properties / praemeter PEP_COUNT 42 Validation Results 2 44 Validation Results 0 45 Peptide_results / decoy_matches_count 0 46 Peptide_results / decoy_matches_count 0 47 Peptide_results / decoy_matches_count 0 48 Opeptide_results / target_matches_count 0 49 Opeptide_results / target_matches_count 0 40 Opeptide_results / target_matches_count 0 41 Opeptide_results / target_matches_count 0 41 Opeptide_results / target_matches_count 0 42 Opeptide_results / target_matches_count 0 44 Opeptide_results / target_matches_count 0 44 Opeptide_results / target_matches_count 0 45 Opeptide_results / target_matches_count 0 46 Opeptide_results / target_matches_count 1 47 Opeptide_results / target_matches_count 1 48 Opeptide_results / target_matches_count 1 494 Opeptide_results / target_matches_count 1 495 Opeptide_results / target_matches_count		45		peptide_filters#2 / properties / threshold_value	25.0
47 COUNT 46 protein_filters / parameter 47 protein_filters / properties / threshold_value 49 Validation Results 40 Validation Results 41 Peptide_results / decoy_matches_count 42 Validation Results 43 Peptide_results / decoy_matches_count 44 Peptide_results / decoy_matches_count 45 Peptide_results / decoy_matches_count 46 Protein_results / decoy_matches_count 47 Protein_results / decoy_matches_count 48 Protein_results / decoy_matches_count 49 Protein_results / decoy_matches_count 49 Protein_results / decoy_matches_count 40 Protein_results / decoy_matches_count 40 Protein_results / decoy_matches_count 41 Protein_results / decoy_matches_count 42 Protein_results / decoy_matches_count 43 Protein_results / decoy_matches_count 44 Protein_results / decoy_matches_count 45 Protein_results / decoy_matches_count 46 Protein_results / decoy_matches_count		46		protein_filters / description	protein set filter on peptide count
40 protein_filters / properties / threshold_value 2 40 Validation Results 6 6 6 6 6 6 6 6 6 6 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1<		47		protein_filters / parameter	PEP_COUNT
Validation Results peptide_results / decoy_matches_count 60 60		48		protein_filters / properties / threshold_value	2
60		49	Validation Results		
61 peptide_results / tdr 1.1553381 62		50		peptide_results / decoy_matches_count	60
02 Construits / target_matches_count 999 03 Protein_results / target_matches_count 00 04 Construction_results / target_matches_count 00 05 Protein_results / target_matches_count 00 06 Sigl Ids 00 07 Option_results / target_matches_count 01 08 Project id 11 09 Option_results / target_matches_count 00 00 Option_results / target_matches_count 01 01 Option_results / target_matches_count 01 02 Option_results / target_matches_count 01 03 Option_results / target_matches_count 01 04 Option_results / target_matches_count 01 05 Option_results / target_matches_count 01 06 Option_results / target_matches_count 01 07 Option_results / target_matches_count 01 08 Option_results / target_matches_count 01 09 Option_results / target_matches_count 01 01 Op		51		peptide_results / fdr	1.1953381
03 0 0 0 64 0 0 0 65 0 protein_results / far 0.0 66 0 protein_results / far 0.0 67 0 0 0 68 0 Project id 10 69 0 0 121 60 0 0 121 61 0 0 35 62 0 0 35 63 0 0 10 64 0 0 14 65 0 0 14 66 0 0 14 67 0 0 14 68 0 0 14 69 0 0 14 60 0 0 14 61 0 0 14		02		pepude_results / target_matches_count	9979
Direction_results / rui 0.01 66		03		protein_results / decoy_matches_count	0
Book Book <th< td=""><td></td><td>54</td><td></td><td>protein_results / fdr</td><td>0.0</td></th<>		54		protein_results / fdr	0.0
Operation Operation <thoperation< th=""> <thoperation< th=""> <tho< td=""><td></td><td>56</td><td>Sal Ida</td><td>protein_resuits / target_filatches_count</td><td>494</td></tho<></thoperation<></thoperation<>		56	Sal Ida	protein_resuits / target_filatches_count	494
Inspection Inspeci		67		Project id	1
Constraint Constra		58		Projectio Datacatid	121
00 ResultSummary id 108 01 Mis Search id 14 02 Peaklist Search id 14 03 Peaklist Software id 11 04 Search Software id 14		59		ResultSet id	35
81 Mis Search id 14 02 Peaklist Search id 14 03 Peaklist Software id 14 04 Search Settings id 14		60		ResultSummarv id	108
62 Peaklist Search id 14 63 Peaklist Software id 1 64 Search Settings id 14		61		Msi Search id	14
63 Peaklist Software id 1 64 Search Settings id 14		62		Peaklist Search id	14
84 Search Settings id 14		63		Peaklist Software id	1
		64		Search Settings id	14

Property window opened with multiple Identification summaries selected:

The color of the type column indicates if the values are the same (white) or different (yellow)

Properties 8			4
Group	Туре	F083066	F083064
	PSM Decoy Number	342	346
	Peptide Decoy Number	192	194
	is_coverage_updated	true	true
Validation Parar	neters		
Come Malues	peptide_expected_fdr	5.0	5.0
Same values	peptide_filters#1 / description	peptide match rank filter	peptide match rank filte
<u> </u>	peptide_filters#1 / parameter	RANK	RAN
	peptide_filters#1 / properties / threshol	1	
	peptide_filters#2 / description	peptide match score filter	peptide match score filte
Different Values	peptide_filters#2 / parameter	SCORE	SCOR
Different values	peptide_filters#2 / properties / threshol	15.69	15.6
Validation R	esults		
	peptide_results / decoy_matches_count	342	34
	peptide_results / fdr	4.980341	4.994226
	peptide_results / target_matches_count	13392	1351
	protein_results / decoy_matches_count	186	18
	protein_results / fdr	33.0373	32.32497
	protein_results / target_matches_count	940	97
	Sql Ids		



Display a Spectral Count

You can display a generated Spectral Count by using the right mouse popup.

Quantitations			
SC-ds2	Display Abundances	ndances Peptides Ions tification Summary Peptides Design Proteins Sets	
🕀 👷 XIC	Display Identification Summary	y Abundances Peptides Ions y Identification Summary Peptides y Exp.Design Proteins Sets	
SC - ds	Display Exp.Design	Display Abundances Peptides Ions Display Identification Summary Peptides Display Exp.Design Proteins Sets	
	Rename Delete		New User Window Manage User Windows
	Refine Protein Sets Abundances Clone & Extract Abundances		
	Export Excel		
	Properties		

To have more details about the results, see spectral count result

Protein Set	Overviev	Descrip	tion	#Peptide	#Quant. Peptide	Peptides Coun YOC_SP-	Basic SC YOC_SP-	Specific SC YOC_SP-	Weighted S YOC_SP-	Peptides Count YOC_SP-	Basic SC YOC_SP-	Specific SC YOC_SP-	Weighted SC YOC_SP- Y
1 B8YE68_HCM	/A	tr B8YE6	68 B8	233	233	161	1044	1044	1044.00	164	1020	1020	1020.00 Ty
2 B8YEA8_HCM	VA	tr B8YE	A8 B8	104	104	73	398	398	398.00	73	338	338	338.00 Ty
3 B8YE61_HCM	/A	tr B8YE	61 B8	134	134	73	204	204	204.00	73	195	195	195.00 Ty
4 🍃 PP71_HCMVA		sp P067	726 P	98	98	63	242	11	217.94	52	226	1	202.56 Ty
5 B8YEA7_HCM	VA	tr B8YE	A7 B8	95	95	i 52	231	0	24.06	51	225	0	23.44 Su
6 🔓 B8YEB1_HCM	/A 📲	tr B8YEE	B1 B8	60	60	37	73	73	73.00	32	61	61	61.00 Ty
7 6 B8YE83_HCM	/A	tr B8YE8	83 B8	58	58	31	71	71	71.00	29	67	67	67.00 Ty
8 B8YEA5_HCM	VA	tr B8YE	A5 B8	51	. 51	. 23	39	39	39.00	29	51	51	51.00 Ty
9 👌 B8YE91_HCM	/A	tr B8YE9	91 B8	34	34	20	43	43	43.00	21	36	36	36.00 Ty
0 B8YE82_HCM	/A	tr B8YE8	82 B8	27	27	15	36	36	36.00	13	35	35	35.00 Ty
11 🔓 A0A024R1N1	HU	tr A0A0	24R1	26	26	i 15	21	20	20.99	14	23	21	22.75 Ty
2 🔓 FLNC_HUMAN		sp Q143	315 F	31	. 31	. 7	7	7	7.00	9	9	9	9.00 Ty
3 🍃 #C#P60712		SWISS-F	PROT	23	23	14	32	9	25.49	15	43	11	33.21 Ty
14 🖾 ACTG HUMAN	l <mark> </mark>	sp P632	261 A	23	23	13	28	5	10.02	14	34	2	8.76 Ty
replue sequence	PTMS	Score	Charge	m/z	Protein P.	Overview Y	OC_SP- Y	DC_SP- YOC_S	SP-	•		-	
	PTMS	Score	Charge	m/z	Protein P.	Overview Y	OC_SP- Y	DC_SP- YOC_S	sp-	97			-52
LVAFLER	PTMs	Score 50.36	Charge	m/z 424.2556 609.9552	Protein P. B8YE91 B8YE91	Overview Y	OC_SP- YO	DC_SP-YOC_S	SP- 🛡			$\overline{\mathbf{N}}$	-52
I LVAFLER I TIRSEAEDSYHFSSA DTSLOAPPSYEESV.	WK	Score 50.36 60.58 100.35	Charge	m/z 2 424.2556 3 609.9552 2 1050.4804	Protein P. B8YE91 B8YE91 B8YE91	Overview Y 1 1 1	OC_SP- Y(0 7	DC_SP-YOC_S	sp-	9			-52
LVAFLER TIRSEAEDSYHFSSA DTSLQAPPSYEESV. SSNVFDLEEIMR	K	50.36 60.58 100.35 77.28	Charge 2 3 3 2 4 2 2	m/z 2 424.2556 3 609.9552 2 1050.4804 2 720.3463	Protein P. 88YE91 88YE91 88YE91 88YE91	Verview Y 1 1 1	OC_SP- Y0 0 7	DC_SP- YOC_S 1 0 7	SP-	9			-50
IVAFLER ITIRSEAEDSYHESSA DTSLQAPPSYEESV. SSNVFDLEEIMR MTATFLSK	K	Score 50.36 60.58 100.35 77.28 23,56	Charge 2 3 3 3 2 3 2 3 2 3 2 3 2 3 2 3 3 2 3	m/z 2 424.2556 3 609.9552 2 1050.4804 2 720.3463 2 449.7386	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview Y 1 1 1 1 1 1 1 1	OC_SP- Y(0 7 1 0 7 1 0	0 7 1 0 7	SP- 🗗 🖉	9 8 7			-52 -50 -48
IVAFLER ITIRSEAEDSYHFSSA DTSLQAPPSYEESV. SSNVFDLEEIMR MTATFLSK GLDDLMSGLGAAGK	VK Oxidation (50.36 60.58 100.35 77.28 23,56 100.74	Charge 2 3 3 3 2 3 2 3 2 3 2 3 2 2 3 2 2 3 2 2 3 3 2 2 3 3 3 3 2 2 3 3 3 3 2 2 3	m/z 2 424.2556 3 609.9552 2 1050.4804 2 720.3463 2 449.7386 2 660.8271	Protein P. B3YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	1 1 1 1 1	OC_SP- Y(0 7 1 0 7 1 0 2	0 7 1 0 2	SP- C	9 8 7 7 6			-52 -50 -48
LVAFLER TIRSEAEDSYHFSSA TIRSEAEDSYHFSSA SSNVFDLEEIMR SSNVFDLEEIMR MTATFLSK GLDDL ^M SGLGAAGK NIICTS ^M KPINEDLD	Coxidation (Score 50.36 60.58 100.35 77.28 23,56 100.74 32.05	Charge 2 3 3 3 2 3 2 3 2 3 3 3 3 3 3 3	m/z 424.2556 609.9552 1050.4804 720.3463 449.7386 2660.8271 900.1106	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview y 1 1 1 1 1 1 1 1 1 1 1 1 1	OC_SP- Y(0 7 1 0 7 1 0 2 0	DC_SP- YOC_9 1 0 7 1 0 2 0		9 - · · · · · · · · · · · · · · · · · ·			-52 -50 -48 -46
EVAFLER TIRSEAEDSYHFSSA TIRSEAEDSYHFSSA STRUCTURE STRUELEIMR MTATFLSK GLDDUNSGLGAAGK NIICTSMKPINEDLD VLELYSQK	Coxidation (Score 50.36 60.58 100.35 77.28 23.56 100.74 32.05 50.19	Charge 2 3 3 3 2 2 3 2 3 2 3 3 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 3 2 2 3 3 3 3 2 2 3	m/z 424.2556 609.9552 1050.4804 720.3463 449.7386 660.8271 900.1106 490.2770	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview y	OC_SP- Y(0 0 0 1 0 2 0 1		8			-52 -50 -48 -46
EVALUATION EVALUATION	Coxidation (Coxidation (Carbamido	Score 50.36 60.58 100.35 77.28 23.56 100.74 32.05 50.19 50.22	Charge 2 3 3 3 2 2 3 3 3 2 2 3 3 3 2 2 2 2 2	m/z	Protein P. 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91	Overview y	Dasic 3C OC_SP- Y(0 7 1 0 2 0 1 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 7 1 0 2 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0		idance			-52 -50 -48 -46 -44
PEPULE SEQUENCE PEPULE SEQUENCE TISLQAPPSYEESV. SSNVFDLEEIMR MTATFLSK GLDDLMSGLGAAGK NILCTS ^M KPINEDLD VLELYSQK DTSLQAPPSYEESV1 FAQCYSSYSR	Coxidation (Coxidation (Carbamido Carbamido	Score 50.36 60.58 100.35 77.28 23.56 100.74 32.05 50.19 50.22 50.22 72.08	Charge 2 3 3 3 2 2 2 3 2 2 2 3 3 3 2 2 2 2 2 2	m/z	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview y	Dasic 3C OC_SP- Y(0 7 1 0 2 0 1 0 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	C_SP- VOC_S 1 0 7 1 0 2 0 1 0 1 0 1 0 1					-52 -50 -48 -46 -44
VAFLER IVAFLER TIRSEAEDSYHFSSA DTSLQAPPSYEESV. SSNVFDLEEIMR SMTATLSK GLDDM/SGLGAAGK NIICTS/KPINEDLD 3 VLEL/SQK DTSLQAPPSYEESV/ AQQNGTDSLDGQ	Coxidation (Coxidation (Carbamido Carbamido	Score 50.36 60.58 100.35 77.28 23.56 100.74 32.05 50.19 50.22 72.08 143.66	Charge 2 2 2 3 3 2 2 3 3 2 2 3 4 2 2 3 5 3 3 9 2 2 2 2 2 2 2 3 5 3 3 6 2 3 7 4 2 4 3 7 4 4 3 7 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	m/z 424.2556 609.9552 1050.4804 720.3463 449.7386 660.8271 900.1106 900.1106 2 490.2770 1090.4628 6 634.7721 962.4702	Protein P. B8YE91 B8YE91 B	Overview y	Dasic 3C OC_SP- Y 1 0 7 1 0 2 0 1 0 1 0 1 9	C_SP- VOC_S 1 0 7 1 0 2 0 1 0 0 1 9		9			-52 -50 -48 -46 -44 -42
EVAPLER E	Coxidation (Coxidation (Carbamido NPhospho (S Carbamido Phospho (S Phospho (S	Score 50.36 60.58 100.35 77.28 23,56 100.74 32.05 50.19 50.22 72.08 143.66 74.94	Charge 2	m/z 424.2556 609.9552 1050.4804 720.3463 449.7386 660.8271 900.1106 490.2770 1090.4628 634.7721 962.4702 1090.4615	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview Verview 1 Image: Constraint of the second secon	Dasic 3C OC_SP- 1 0 7 1 0 2 0 1 0 1 0 1 9 0 0	C_SP- VOC_S 1 0 7 1 0 2 0 1 0 1 0 1 0 1 0 1 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0		9			-52 -50 -48 -44 -44 -44
VAPLER VAPLER VAPLER VTRSEAEDSYHPSSA DTSLQAPPSYEESV. SSUVPDLEEIMR GLDDLWSGLGAAGK MTATFLSK GLDDLWSGLGAAGK NILCTSWRPINEDLD VLELYSQK DTSLQAPPSYEESV. FAQCYSSYSR AQQNGTDSLDGQ. DTSLQAPPSYEESV. KQEVNMSDSALDCV	Coxidation (Coxidation (Coxidation (Carbamido Thospho (S Carbamido Thospho (S R Oxidation (Score 50.36 60.58 100.35 77.28 23.56 100.74 32.05 50.19 50.22 72.08 143.66 74.94 77.28	Charge 2 3 2 3 2 3 2 3 2 4 2 2 2 2 2 3 2 3 2 4 2 3 3 4 2 3 3 4 2 3 3 4 2 3 3 4 2 3 3 5 2 6 2 7 4 7 5 7 7 8 3 7 7 7 7 8 3 7 7 8 3 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	m/z 2 424.2556 3 609.9552 1050.4804 2 720.3463 2 449.7386 2 660.8271 3 900.1106 2 490.2770 1090.4628 2 634.7721 3 962.4702 2 1090.4615 3 813.3811	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview Verview 1 Image: Constraint of the second secon	Dasic 3C OC_SP- 1 0 7 1 0 2 0 1 0 1 9 0 1 1 0 1 1 0 1 0 1 0 0 1 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	0 C_SP- VOC_3 0 7 1 0 0 2 0 1 1 0 1 0 1 9 0 1 1 0 1 1	SP- Image: Constraint of the second sec	9 8 6 9 6 9 9 9 6 9			-48 -44 -44 -42
VAPLER VAPLER VAPLER VTRSEAEDSYHPSSA DTSLQAPPSYEESV. SSNVFDLEEIMR MTATFLSK GLDDUNSGLGAAGK NIICTSMKPINEDLD VLELYSQK FAQCYSSYSR AQQNGTDSLDQQ DTSLQAPPSYEESV. AQQNGTDSLDQC SQEVMSSALDCV VCSMAQGTDLIR	Coxidation (Coxidation (Carbamido Phospho (S Phospho (S N Phospho (S R Oxidation (Carbamido	Score 50.36 60.58 100.35 77.28 23,56 100.74 32.05 50.19 50.19 50.22 72.08 143.66 74.94 77.28 75.88	Charge 2 2 3 3 5 2 5 2 5 2 5 2 5 2 5 2 5 2 5 2	m/z 424.2556 3609.9552 1050.4804 720.3463 449.7386 660.8271 3900.1106 2490.2770 1090.4628 2634.7721 3962.4702 1090.4628 3962.4702 1090.4615 3813.3811 2683.8245	Protein P. 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91 88YE91	Overview Verview 1 Image: Constraint of the second secon	Dasic 3C OC_SP- 1 0 7 1 0 7 1 0 1 0 1 9 0 1 1 9 0 1 1 1 1 1 1 1 1 1 1 1 1 1	C_SP- VOC3		9 8 7 6 9			+52 +50 +48 +46 +44 +42 +40
EVAPLER E	Coxidation (Coxidation (ECCarbamido NPhospho (S Carbamido Phospho (S R Oxidation (Carbamido	Score 50.36 60.58 100.35 77.28 223,56 100.74 32.05 50.19 50.22 72.08 143.66 74.94 77.28 75.88 89.70	Charge 2 2 3 3 5 2 5 2 5 2 5 2 5 2 5 2 5 2 5 2	m/z 2 424.2556 3 609.9552 2 1050.4804 2 720.3463 2 449.7386 3 660.8271 3 900.1106 2 490.2770 2 490.2770 2 1090.4615 3 933.8245 2 652.8307 3 652.8307	Protein P. B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91 B8YE91	Overview Verview 1 Image: Constraint of the second secon	Dasic 3C OC_SP- 1 0 7 1 0 7 1 0 2 0 1 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	CC_SP- VOC_3 V		9 8 7 6 9 6 9			-52 -50 -48 -46 -44 -42 -40 -38
Vighter Sequence Varler Varler Vighter Sequence Vighter Sequence Sinverbleeing Vighter Sequence Sinverbleeing Vighter Sequence Vighter S	Oxidation (Oxidation (Carbamido YPhospho (S Carbamido Phospho (S Carbamido Carbamido Carbamido .	Score 50.36 60.58 100.35 77.28 23,56 100.74 32,05 50.19 50.22 72.08 143.66 74.94 77.58 89.70 87.25	Charge 2 3 3 3 3 4 2 2 2 2 2 2 2 2 2 2 2 2 2	m/z 2 424.2556 609.9552 2 1050.4804 2 720.3463 2 449.7386 2 660.8271 9 00.1106 2 490.2770 2 1090.4628 2 634.7721 9 652.4702 2 1090.4615 8 813.3811 9 652.4702 2 633.8245 2 632.83245 3 1068.1981	Protein P. B8YE91 B8YE91	Overview y	Dasi SC (SP- (V) C_SP- 1 0 7 1 0 2 0 1 1 0 1 1 0 1 0 1 0 0 1 1 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	C_SP- VC_3		9 8 7 6 9			-52 -50 -48 -46 -44 -44 -42 -40 -38
VEPENDE SEQUENCE VAFLER UNFLER TITRSEAEDSYNFSSA DTSLQAPPSYEESV. SMTNFLSK GLDUNSGLGAAGK VIIICTSWRPINEDLD GLDUNSGLGAAGK VIIICTSWRPINEDLD TSLQAPPSYEESV. FAQCYSSYSR AQQNGTDSLDQQ DTSLQAPPSYEESV. KQEVNWSDSALDCV VIIICTSWAQGTDLIR GLDDLMSGLGAAGK KGPGPPSDASTA GSTMLYR	Oxidation (Coxidation (ECCarbamido N Phospho (S Carbamido Phospho (S R Oxidation (Carbamido arbamido	Score 50.36 60.58 100.35 77.28 23.55 100.74 32.05 50.19 50.22 72.08 143.66 74.94 77.28 89.70 87.58 89.70 87.25 89.70 87.25	Charge 2 3 3 3 4 2 5 2 2 5 2 2 5 2 2 5 2 2 5 2 2 5 2 2 5 2 2 2 2 2 2 2 2 2 2 2 2 2	m/z 2 424.2556 3 609.95522 2 1050.4804 2 720.3463 2 449.7386 6 60.8271 3 900.1106 4 490.2770 2 1090.4628 6 64.7721 3 952.4702 1 090.4613 3 813.3811 6 83.8245 6 52.8307 3 1058.1981 2 441.7267 3 412.7267 3 412.7267 4 412.7267 3 412.7267 4	Protein P. B8YE91 B8YE91	Overview y 1 1 1 1 1 1 1 1 1 1 1 1 1	Data 50 (C_SP- 7 7 1 0 7 7 1 0 0 0 1 1 9 0 1 1 0 0 1 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	C_SP- VC_3		9 8 7 6 9 6 9 6 9 6 9 6 9 9 9 6 9			-52 -50 -48 -46 -44 -44 -42 -40 -38
VAPLER VAPLER VAPLER VAPLER VTRSEAEDSYHPSSA DTSLQAPPSYEESV. SSNVFDLEEIMR MTATFLSK GIADDI/SGLGAAGK NIICTS*/KPINEDLD VLELYSQK OTSLQAPPSYEESV. DTSLQAPPSYEESV. DTSLQAPPSYEESV. CSMQQGTDLIR GQDUMSGLGAAGK KGPGPPSDADTA GSTWLYR SSYSQHVTS:EAVSI	Coxidation (Coxidation (Coxidation (Carbamido Phospho (S Carbamido Carbamido Carbamido Carbamido t Phospho (S t	Score 50.36 60.58 100.35 77.28 23.55 100.74 32.05 50.19 50.22 72.08 143.66 74.94 77.28 75.88 89.70 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 28.60 87.25 97.28 97.29 97.28 97.29 97.28 97.29 97.28 97.29 97.28 97.29 97.28 97.29 97.28 97.29 97.28 97.29 97.20 97.	Charge 2 3 3 3 4 2 5 2 2 5 3 3 4 2 2 2 2 3 3 2 2 2 2 2 3 3 2 2 3 3 2 2 3 2 2 3 2 2 3 2 2 3 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2	m/z 424.2556 609.9552 1050.4804 2720.3463 449.7386 660.8271 900.1106 490.2770 1090.4628 634.7721 962.4702 1090.4615 813.3811 813.3811 663.8245 652.8307 1068.1981 441.7267 6146.199	Protein P. B8YE91 B8YE91	Overview Verview 1 Image: Constraint of the second secon	Dask 50 (C_SP- (Y) 7 7 7 1 0 7 7 7 7 7 1 0 0 1 0 1 0 1 0 1 0 0 1 0 0 1 0 0 7 7 7 7 7 7 7 7 7 7 7 7 7	C_SP- VOC3		9 8 6 9			-46 -44 -42 -40 -38 -36
PEpulae Sequence PEpulae Sequence IVAFLER ITRSEAEDSYNFSS/A DTSLQAPPSYEESV, STNVPDLEEIMR MTATELSK GLDDLMSGLGAAGK MTLTSK VERVENED TSLQAPPSYEESV DTSLQAPPSYEESV FAQCYSSYSR AQQNGTDSLDGQ, DTSLQAPPSYEESV VCSYAQGTDLR GLDDLMSGLGAAGK KOSPOPSSDASTA, GSTWLYR SVYSQHVTSSEAVSI TMQLIPDDYSNTH,	Oxidation (Carbamido N Phospho (S Phospho (S R Oxidation (Carbamido H Phospho (S I H Phospho (S	Score 50.36 60.58 100.35 77.28 23,56 100.74 32,05 50.19 50.22 72.08 143.66 74.94 77.28 89.70 87.25 28.60 46.22 28.60 46.22 29.43,17 29.22 29.	Charge 2 3 3 2 4 2 5 3 2 4 2 2 5 3 3 2 2 4 2 2 2 2 2 2 2 2 2 2 2 2 2	m/z 2 424.2556 609.9552 1050.4804 2 720.3463 2 449.7386 2 660.8271 1 090.1106 2 490.2770 1 090.4628 2 634.7721 3 962.4702 2 1090.4628 6 634.7721 8 813.3811 6 638.8245 2 652.8307 1 068.1981 2 441.7267 6 18.61961 6 18.61991 6 18.61991 6 18.61991 6 18.61991 6 18.61991 1 6 18.61991 1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Protein P. 88YE91 88YE91	Overview y 1 1 1 1 1 1 1 1 1 1 1 1 1	Data SCP Y	C_SP- VC_3		9 8 6 9 6 9		koc vice	-44 -44 -42 -40 -38 -36 -50 -48 -44 -44 -44 -42 -40 -38 -36 -36 -36 -36 -36 -36 -36 -36 -36 -36
VEPUAL SEQUENCE VAFLER UTRSEAEDSYNFSSA DTSLQAPPSYEESV. SONVFDLEEIMR GLDDLWSGLGAAGK MTATFLSK GLDDLWSGLGAAGK VIELYSQK DTSLQAPPSYEESV FAQCYSSYSR AQQNGTDSLDGQ. DTSLQAPPSYEESV KQEVN/SDSALDCV GLDDLWSGLGAAGK KGPGPPSSDASTA GSTVLYR SVYSQHVTS:EAVSJ SVYSQHVTS:EAVSJ VIQLIPDOYSNTH	Coxidation (Coxidation (ECCarbamido N Phospho (S Carbamido Phospho (S Carbamido 1 Phospho (S Carbamido	Score 50,36 60,58 100,35 77,28 23,56 100,74 32,05 50,19 50,22 72,08 143,66 74,94 77,28 75,88 89,70 87,25 28,60 46,29 43,17 87,23 28,60 46,29 43,17 28,28 28,50 29,50 29,50 29,50 29,50 20,50 29,50 29,50 20,	Charge Charge	m/z 424.2556 609.9552 1050.4804 2720.3463 449.7386 660.8271 3900.1106 490.2770 1090.4628 2090.4615 813.3811 633.8245 652.8307 1068.1981 241.7267 618.6109 625.9638 520.8361 241.7267 521.502	Protein P. B8YE91 B8YE91	Overview Verview 1 Image: Constraint of the second secon	Data 52 (C_SP- Y 1 0 7 1 0 7 1 0 0 0 1 1 0 0 1 1 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	C_SP- VC_3		9 8 7 6 9 6 9 6 9 6 9 6 9 6 9 6 9 9 6 9 9 6 9	KOC_SB-JFC S		

The overview is based by default on the weighted spectral count values. (Note: if you sort on the overview column, the sort is based on max (value-mean (values))/mean (values). So, you will obtain the most homogenous and confident rows first)

For each compared dataset, are displayed:

- status (typical, sameset, /)
- peptide numbers



- the basic spectral count
- the specific spectral count
- the weighted spectral count
- the selection level

User can change the information displayed by the overview using the table settings icon (\gtrsim) .

Display a quantitation result

To display a quantitation, other than spectral count, right click on the selected node in the Quantitation tree, and select "Display Abundances", and then the level you want to display:

Uuantitations		
🗝 🕪 Ide	Display Abundances	Peptides lons
🕀 💼 Gro	Display Identification Summary	Peptides
🗄 💼 Gro 🕀 👷 Phosph	Display Exp. Design	Proteins Sets
🕀 间 Quant /	Add Quantitation Folder	Modification Sites
sc re	Rename	Modification Clusters
🕀 🤠 Quant	Delete	New User Window
🕀 🧏 Quant 👚 👘 Trash	Compute Post Processing on Abundances	Manage User Windows
	Clone & Extract Abundances	

Note: You can also display the identification summary used as reference for the quantitation from the popup menu in the quantitation tree:



Display Protein Sets

Protein Sets

By clicking on "Display Abundances" / "Protein Sets", you can see all quantified protein sets. For each quantified protein set, you can see below all peptides linked to the selected protein set and peptides lons linked to the selected peptide. For each peptide lon, you can see the different features and the graph of the peakels in each quantitation channel.



Logs	×	🛛 📩 Qua	antSmall Protein	Sets x	🛃 quant_100	vs10 Protein	Sets ×										•	
M T		Protein S	iet	Overview	Description	ı	#Peptide	#Quan Peptide	t. = #Quant F083064	. PSMs	Abundance F083064	= #Qua F083066	nt. PSMs	Abundance F083066	 #Quant. PSMs F083067 	 Abundance F083067 	 #Quant. PSMs F083068 	E i
2	16	🔓 G3P2	_YEAST		sp P00358	G3P2_YEA		31	3	9	12 056 782		15	11 491 365	17	11 211 114		^
	17	ALBU	_HUMAN_UPS		P02768ups	ALBU_HU		36	24	12	7 190 832		10	7 328 332	10	7 633 882		
	18	🔓 HSP7	5_YEAST		sp P11484	HSP75_YE		31	22	61	172 525 136		57	174 860 400	49	174 372 976		
먍	19	ISP7	6_YEAST		sp P40150	HSP76_YE		30	1	3	2 624 544		3	2 802 476	2	2 758 012		-
•	20	🔓 METE	_YEAST		sp P05694	METE_YE		31	24	45	60 299 724		40	58 647 992	34	57 221 908		
-	21	TRFE	_HUMAN_UPS		P02787ups	TRFE_HU		28	20	0	3 597 271		0	3 780 158	1	3 451 923		-
	22	🔓 EF3A	_YEAST		sp P16521	EF3A_YEA		30	23	73	94 451 056		60	94 658 392	66	95 807 080		
	23	HS 10	4_YEAST		sp P31539	HS104_YE		31	25	25	59 844 576		28	60 295 892	28	60 316 072		
	24	6PGD	1_YEAST		sp P38720	6PGD1_YE		23	18	39	76 439 632		32	71 455 272	33	69 911 080		
	25	HSP6	0_YEAST		sp P 19882	HSP60_YE		24	19	29	85 596 496		24	82 816 000	31	81 494 104		_ 117
	26	GPF:	1_YEAST		sp P16861	K6PF1_YE		25	23	34	36 112 208		34	34 291 688	30	35 702 136		~
		<															>	<u>۲</u>
M T	19	Status	Peptide Seque.	PTMs	Score	Charge m	n/z	Overview	Abundance F083064	 Abun F08306 	dance = Abun 6 F08306		Gra	Linear Plot 🗸	X Axis: Quant. C	ihannel 🗸 Y A	xis: Abundance	e
2	20	₫ V	FNLPLFPTTTIG		76.92	2 1	1054.5780		5 401 614	5 49	5 122 4 99 ^		0,0E	°				
	21	P 6	ESVYAQSITSKPI	VK	70.14	2	768.9161		1 022 190	111	9 563 9:	2	7,0E	6		/	6,8	E/
	22	6 A	AVDVTALEMVK		82.43	2	588.3205		2 005 457	1 92	3 550 2 09		6.0E	6			-6.6	SE7 8
뿧	23	🧉 I	TVDELFK		37.21	2	482.7714		2 620 569	2 78	4 6 3 2 5 7	1567	U					Ē
	24	🖒 P	PVLLGPVSYLFLG	к	63.58	2	751.9542		3 370 486	3 09	4 900 2 76	[390]	2 5,0E	6-	- /		-6,4	4E7 🖥
6 QP	25	<u>6</u>	GLPVAALHVDFVF	R	61.04	2	697.4023		791 030	66	8 623 77		P 4,0E	6-				ą
0	26	🖒 Y	DLSPIDTLFAMO	GR	73.91	2	799.8994		1 799 523	1 80	1 183 1 77		3,00				-6,2	2E7
	27	P 1	TQAMQLALALR.		59.51	3	824.1059		1 300 722	1 37	4616 13		< 3,0℃	°				ę
	28	₽ /			41.5	3	1019.5620		704 059	61	6 967 6l		2,0E	6 -			-6,0	E7 着
	29	🖒 L	DEVVVITK		58.13	2	508.3057		2 163 346	2 24	4014 22:		1,0E	6-				007
	30	ତ ।			28.85	3	570.6732		587 240	38	5 172 3.					<u>a</u>	i >,°	/E/
	31	💎 I	ELGIHTRPVLLGP	2	32.16	4	578.0911		212 220	21	5340 2. V			F083064 F	083066 F083067	F083068 F08306	9 F083070	
		<									>				Quant. 0	hannel		
_	_											<u> </u>		-	-			
Qu	anti.	Peptides i	Ions XIC Feat	tures								Graj	nic: Li	near Plot 🗸 X	AXIS: RI	Y Axis: Inter	nsity 🗸	e
iii		Destide	Chathan		characteristic		DT Due	Destai	Abundance	e 🛛 Abu	ndan (📻		.5E6					
T		Peptide	Status	PTMS 5	core Charge	m/z	RI Pro	Protei	F083064	F0830	66 🗳 👍	œ						
2	1	ITVDELFK	< 🅢 🛛		37.21	2 482.7710	75.97	1 METE_Y	2 620 56	9	<u>^</u>	III 3	.0E6		M			
			, i i									200				M		
												2	,5E6		MALLAM			
25												s i,	056		/ . //'\'.\\	NIV		
												Sus .	,020					
												Ē	,5E6 - ···	1	[M.	∧ /// [™]		
												-		IR CO		·y/		
												1	,0E6	N/V		/ <i>Y</i> N	M	
												_		Na	XX	V 'I	Λh	
												5	.0E5 -	1P		Kr.	L.	
											~			- Alle		man	WA	<u> </u>
		<									>			73,5 74	74,5 75	75,5 76	76,5 77	77,5
																рт		

The overview is based by default on the abundance values.

Note: if you sort on the overview column, the sort is based on max (value-mean (values))/mean (values). So, you obtain the most homogenous and confident rows first.

For each quantitation channel, are displayed:

- the raw abundance
- the peptide match count
- the abundance
- the selection level

By clicking on the using the "table setting" icon \aleph , you can choose the information you want to display or change the overview.

Peptides

The middle part of the window lists all peptides of the selected Protein set with the same kind of quantitative data. The status column indicates whether the peptide was used or not for protein set abundances. On the right, a graph allows you to see the variations of the abundance (or raw abundance) of a peptide in the different quantitation channels.



Features and Peakels

You can see the different features in the different quantitation channels and the graph of the peakels:

XI	C Pe	Peptides Ions XIC Features														
Fea	ature	s (4)														
T		Мар	Quant. Channel	m/z	Charge	Elution Time (min)	Apex Intensity	Intensity	Duration (sec)	Predicted El. Time (min)	F					
		OEMMA121101_45b	F067909	727.9713	3	18.56	315 826	545 185	37	18.50						
		OEMMA121101_58b	F067911	727.9704	3	18.11	575 144	1 114 286	47							
		OEMMA121101_47b	■ F067901	727.9710	3	18.49	287 700	615 248	40	18.31						
[<u></u>		OEMMA121101_43b	F067900	727.9710	3	18.95	372 579	695 966	43	18.71						
				1							1					

By clicking on 💷 you can display either:

- the peaks of isotope 0 in all quantitation channels
- all isotopes for the selected quantitation channel:

Quanti	Peptides Ions XIC Feat	tures						Graphic :	Linear Plot 🕔	/ X Axis:	RT	✓ Y Axis: Intensity ✓		C
Feature	s (6)						1						1	-
T	Мар	Quant. Channel	m/z	Charge	RT	₽	€	6,0E6 -						
X 1	OEMMA121101_36b	F083064	1054.5767		2	A	Ⅲ	E 0E6 -			\sim			
2	OEMMA121101_38b	F083066	1054.5762		2		1000	5,020						
3	OEMMA121101_40b	F083067	1054.5759		2		<u></u>	4.056 -			$N \frown$			
4	OEMMA121101_61b	F083068	1054.5762		2			Ϋ́, Ϋ́, Ϋ́, Ϋ́,			1	\backslash		
1 5	OEMMA121101_63b	F083069	1054.5783		2			S		1	\sim			
- e	OEMMA121101_65b	F083070	1054.5776		2			9 3,0E0			_			
	<		_			× >		2,0E6 - 1,0E6 -	96,2 S	ime: 96,2	96,6	F083064Isotope index: Apex Int.: 1 601 330	7,31 3 97,2	- +
												PT . 06 7072		1
							_	_				Intensity : 1070202 6250		1

By clicking on $\boxed{100}$ you can see the chromatograms of the features and their first time scan and last time scan in mzScope. For more details see the mzScope section.

It is also possible to extract the corresponding chromatogram for one or all of the features.

Quar	nti.	Peptides Ions	XIC Feat	ures				
Featu	res	(6)						
T		Мар		Qua	nt. Channel		m/z	
X	1	OEMMA1211	01_36b	- F04	83064			482.7711
	2	OEMMA1211	01_38b	F0	83066			482.7710
	3	OEMMA1211	01_40b	F04	83067			482.7710
	4	OEMMA1211	01_61b	■ F04	83068			482.7713
	5	OEMMA1211	01_63b	■ F04	83069			482.7717
1	6	OEMMA1211	.01_65b	= F0	83070	Copy	cell	712
						Selec	t All	
						Displ	ay	>
					_	View	Selected Da	ata
		<				View	All Data	
						Extra	ct Selected	XIC
						Extra	ct All XIC	

The resulting chromatograms will be displayed in the same windows as peakel.





Display Peptides

By clicking on "Display Abundances" / "Peptides", you can see:

- identified and quantified Peptides
- non identified but quantified peptides
- identified but not quantified peptides (linked to a quantified protein)

Logs	.ogs x 👷 quant_100vs10 Protein Sets x 👷 quant_100vs10 Peptides x																	
M T		Sta	Peptide Sequence	PTM	Score	Charge	m/z	RT	Prot	Protei.	Overvie	w Qua PSMs	int.	Abundance F083064	 Quant. PSMs 	Abundano F083066	e Quant. PSMs count	Abundance F083067
2	2	4 🖒	NLSVEDAAR		49.59	2	487.7487	23.34	. 1	1 CATA_			0	643 183		641 974	+ 0	628 37
	2	5 🖒	DLFNAIATGK		42.76	2	525.2849	69.99	1	1 CATA_			0	88 747	(205 920	0 0	173 47
	2	6 🖒	DPASDQMQHWK		68.12	2	671.7960	27.77	1	1 CATA_			0	C	() () 0	
122	2	7 🖒	NFTEVHPDYGSHIQAL		35.75	3	728.6957	74.97	1	1 CATA_			0	C	(97 623	3 0	
	2	8 🖒	DPILFPSFIHSQK		23.95	3	510.2776	84.59	1	1 CATA_			0	372 169	(261 540	0 0	324 51
	2	9 🖒	GPLLVQDVVFTDEMA.		53.71	3	730.3622	91.96	1	1 CATA_			0	C	() (0 0	
	3	0	NPVNYFAEVEQIAFDF	SN Oxi	41.53	3	1064.8340	96.29	1	1 CATA_			0	206 530	(185 369) 0	193 97
	3	1	DPASDQMQHWK	Oxi	68.05	2	679.7931	. 13.94	- 1	1 CATA_			0	98 339	(122 317	<u>ر</u> 0	95 93
	3	2 👲 📃	FYTEDGNWDLVGNNT		53.12	3	840.0742	97.96		1 CATA_			0	129 663) (133 165	5 0	
	3	3	GAGAFGYFEVTHDITK		71.78	2	856.9185	76.92		1 CATA_			0	92 758	(0 0	0 0	
	3	4 🖒 🔄	LNVITVGPR		55.22	2	484.7981	48.58		1 CATA_			1	729 576	(865 704	1 0	913 02
		<																>
M		Peptide S	equence Status	PTMs	Score	Charg	ge m/z	RT	P	rot F	Protein Sets	Pep. match count	nt F	Abundance P	ep.	Abundance	Pep. match c =083067	ount Abundance
TT I	1	VTEDGNN			32	51	2 1250 6	5108	97.96	10	ATA HUM	Factor	0	125 180		185 452		0
X	2	YTEDGN			53	12	3 840.0	1740	97.94	10			0	129 663	0	133 165		0
	~	1120014					0 01010		571.51	10				125 505	•	100 100		•

The lower view lists all peptide ions (specific charge) of selected peptide. The status column indicates if the ion is valid or not and if it was used for peptide quantitation.

Display Peptides Ions

By clicking on "Display Abundances" / "Peptides Ions", you can see:

- all identified and quantified Peptides Ions
- non identified but quantified peptides lons



Logs 🗴 👷 quant_100vs10 Protein Sets 🗴 👷 quant_100vs10 Peptides 🗴 號 quant_100vs10 Peptides Ions 🗴

Peptide Sequence	PTMs	Score	Charge	m/z	RT	Protein S	Protein Sets	Pep. match	Abundance F083064	Pep. match count	Abundance F083066	Pep F0830
VTSNVVLVSGEGER		32.56	2	723.3838	39.10	1	SKP1_YEAST	0	1 065 472	0	1 028 804	ł
ETYGEMADCCAK	Carbami	70.19	2	717.7703	23.53	1	ALBU_HUMAN	0	0	0	0)
TFESEAAHGTVTR		100.21	2	703.3387	20.95		IDHC_YEAST,	4	1 405 549	6	1 185 391	
KLNEIDNKR		43.48	2	565.3196	10.08	1	YP260_YEAST	0	0	0	0)
YFLDALPVALLGMNAD		57.91	3	1063.5338	111.69	1	RIR2_YEAST	0	1 674 545	1	1 510 852	2
GDTPDQGHLQTR		44.99	2	662.8149	11.26	1	CLH_YEAST	1	104 071	1	129 788	\$
VEFLEDTSR		45.63	2	548.2687	42.03	1	RS28A_YEAST	2	5 523 036	4	5 571 094	ł
NANPWGGYSQVQSK		65.91	2	768.3644	41.60	1	COX5A_YEAST	0	387 750	2	494 283	6
NDLTASQLSDKINDVR		29.19	2	894.9583	53.25	1	BGL2_YEAST	0	293 968	0	239 616	6
VGQAVETVGQAGRPK		26.44	3	499.6104	18.59	1	RPN1_YEAST	1	1 446 326	0	1 192 612	2
VGQAVETVGQAGRPK		81.99	2	748.9123	18.56	1	RPN1_YEAST	0	278 058	2	341 131	L
ADIHLVELLYYVEELDS		41.68	3	1036.2253	115.45	1	GSTA1_HUMA	0	64 956	0	86 812	2
NATFPGVQMK		38.70	2	546.7785	45.86	1	PDC1_YEAST	2	33 662 596	2	33 767 420	
WAGNANELNAAYAAD		106.89	2	999.4583	62.99	1	PDC1_YEAST	10	36 896 336	7	33 997 268	\$
WAGNANELNAAYAAD		43.12	3	666.6412	63.14	1	PDC1_YEAST	1	3 922 132	2	4 059 540	
LQLLKPFKPWDGK		24.11	4	393.2344	82.01	1	ACON_YEAST	0	0	0	0)
QLQLIQVEIK		3 <mark>8.78</mark>	2	606.3718	79.83	1	HS104_YEAST	0	899 909	0	1 005 325	5
RKPVTEAR		27 <mark>.94</mark>	2	478.7854	7.06	1	TRFL_HUMAN	0	18 459	0	18 459)
LYQTEPSGIYSSWSAQ		91.45	2	1122.5495	79.47	1	PSA7_YEAST	1	605 001	1	598 297	1
TNANLPEGEKK		46.44	2	600.8143	10.06	1	THRC_YEAST	1	417 456	2	396 757	'
TNANLPEGEKK		43.02	3	400.8786	10.06	1	THRC_YEAST	2	777 439	0	640 429	
LPNSNVNIEFATR		79.11	2	737.8857	60.35	1	VDAC1_YEAST	4	2 909 117	5	2 801 511	

Display Modification Sites/Clusters

By clicking on "Display Abundances" / "Modification XXX" (Site, Clusters or Annotated Clusters), you can see the same Clusters/Sites information as in "Identification Summary/Display / Modification XX", See <u>Display Modification</u> description

In addition, quantitative information is computed and displayed.



Upper View: This view lists all Modification Sites or Clusters. For each of them, in addition to cluster information, quantitative data is given. Abundance is calculated using peptide's abundance sum.

2nd view: Cluster/Site peptides list. The PSMs list is accessible in a hidden tab. Peptide quantitative information are displayed the same way it is done in other quantitative views.



3rd view : Graphic illustration of the cluster/site peptides mapped to the protein sequence is shown on the left. On the right, a graph allows you to see the variations of the abundance (or raw abundance) of these peptides in the different quantitation channels.

Bottom Right view: This view is the same as the "Peptide" view in "<u>Display Xic Protein Sets</u>" above.

Display Experimental Design and Parameters

By clicking on "Exp. Design > Parameters", you can see the experimental design and the parameters of the selected XIC.

Task	s Log 🛛 🛚 📩 XIC Exp.	Design 🛛
	Exp.Design Exp. Para	meters
	XIC Group ds 1 Group ds 1 Group ds 1 Group ds 1 Group ds 1 Group ds 1 Group ds 2 Group ds 2 G	l)1 w File : OEMMA 12110 1_47b.mzdb)0 w File : OEMMA 12110 1_43b.mzdb)2 w File : OEMMA 12110 1_56b.mzdb 2)9 w File : OEMMA 12110 1_45b.mzdb 11 w File : OEMMA 12110 1_58b.mzdb 10 w File : OEMMA 12110 1_50b.mzdb
Tasks	Log 🛛 👷 XIC Exp. Des	sign %
	Exp.Design Exp. Paramet	ers
	XIC Parameters	
	Extraction moz tolerance	(ppm): 5.0
	Extract XIC from:	All detectable features
	Deisotoping Identifica	ation Based
	Clustering Alignment 1	Normalization Master Map
	Feature Clustering	.
	moz tolerance (ppm):	5.0
	time tolerance (s):	15.0
	time computation:	Most Intense
	intensity computation:	Most Intense

If you have launched the "compute post processing ..." on the XIC, you can also display the corresponding parameters.



Logs	🗴 📩 quant_100vs10 Exp. Design 🔹 📩 quant_100vs10 Protein Sets 🔹 🌺 quant_100vs10 Peptides		٥
	Exp.Design Exp. Parameters Compute Post Processing		招 単
	Pep. selection Pep. configuration Prot. configuration	1	
	Pentides Selection Method: Razor and Specific Specify peptides to consider for quantitation	\sim	
	Discard Miss Cleaved Peptides		
	Discard Modified Peptides		
	✓ Acetyl (Protein N-term)		
	Carbamidomethyl (C)		
	Oxidation (M)		
	Modified Peptide Filtering Method: Discard all forms	\sim	
	Discard Pep Ions Sharing Peakels		

Display Map Alignment

By clicking on "Exp. Design > Map Alignment", you can see the map of the variation of the alignment of the maps compared to the map alignment of the selected XIC. You can also calculate the predicted time in a map from an elution time in another map.





Frame Toolbars Functionalities



A: Display Decoy Data.

- B: <u>Search</u> in the Table. (Using * and ? wild cards)
- C: Filter data displayed in the Table
- D: Display settings dialog (you can modify displayed columns and perform double sorting)
- E: Export data displayed in the Table
- F: Send to Data Analyzer to compare data from different views
- G: Create a Graphic : histogram or scatter plot . Only on PSMs table
- H: Display number of entities in the table (number of PSMs / Peptides / Proteins...)
- I: Right click on the marker bar to display Line Numbers or add Annotations/Bookmarks
- J: Export view as an image
- K: Generate Spectrum Matches (specific to spectrum grahic)
- L: Expands the frame to its maximum (other frames are hidden). Click again to undo.
- **M**: Gather the frame with the previous one as a tab.
- N: Split the last tab as a frame underneath
- O: Remove the last Tab or Frame
- P: Open a dialog to let the user add a View (as a Frame, a Tab or a splitted Frame)
- Q: Save the window as a user window, to display the same window with different data later



Create a custom User Window

You can lay out your own user window with the desired views.

You can do it from an already displayed window, or by using the right click mouse popup on a dataset like in the following example (Use menu "Search Result>New User Window..." or "Identification Summary>New User Window...")

ojects %			6	-	Tasks	Log	
ProjectTest(63)		-		₹ ⊘		i0
Identification	ns					2	1
(F 067 ^{ee})		_				V	1
🗄 🗂 Trasl	Search Result					•	10
	Identification Summary		PSM				9
	Properties		Peptides				8
	Add		Protein Sets				6
	Merge		New User Wind	low	,		5
	Validate		Manage User W	lin	dows		4
	Change Trainel Destain	-	Manage Oser W	111	I		3
	Change Typical Protein					×	2
	Generate Spectrum matches					-	1
	Compare With SC					Ê	<u> </u>
	Export						
	Compute Statistical Reports (MSDiag)						
	Change Description						
	Rename						
	Delete				Task:		
					r Tim	esta	amp
				.	Ask	Tim	ne:

In the example, the user has clicked on "Identification Summary>New User Window..." and selects the Peptides View as the first view of his window.

8-	User Defined Window	×
Window Title		
Title : Peptides		
Views		
name	description	I ₽
PTM Protein Sites	PTM Protein Sites of an Identification Summary	^
MSQueries	All MSQueries of an Identification Summary	
Protein Set	All Protein Sets of an Identification Summary	
Peptides	All Peptides of an Identification Summary	
PSM	All PSM of an Identification Summary or corresponding to a Peptide Ins.	
		~
	🖌 ОК 🛛 🗶 С	ancel

You can add other views by using the '+' button.



isks Log	🛿 🕕 F06789	7 Peptides 🛛 🕅								4		•
	Peptide	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Protein Set Count	RT	PTM	E.	П
1	GVLGYTEDAV	172.61	3568.72	1190.58	3.25	3	0	1	. 110.56			Ш
2	VAVDDPSVLA	164.99	2309.20	1155.61	1.92	2	0	1	. 105.21			
3	AANLGGVAVS	162.46	1971.98	987.00	0.02	2	0	1	. 76.36			
4	QLSLWGADN	162.36	2091.92	1046.97	0.36	2	0	1	. 74.59			
5	RYGASAGNV	159.58	3412.71	1138.58	2.36	3	1	2	111.50			
6	IIYVDDGVLSF	156.29	2265.18	1133.60	-0.71	2	0	1	. 130.23			
7	FAIPAINVTSS	153.57	2159.16	1080.59	2.34	2	0	1	. 94.27			
8	VILFIGDGSLQ	152.44	2432.33	1217.18	0.72	2	0	1	. 122.24			
9	TPGLSNATQV	149.59	2121.07	1061.54	0.87	2	0	1	. 76.14			
10	FIAEGSNMGS	149.52	2297.10	1149.56	2.21	2	0	1	. 87.82			
11	FQYIAISQSD	149.20	1947.85	974.93	-0.59	2	0	1	. 54.46	Carbamidom		
12	VINDAFGIEE	149.11	2574.30	1288.16	2.05	2	0	1	. 96.50			
13	SEGITDIEES	148.57	2110.95	1056.48	0.30	2	0	1	. 73.00	Acetyl (Prote		
14	AVGDNDPIDV	147.54	2516.26	1259.14	1.68	2	0	1	. 96.63			
15	SIVPSGASTG	146.03	1839.91	920.96	0.10	2	0	2	64.56			
16	EAQADAAAE	145.20	2370.08	1186.05	0.10	2	1	1	. 70.72			
17	SINPDEAVAY	144.42	2576.26	1289.14	4.15	2	0	2	93.19			
18	AAEEADADA	142.60	2340.97	1171.49	0.67	2	0	1	. 82.40			
19	HVDELLAECA	142.13	1611.76	806.88	-0.69	2	0	1	. 48.23	Carbamidom		
20	GYLADDIDAD	141.97	2652.22	1327.12	2.55	2	0	1	. 90.14			
21	LNLPTNSSISV	141.06	2072.07	1037.05	0.91	2	0	1	. 84.74			
22	VGQFDQVLNL	140.29	3580.70	1194.57	1.57	3	0	1	. 98.42	Carbamidom		
23	AVGSLTFDEN	139.62	2213.08	1107.55	1.47	2	0	1	. 86.63			
24	SGETEDTFIA	139.06	1820.92	911.46	-0.14	2	0	2	130.29			Ν
25	QAFDDAIAEL	139.04	2143.98	1073.00	2.03	2	0	2	92.62		_	
24	NIL VAINETTA AL	100.00	1727.00	070.00	4 74	-	0		100.01		1 -	Ш

In this example, the user has added a Spectrum View and he saves his window by clicking on the "Disk" Button.

Task	s Log	🔉 🌔 F06789	7 Peptides 🛛 🕅										
		Peptide	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Protein Set Count	RT	РТМ	Ę	53
m in i	1	GVLGYTEDAV	172.61	3568.72	1190.58	3.25		3 0	1	. 110.56		-	暭
T	2	VAVDDPSVLA	164.99	2309.20	1155.61	1.92	2	2 0	1	. 105.21			
	3	AANLGGVAV	162.46	1971.98	987.00	0.02	2	2 0	1	. 76.36			
	4	QLSLWGADN	162.36	2091.92	1046.97	0.36	2	2 0	1	. 74.59			
•	5	RYGASAGNV	159.58	3412.71	1138.58	2.36	3	3 1	2	111.50			
	6	IIYVDDGVLSF	156.29	2265.18	1133.60	-0.71	2	2 0	1	. 130.23			
	7	FAIPAINVTS	153.57	2159.16	1080.59	2.34	2	2 0	1	. 94.27			
	8	VILFIGDGSL	152.44	2432.33	1217.18	0.72	2	2 0	1	. 122.24			
	9	TPGLSNATQV	149.59	2121.07	1061.54	0.87	2	2 0	1	. 76.14			
	10	FIAEGSNMGS	149.52	2297.10	1149.56	2.21	2	2 0	1	. 87.82			
	11	FQYIAISQSD	149.20	1947.85	974.93	-0.59		2 0	1	. 54.46	Carbamidom.	· _	
	12	VINDAEGIEE	149.11	2574.30	1288.16	2.05		0	1	96.50		Ţ.	
-					Query 2836	8 - GVLGYTEDAV	/SSDFLGDSHSS	FDASAGIQLSPK					53
199	:	250 000 -											中
						1							
		200 000 -											
	t	150.000											
	nsi	150 000 1											
	Ite	100 000 -											
	.=												
		50 000 -											
		0 1	250 5		1,000, 1	250 1 500	1 750	2,000 2,00	0 2 5 00 2 3	2000	2.250		_
		U	200 5	00 /50	T 000 T	.250 1500	1/50	2 000 2 25	0 2500 2.	/50 3 000	3 200	3 200	4
							m/z						

The user selects 'Peptides Spectrum' as his user window name



Та	sks	Log	🔉 🕕 F06789	7 Peptides 🛛 🕺									• • •	
) [Peptide	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Protein Set Count	RT	РТМ	F.	器
11		1	GVLGYTEDAV	172.61	3568.72	1190.58	3.25		3	D	110.56 ا		-	-
T		2	VAVDDPSVLA	164.99	2309.20	1155.61	1.92		2	1 0	105.21			
	,	3	AANLGGVAV	162.46	1971.98	987.00	0.02		2	1 C	76.36			
-		4	QLSLWGADN	162.36	2091.92	1046.97	0.36		2	1 1	74.59			
1		5	RYGASAGNV	159.58	3412.71	1138.58	2.36		3	1 2	111.50			
		6	IIYVDDGVLSF	156.29	2265.18	1133.60	-0.71		2	1 1	130.23			
	-	7	FAIPAINVTS	153.57	2159.16	1080.59	2.34		2	1	94.27			
	S	elect	Window Name				0.72		2	1 1	122.24			
							0.87		2	2 1	. 76.14			
		Wind	dow Name : Pep	otides Spectrum			2.21		2	0 1	87.82			
				_			-0.59		2	0 1	. 54.46	Carbamidom.	··	
		✓ OK ✓ Cancel GVI GYTEDAWSSDEI GDSHSSTEDASAGTOL SPK												
	lŀ						GVLGYTEDAV	SSDFLGDSHSS	IFDASAGIQLSPK					22
	UL	_												цф
			200.000 -	_	_	_								*
		~												
		sity	150 000 -											
		intel	100 000 -											
		-	50 000					.1						
						ير الإ بال								
			0	250 5	0 750	1000 1	250 1 500	1 750	2 000 2 2	50 2 500 2	750 3 000	3 250	3 500	-
								m/z						

Now, the user can use his new 'Peptides Spectrum' on a different Identification Summary.



Filter Tables

You can filter data displayed in the different tables thanks to the filter button at the top right corner of a table.



Logs	×	F024297 Proteins ×
\bigcirc		Protein
M	1	ap P 19097 FAS2_YEAST
$\overline{\nabla}$	2	spiP07149 FAS1_YEAST
50	3	ap P 10592 HSP 72_YEAST
× 1	4	sp P16521 EF3A_YEAST
	5	sp P00549 KPYK1_YEAST
	6	sp P10591 HSP71_YEAST
•	7	ap P32324 EF2_YEAST
	8	sp P07259 PYR1_YEAST
	9	sp P11484 HSP75_YEAST
	10	sp P40150 HSP76_YEAST
	11	DIPODSEDIPCK VEAST

When you have clicked on the filter button, a dialog is opened. In this dialog you can select the columns of the table you want to filter thanks to the "+" button.

In the following example, we have added two filters:

- one on the Protein Name column (available wildcards are * to replace multiple characters and ? to replace one character)

- one on the Score Column (Score must be at least 100 and there is no maximum specified).

Filters
Filter(s)
Protein = V GLPK*
AND 100.0 <= Score <=
Peptides 🗸
V OK Cancel

The result is all the proteins starting with GLPK (correspond to GLPK*) and with a score greater or equal than 100.

Note: for String filters, you can use the following wildcards: * matches zero or more characters, ? matches one character.

📄 F0	71423 Proteins 🛚 🕿	
	Protein	Score F
	GLPK_ECODH	2503.88
10 10	GLPK_ECOLI	2503.88
	GLPK_ECOBW	2503.88

Search Tables

In some tables, a Search Functionality is available thanks to the search button at the top right corner.



Logs	×	F024297 Protein Set ×	
		Protein Set	Description
🛍 <	64	SP 1P00549 KPYK1_YEAST	Pyruvate kinase 1 OS=Saccharom
	5	sp P16521 EF3A_YEAST	Elongation factor 3A OS=Sacchar
a l	6	sp P10591 HSP71_YEAST	Heat shock protein SSA1 OS=Sac
×	7	sp P32324 EF2_YEAST	Elongation factor 2 OS=Saccharo
	8	sp P07259 PYR1_YEAST	Protein URA2 OS=Saccharomyces
	9	sp P11484 HSP75_YEAST	Heat shock protein SSB1 OS=Sacc
•	10	sp P40150 HSP76_YEAST	Heat shock protein SSB2 OS=Sacc
	11	sp P00560 PGK_YEAST	Phosphoglycerate kinase OS=Sac
	12	sp P38972 PUR4_YEAST	Phosphoribosylformylglycinamidin
	10	IDDOODTICNICO, VEACT	Feelen 200 Ceelenness

When you have clicked on the search button, a floating panel is opened. In this panel you can select the column searched and fill in the searched expression, or the value range.

For searched expressions, two wild cards are available:

- '*' : can replace all characters
- '?' : can replace one character

In the following example, the user searches for a protein set whose name contains "PGK".

Log	s ×	F024297 Protein Set	×	
		Protein Set		Description
M		Protein Set	Protein S	et = v *PCK*
T			Troteins	
	7	sp P32324 EF2_YEAST		Elongation factor 2 OS=Saccharo
	8	sp P07259 PYR1_YEAST		Protein URA2 OS=Saccharomyces
99	9	sp P11484 HSP75_YEAST		Heat shock protein SSB1 OS=Sacc
🕶	10	sp P40150 HSP76_YEAST		Heat shock protein SSB2 OS=Sacc
	- 11	sp P00560 PGK_YEAST		Phosphoglycerate kinase OS=Sac
	12	sp P38972 PUR4_YEAST		Phosphoribosylformylglycinamidin
	13	sp P00925 ENO2_YEAST		Enolase 2 OS=Saccharomyces cer

You can do an incremental search by clicking again on the search button of the floating panel, or by pressing the Enter key.

Graphics

Create a Graphic

There are two ways to obtain a graphic from data:

- 1. In the windows with PSM of a Search Result or of an Identification Summary, you can ask for the display of a histogram in a new window to check the quality of your identification.
- 2. In any window, you can click on the '+' button to add a graphic (Scatter Plot or Histogram) as a view in the same window



Tasks	Tasks Log 🛛 🕅 F067897 PSM 🕫														
		Peptide	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion	PTM	Protein Sets	F\$	23 1
m	1	ILFQK	21.38	113	2	647.40	648.41	-0.42	1	0					-
T	2	LIFAGK	28.56	113		647.40	648.41	-0.42					RS27A_YEAST		
	3	LLFQK	21.38	113		647.40	648.41	-0.42							
	4	LVDLIK	25.99	182		699.45	350.73	-0.28					PHSG_YEAST		
	5	LVDLLK	25.99	182		699.45	350.73	-0.28							
	6	LVDLIK	24.51	185	1	699.45	350.73	0.63	2	0			PHSG_YEAST		
	7	LVDLLK	24.51	185	1	699.45	350.73	0.63	2	0					
1	8	LVDLIK	22.23	187	1	699.45	350.73	1.17	2	0			PHSG_YEAST		
	þ	LVDLLK	22.2 <mark>3</mark>	187	1	699.45	350.73	1.17	2	0					
	10	VPFGGVK	31.28	200	1	702.41	352.21	0.24	2	0			ALDH6_YEAST		
	11	AAFIER	28.59	223		705.38	353.70	-0.37	2	0			RIR4_YEAST		
	12	AAFIER	23.5 <mark>3</mark>	225	1	705.38	353.70	1.47	2	0			RIR4_YEAST		
	13	LADFLK	24.19	227	1	705.41	353.71	-0.91	2	0			SYYC_YEAST		
	14	AIDLFK	25.18	230	1	705.41	353.71	2.06	2	0			SYRC_YEAST		
	15	AIDLFK	25.12	232	1	705.41	353.71	2.65	2	0			SYRC_YEAST		
	16	AGAFITK	34.03	239	1	706.40	354.21	-0.79	2	0			PYRD_YEAST		
	17	AGAFITK	41.53	240	1	706.40	354.21	0.20	2	0			PYRD_YEAST		
	18	KAMITK	27.74	240	2	706.40	354.21	-4.54	2	1		Oxidation (M3)			
	19	FGMDLK	34.44	256	1	709.35	355.68	-0.34	2	0			RIR4_YEAST		
	20	FGMDLK	23.01	258	1	709.35	355.68	0.61	2	0			RIR4_YEAST		
	21	DHLLGR	24.18	264	1	709.39	355.70	-0.91	2	0			RL16B_YEAST		
	22	HILSIK	22.97	268	1	709.45	355.73	-0.69	2	0			PYR1_YEAST		
	23	HLISIK	22.97	268	1	709.45	355.73	-0.69	2	0					
	24	HILSIK	23.16	269	1	709.45	355.73	-0.58	2	0			PYR1_YEAST		
	25	HLISIK	23.16	269	1	709.45	355.73	-0.58	2	0					۹
	26	HILSIK	24.81	272	2	709.45	355.73	-0.18	2	0			PYR1_YEAST	-	+
	27		74.81	272	2	700.45	355 73	_0.18	2	1 0		1	1		

If you have clicked on the '+' button, the Add View Dialog is opened and you must select the Graphic View

rasks Log		🛚 🕕 F067	7897 PSM 🛛 🗱										4		- 0
D	P	eptide	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion	РТМ	Protein Sets	₽	23 1
fii 1	IL	FQK	21.3 <mark>8</mark>	113	2	647.40	648.41	-0.42		L C					- 単
2 2	LI	FAGK	28.56	113		647.40	648.41	-0,42					RS27A_YEAST		
3	: LL	.FQK	21.38	113		647.40	648.41	-0.42							
2 1			75.00	107		<u> 600 45</u>	250 72	0 10							
	Ac	dd a View					100.00								
-	l r	Views													
		name						description					F		
		Graphic					G	raphics : Histo	ogram / Scatte	er Plot					
1		Proteins					P	roteins for a P	eptide Match						
1		Spectrum					S	pectrum of a F	Peptide						
1													+		
1 1 1		View Positio	on: 🔘 Bel	ow 🔘 Tab	bed 🧿 Sp	litted									
1 2											[🖌 ОК	🖌 Cancel		
2	L										_	_			
23	H	ISIK	22.97	268	1	709.45	355.73	-0.69	2	2 0					
24	1.00		22.16	260	1	709.45	355.73	-0.58		0					
	H	ILDIN	23,10	205	-			0.00					PYR1_YEAST		
25		LISIK	23.16	269	1	709.45	355.73	-0.58	-	2 0			PYR1_YEAST	-	
25 26			23.16 23.16 24.81	269	1	709.45 709.45	355.73 355.73	-0.58		2 0 2 0			PYR1_YEAST PYR1_YEAST		+



Graphic options

Graphic Toolbar



A: When this button is selected, you have the "Pointer Mode" activated.

In this mode :

- If you move with the left mouse button pressed on the middle of the graphic, you can scroll along the X and Y Axis.

- If you move with the right mouse button pressed from the top/left corner to the bottom/right corner, a zooming rectangle is displayed. When you release the mouse button, a zoom in according to the zooming rectangle is performed.

- If you move with the right mouse button pressed from the bottom/right corner to the left/top corner, a view all is done.

B: When this button is selected, you have the "Selection Mode" activated.

By clicking on the black right arrow, you can switch between the square selection mode and the lasso selection mode.



In this mode:

- Use the left mouse button to do a square or lasso selection.
- The right mouse button works as with the "Pointer Mode"

C: Zoom out / Zoom in / View all. Click on the black right arrow to select the zooming mode.

D: Display/Remove Grid toggle button

E: Display/Hide View All Map. The goal of this map is to display the whole graphic in a small zone even when you have zoomed

F: Open a settings dialog for the graphic. You can modify for example colors or bins of an histogram.

G: Lock/Unlock incoming data. If it is unlocked, the graphic is updated when the user applies a new filter to the previous view (for instance Peptide Score >= 50) If it is locked, changing filtering on the previous view does not modify the graphic.

H: Select Data in the graphic according to data selected in the table in the previous view.

I: Select data in the table of the previous view according to data selected in the graphic.

J: Export graphic to image

K: Select the graphic type: Scatter Plot / Histogram

L/M: Select data used for X / Y axis.



Popup Menu on Graphic Area

By right mouse click on the graphic area, you get a popup with several menus:

- Add Group: after selecting some data, you can create a group defined by a name and a color (there are two groups in the example : PSM xyz and PSM abc)
- Select Group: Right click on a data group to select them.
- Delete Group: Right click on a data group and delete it
- Add Vertical Cursor: Add a vertical cursor, its X position is displayed on the X Axis.
- Add Horizontal Cursor: Add an horizontal cursor, its Yposition is displayed on the Y Axis.



- Delete Cursor: Right click on a cursor and delete it.



Popup Menu on Axis

By right mouse click on an axis, you get a popup with several menus:

- Log 10 / Linear Axis : toggle the display of the axis between log and linear.
- Hide / Display Title : hide the axis title if you want more space for the graphic
- Remove / Add Vertical Grid
- Set Visible Range : to set the minimum and maximum values of the axis
- Width measurement : display 2 cursors and the measurement of the space between the cursors.

Zooming

There are several ways to perform zoom actions.

Zoom in:

- Click on the Zoom in button in the toolbar.
- Press the right mouse button and drag to the right bottom direction. A red box is displayed. Release the mouse button when you have selected the area to zoom in.
- Put the mouse over an axis and roll upward the mouse wheel. The zoom in is centered around the middle of the axis. If you press on Ctrl Key at the same time, the zoom in is performed around the mouse position.

Zoom out:

- Click on the Zoom out button in the toolbar.
- Put the mouse over an axis and roll downward the mouse wheel. The zoom out is centered around the middle of the axis. If you press on Ctrl Key at the same time, the zoom out is performed around the mouse position.



View All:

- Click on the View All button in the toolbar.
- Press the right mouse button and drag to the left top direction. When you release the mouse button, the zooming is reset to view all.

Selecting

To be able to select data, you must be in "Selection Mode"

Select: You can select with a rectangle area or with a lasso according to the selected button. Press the left mouse button and drag the mouse to surround the data you want to select. When you release the button, the selection is done. Or left click on the data you want to select. It is possible to use the Ctrl key to add to the previous selection.

Unselect: Left click on an empty area to clear the selection.



View All Map



Click on View All Map button to display the map. This map always displays the whole graphic and the zoomed area. You can directly zoom on the view all map. You can resize it and move it.



Quality Control

Search Result QC

You can run a Quality Control on any leaf Search Result, that is to say an imported Result File not a merged search result. It consists in a transversal view of the imported data: rather than visualising the results per PSM or Proteins, results are sorted according to the score, charge state...

Choose the menu option:

Projects × MzDB	Files	- Task	s Log X Tutoriel Formation D
in Tutoriel Format	on Déc 2016		Exp. MoZ per charge and score
 Identifications Al Importe Vald and n Posso Posso Posso Posso Posso 	d erge 14 16 17 Valid	5	Resultset Target PSM Decoy PSM
- C FOR	Display Search Result		MSQueries
0 108	Display Identification Summary	1	PSM
I Trash	Add Dataset		Proteins
	Rename		Quality Control
	Clear Delete		New User Window Manage User Windows
	1	-	

Settings

Configure some settings before launching the process

- Score windows: you can split your data in different groups based on the score. The default groups are : less than 20, between 20 and 40, between 40 and 60, over 60
- Max rank: data can be filtered to get a view focused on the best ranks. Default is to consider only the first rank.

*	Define settings for Statistical Reports	×
Statistical	Reports Settings	
General	Settings	¥
Statistic	al Settings	
Score	vindows (ex: 20-40-60) : 20-40-60	
	Max rank : 1	
Save	👔 Load 🔮 OK 👗 Cancel	U

QC results

The report will appear in a matter of seconds (depending on the amount of data to be processed). You will get the following tabs:


Assigned and unassigned spectra: Pie chart presenting the ratio of assigned spectra



Score repartition for Target/Decoy data: Histogram presenting the amount of PSM per group of score, separating target and decoy data





PSM per charge and score: Histogram presenting the amount of PSM per group of score and charge state



Experimental M/z per charge and score: Box plot presenting M/z information for each category of score and charge state





Number of matches per minute of RT and score: histogram presenting the amount of PSM per score and retention time. This view is only calculated when retention time is available.



Each graph is also available in a table view

Exp. MoZ per charge and score Mumber of matches per minute of retention time and score							
🌔 Assigned and unassigned spectra 🛛 🌔 PSM per charge and score 🖉 🌔 Score repartition for Target/Decoy data							
	Resultset	Score <= 20.0		20.0 < score <= 40.0	40.0 < score <= 60.0	Score > 60.0	
В	Target PSM	19	918	3 652	3 306	2 3	04
	Decoy PSM	5	589	14	0		0



Ms Files Tab

In order to facilitate different actions on Ms Files, Proline Studio contains an homonym tab providing the end user with a view over his local and server remote file system, called Local File System and Proline Server File System respectively.

Furthermore, on local file system a series of actions can take place, through an appropriate popup menu, on the encountered .mzdb and .raw files, including among others the:

- Conversion of a .raw file to an .mzdb file
- The upload of an .mzdb file
- View .mzdb files
- Detect its peakels



Apart from the popup menu supported functionality, since Proline Studio 1.5, uploads can be triggered via drag and drop mechanism.



mzDB File Upload

As mentioned earlier, after selecting a number of files, the user can either drag and drop them inside the remote site, or use the popup menu as shown in the following screenshot. It is important to precise that both approaches are not compatible with a selected group consisting of different file types.



As we can see, clicking on upload opens a dedicated dialog packing a series of uploading options:

Files Selection	
C:\Users\AK249877\Documents\Andreas\QEx2_007990b.mzdb	
Upload Options	
Delete mzdb file after a successful upload	
Create Parent Directory in Destination	
Server's mounting point : mzdb_PROD	•
V OK K Cancel	0

• The deletion of the file(s) after the successful upload

• The creation of the file(s) parent directory in destination: only the direct parent folder will be created. Otherwise, the file will be uploaded at the mount point root.

• The mounting point at the server: depending on server mounting points configuration.



Furthermore, the dialog permits us to add or remove .mzdb files to upload. The uploading tasks status could be viewed in the Logs tab.

Raw File Conversion

In the same way, when the user desires to convert and upload a raw file, he or she can use the respective dialog through the popup menu.

Files Selection		
O:\301-Projets_BGE\301.6	5-EDYP-Echange\Andrea\raw files\OEKAC141027_25.raw	
01,001110,002,002,001		
Conversion & Upload Optic	ons	
Converter (.exe) :	D:\mzdb_x64_0.9.8d\raw2mzDB.exe	
Output Path :		
	Delete raw file after a successful conversion	
	Upload myth file successful conversion	
	V opideu inizab nie successiai conversion	
Server's mounting point :	mzdb_PROD	•
	Delete, made file after a successful upload	
	V Delete Inzab ne arter a succession apload	
	Create Parent Directory in Destination	
		•

For the upload step, the same options as described above are shown.

For the conversion, the path to the converter exe file should be specified. This value will be saved upon different executions. A default path may be specified in the <u>general settings</u> dialog. The same way mzdb file may be deleted after a successful upload, raw files could be deleted after a conversion.

TIC or BPI chromatogram

When the user chooses to "View" an mzDB file, the MzScope window is opened.

By default, the TIC chromatogram is displayed. You can click on "BPI" to see the best peak intensity graph.

By clicking in the graph, you can see below the scan at the selected time.





You can choose to display 2 or more chromatograms on the same graph, by selecting 2 files and clicking on "View"



chromatogram at a given mass by entering the specified value in the panel above.

Scan



You can navigate through the scans

- by increasing or decreasing the scan Ids
- by entering a retention time
- by clicking the keys arrows on the keyboard (Ctrl+Arrows to keep the same ms level)



By double clicking on the scan, the corresponding chromatogram is displayed above (The Alt key or the check box "XIC overlay" allows you to overlay the chromatograms in the same graph).





Peakels

By selecting a file, you can click on "Detect Peakels" in the popup menu.

Projects	MzDB File	s %			
MzDB Files L	ocation				
D:\Jocal					
Raw files					
OEMMA121	101_45b.mz	zdb			
OEMMA121	OEMMA121101_47b.mzdb				
OEMMA121	101_50b.mz	zdb			
OEMMA121	101_56b.m	zdb			
OEMMA121	101_58b.mz	zdb			
OEMMA121	101_6 <u>1b.m</u> ;	zdb			
OEMMA121	101_6	View data			
OEMMA121	101_6				
		Detect Peakels			

A dialog allows you to choose the parameters of the peakels detection: the tolerance and eventually a range of m/z, or a m/z value:

🗫 Detect Peakels Param	eters ×
Processing Parameters	
Intensity percentile:	0.9
min peaks count:	5
max consecutive gaps:	3
min/max distance (count):	3
min/max ratio (0-1):	0.75
remove baseline	smooth peakels
Precursor mz	
m/z tolerance (ppm): 10.0	
• No m/z bounds	
◯ Enable m/z bounds	
minimum m/z: 0.0	
maximum m/z: 0.0	
O Detect at m/z: 0.0	
Ok	ancel



The results are displayed in a table:

OEM	3MMA121101_61b.mzdb × OEMMA121101_63b.mzdb ×							
T		m/z	Elution	Duration	Apex Int.	Area	MS Count	R.
	1	800.4038	53.88	1.41	30,457,534	1,368,053,504	40	-
	2	800.9050	53.93	1.46	25,768,910	1,132,491,520	41	-
	3	800.4013	84.90	0.85	18, 198, 898	429,421,056	28	
	4	800.4184	93.41	0.88	12,576,231	330,627,712	37	
	5	800.0841	93.44	0.81	10,422,337	276,852,832	33	
	6	800.4133	51.48	0.99	9,245,809	294,092,576	32	
	7	800.5230	35.59	1.63	8,889,166	428,618,624	56	
	8	800.7528	93.44	0.72	8,827,408	223,339,616	29	
	9	800.9026	84.94	0.69	8,689,033	191,712,608	24	
	10	800.8957	55.23	1.21	4,470,434	156,532,816	32	
	11	800.9154	51.50	1.10	4,029,301	130,792,512	36	
	12	800.3785	75.01	1.35	2,855,941	121,684,904	41	-

You can double-click (or through the popup menu) on a row to display the peakel in the corresponding raw file:



Export Data / Image

There are many ways to do an export:

- Export a Table using the export button (supported formats: {xlsx, xls, csv})
- Export data using Copy/Paste from the selected rows of a Table to an application like Excel.
- Export all data corresponding to an Identification Summary, XIC or Spectral Count
- Export an image of a view
- Export Identification Summary data into MzIdentML format (for ProteomeXchange).
- Export Identification Summary spectra list.



1. Export a Table

Projects 🕺 MzDB Files 📃	Tasks I	Log 🛛 🕄 🕕 F0756	05 PSM 88		
💼 Project 1 🔹 🗈 🖉		Peptide	PTM	Score	Calc. Mass
	m	1 FTEGAFK		29.00	798.3912
	T	2 FTEGAFK		10.26	798.3912
	S.	3 LVADLIR		26.56	798.4960
		4 LVIAVER		8. <mark>4</mark> 8	798.4958
		5 RLGVQLD		23.15	799.4549
F075605		6 IEGILEK		8.55	800.4644
i∰ () dsTest		7 LLEGELK		12.21	800.4644
🗄 🗑 dsLocal		8 WANIVAK		0.17	800.4542
		9 TVDVTIR		28.54	802.4543
E054967		10 TVDVTIR		28.01	802.4543
		11 GEPLSFR		20.10	804.4130
		12 GEPLSFR		23.08	804.4130
F083342		13 TVDALMR		8.41	804.4161
		14 LGIDFLK		39.14	804.4745
		15 AVLLSFR		30.85	804.4855
test new version		16 EQFNLR		30.07	805.4083
test old version		17 EQGVAFR		5.07	805.4080
		18 EQVMRK	Oxidation (M4)	5.07	805.4114
Д д д д д д д д д д д д д д д д д д д д		19 MMADVPK	Oxidation (M1)	0.38	806.3664

To export a table, click on the Export Button at the left top of a table.

An export dialog is opened, you can select the file path for the export and the format of the export (supported formats: {xlsx, xls, csv}).

In case that the selected format is either .xls or .xlsx, the user has now the ability to maintain in his exported excel document any rich text format elements (color, font weight etc.) apparent on the original table in Proline Studio. Choice is done using the checkbox shown on the following screenshot.

Export X	Export ×
C:\vero\DATA\Autre\Report.xlsx	C:\vero\DATA\Autre\Report.xlsx
Export Type: Excel (.xisx)	Export Type: Excel (.xlsx) ~
Export Decorated	28 %
✓ Export X Cancel	Export X Cancel

To perform the export, click on the Export Button. The task can take a few seconds if the table has a lot of rows and so a progress bar is displayed.

2. Copy/Paste a Table

To copy/Paste a Table:

- Select rows you want to copy
- Press Ctrl and C keys at the same time. The column titles are also copied

- Open your spreadsheet editor and press Ctrl and V keys at the same time to paste the copied rows. If paste is done in a text editor, the column separator used is the tabulations.



3. Export an Identification Summary, a XIC or a Spectral Count

💼 Test Vero			~	1
🏂 Identification	ns rted			
F123	Display Search Result Display Identification Summary	> >		
() F 130 👚 Trasl	Add Dataset Add Identification Folder			
	Copy Search Result Paste Search Result			
	Rename Clear	>		
	Delete			
	Import MaxQuant Result Validate Search Result			
Quantita	Merge Datasets Filter ProteinSets	>		
Tota	Change Typical Protein Identify Modification Sites			
⊕ []] Quar 	Generate Spectrum Matches Update Spectrum using Peaklist software Retrieve Protein Sequences			
🖶 🐹 Quar	Quantify	>		
	Export	>	Excel	
	Properties	_	Mzldent Spectra I Sequenc	ML ∟ist > e Fasta

To Export all data of a dataset (Identification Summary, XIC or Spectral Count), right-click on the dataset to open the contextual menu and select the "Export" menu and then "Excel..." sub-menu.

You can also export multiple dataset simultaneously, if they have the same type (Identification Summary or XIC or Spectral Count).

An Export Dialog is opened, you can select the file path and the type of the export : Excel (.xlsx) or Tabulation separated values (.tsv).

You can export with the default parameters or perform a custom export. To enable custom export, click on the tick box located on the right of the dialog:

🗫 Export			×
Export to file:	C:\vero\DATA\Autre		
Export Type:	Excel (.xlsx)		\sim
Custom Op	tions	🖌 Export	🗶 Cancel 👔

Custom export allows a number of parameters in addition to the file format to be chosen.

- Sheets to be created. You can **enable/disable** sheets to be exported, **rename** them (by double clicking on the title) or **change their order** by drag and drop. Available sheets are dependent on the identification summary type, but you can export which parameters were used (search settings or import and filters), the list of protein sets, all proteins, PSMs..., modification Clusters or statistics data.
- For each sheet, you can define the fields to be exported: change the title used in the exported file if needed, change the order of the fields using drag and drop of the corresponding row at the desired location.
- Symbol to use for decimal output (comma ',' or point '.')
- Timestamp format to use



- Protein sets to export ('all' or 'validated only')
- Export profile ('best' or 'all)

Description of the exported file is available here.

<u>Note</u>: The parameters could be saved and loaded in further export using the Save / Load buttons of the export dialog.

🗫 Export				×
Export to file: C:\vero\DATA\Autre				
Export Type: Excel (.xlsx)				\sim
 Custom Options 				
Date format: yyyy:MM:dd I	HH:mm:ss 🗸 Protein se	ts: Validated only		~
Number separator: .	 Export pro 	ofile: Best		~
Search settings and infos	☑ Import and filters	Protein sets	Protein matches in protein sets	Bes
Orientation: rows	~		Select/Unselect all fields 🛛 🗸	
Internal field Displayed field n information_p project_name	a Export			^
information_r result_set_name				
information_s search_title				
information_s search_date				
information_r raw_file_name				
information_p peakist_nie_pau	1			
information r result file direct	051/			
information in job number				
information u user name				
information u user email				
information q.,, queries count				
information s searched sequer	nc			
information s software name				
information s software version	1			
information_in instrument_confi	g			
information_d database_names	;			
information_d database_release	es			
information_d database_sequer	nc		\checkmark	
information_t taxonomy			\checkmark	
information_e enzymes			\checkmark	
information_m max_missed_clea	ava		\checkmark	
information_fi fixed_ptms			\checkmark	
information_v variable_ptms			\checkmark	
information p peptide charge	st			¥
Save Load			🖌 Export	Cancel

4. Export an Image

Any graphics in proline can be exported. Click on the Export Image Button 🔤 at the left top of the image.





An Export Dialog is opened where you can select the file path and the export type. Available formats are PNG or SVG formats. SVG format produces a vector image that can be edited and resized afterwards.



5. Export Identification Summary for ProteomeXchange

Actually it is possible to export Identification Summary into MzIdentML, Pride isn't supported any more.

<u>Note</u>: Before exporting data all spectrum matches should have been generated. To do so, right click on the dataset and select "Generate Spectrum Matches".

rest vero			
Identifications			
🖻 🕘 Co	Display Search Result	>	
	Display Identification Summary	>	
Test 0	Add Dataset		
F 1304	Add Identification Folder		
Trash	Copy Search Result		
	Paste Search Result		
	Rename	>	
	Clear		
	Delete		
	Import Search Result		
	Import MaxQuant Result		
	Validate Search Result		
	Merge Datasets	>	
Quantitatic	Filter ProteinSets		
TotalQ	Change Typical Protein		
hospi	Identify Modification Sites		
📳 Quant	Generate Spectrum Matches		
🛃 test	Update Spectrum using Peaklist software		
Quant	Retrieve Protein Sequences		
Trash	Quantify	>	
	Export	> E	xcel
			AzidentMI

Right click on the dataset you want to export and select the "Export" menu and then "MzIdentML..." sub-menu

A dialog is opened where user information may be specified (name, organization ...)



🗫 Export f	to Mzlde	ntML format X				
0	MzIden Spectrum It is also	tML parameters I Matches should have been generated before exporting to MaidentML format. recommended to jave run 'Retrieve proteins sequences'.				
First Name*:		John				
Last Name*:		DOE				
Email:		john.doe@mail.com				
URL:						
Organization	Name*:	org				
URL:						
🕞 Save		🔓 Load 🔿 Next 🔀 Cancel 😢				

The file name and path should be specified in the next step. A progress bar is shown until the file is generated. The generated file contains identification and validation data issues from the dataset. All meta information including instrument configuration as well as search engine parameters are also extracted from dataset associated data.

6. Export Identification Summary spectra list

To export valid PSM Spectra from an Identification Summary or from a XIC Dataset. The exported tsv file is compatible with Peakview.

Note: all Spectrum Matches must be generated first.

Generate Spectrum Matches

When importing a Search Result in Proline, users can view PSM with their associated Spectrum but by default no annotation is defined. Users need to generate (and save) this information explicitly.

- For a single PSM, select the icon near the Spectrum (see <u>Display Peptide and PSM</u>)
 - For a whole Search Result, Identification Summary or Quantitation Result :
 - o right click on a Dataset

•

o select "Generate Spectrum Matches"

In both cases, the following dialog will be opened. User can

- Choose to force spectrum matches to be calculated even if it is defined. This could be useful if previous spectrum matches generation was done with the wrong rules set.
- Use the fragmentation rules set defined at import. In some cases, this information is not accessible and the user should then specify it.
- Specify the fragmentation rules set to use without taking into account the rules specified at import.



Generate Spectrum Matches		×
 Choose Fragmentat With force paramet 	ion Rule Set that will be used to generate spectrum er set, new generated spectrum matches will overwr	matches. ite existing ones.
Force new spectrum matches	; generation	
Fragmentation Rules		
Use fragmentation rule set	defined at import (unknown - see dataset propertie	s)
Select Fragmentation Rule Set	:	~ 📋
	ESI-4SECTOR	^
	ESI-FTICR	
	ESI-QUAD	
	ESI-QUAD-TOF	
	ESI-TRAP	
	ETD-TRAP	
	FTMS-ECD	
	MALDI-ISD	¥

Once executed, the dataset views need to be loaded again to effectively view the spectrum matches.

How to validate a Search Result

See description of Validation Algorithm.

It is possible to validate identification Search Result or merged ones. In the latest case, the filters and validation threshold can be propagated to child Search Results.

Starting Validation





To validate a Search Result:

- Select one or multiple Search Results to validate
- Right Click to display the popup
- Click on "Validate..." menu



Validation Dialog

Identification Validation			×
Validation Parameters Typical Protein Par	ameters		
PSM			
Propagate PSM filtering to child Sear	ch Results		
Prefilter(s)			
Length >=	6	×	
AND Score >=	25.0		
AND Single PSM per MS Ouerv		×	
< Select >		~	
FDR PSM Filter			
I ensure BH V FDR <	= 1.0 %		
Optimisation based on Score	\sim		
Peptide			
Filter(s)			
< Select >	\sim		
FDR Peptide Filter			
BH V Peptide FD	R <= 1.0 %		
Protein Set			
Propagate ProteinSets filtering to ch	ild Search Results (Warning FD	R Validation will not be propagated !	
Filter(s)			
Specific Peptides >= 1		×	
< Salart >			
		~	
FDR Protein Filter			
BH V Protein FD	8 <= 5.0 %		
Scoring Type: Fisher	\sim		
📊 Save 🕕 Load		🖌 OK 🎇 Cancel	8

In the Validation Dialog, fill the different Parameters (see <u>Validation description</u>):

- you can add multiple PSM Prefilter Parameters (Rank, Length, Score, e-Value, Identity p-Value, Homology p-Value) by selecting them in the combobox.

- you can ensure a FDR on PSMs using BH or Taget/Decoy strategy. If using the latest, FDR will be reached according to the variable selected (Score, e-Value, Identity p-Value, Homology p-Value,...)

- you can add a Protein Set Prefilter on Specific Peptides count, peptides or peptides sequence count or on Protein Sets score.



- you can also ensure a FDR on protein Sets or peptides using BH or Target/Decoy strategy. The strategy must be consistent between all levels where an FDR control is done (PSM, Peptide and ProteinSet)

Note: FDR can be used only for Search Results with Decoy Data.

If you run validation on a merged Search Result, you can choose to propagate it to child Search Result. Specified prefilters will be used as defined. For the FDR Filter, it is the threshold found by the validation algorithm which will be used for childs, as a prefilter.

In the second tab, you can define rules for choosing the Typical Protein of a Protein Set by using a match string with wildcards (* or ?) on Protein Accession or Protein Description. (see <u>Change Typical Protein of Protein Sets</u>).

Identification Valid	dation ×
Validation Parameters Typical Protein Parameters	
Set Typical Protein Match	
	✔ Using rules (in priority order):
Rule 0	
Typical Protein Match : *YEAST	on Protein Accession 🗸
advanced RegEx	
Rule 1	
Typical Protein Match : *	on Protein Accession 🗸
advanced RegEx	
Rule 2	
Typical Protein Match : *	on Protein Accession 🗸
advanced RegEx	
(* = any string ; ? = any character)	
Save Doad	V X Cancel

<u>Note</u>: All validation parameters can be saved and loaded using appropriated buttons.



Validation Processing

F	Projects %	-	Tas	ks Log	88	
	💼 ProjectTest 🛛 🗸 👁			id	Category	Task Description
			Ξ	13	Services	Validation of Search Result F067899
	Step Identifications		Ξ	12	Services	Validation of Search Result F067898
	All Imported		•	11	Services	Validation of Search Result F067897
	E-O Replicate 1		2	10	Database A	Load Search Result and Identification Su
				9	Database A	Load Data for Dataset Replicate3
	E-O Replicate2		2	8	Database A	Load Data for Dataset Replicate2
				7	Database A	Load Data for Dataset Replicate1
	E-O Replicate3		2	6	Database A	Load Data for Project ProjectTest
				5	Database A	Load Data for Project ProjectTest
	f Trash		2	4	Database A	Load Projects for User menetrey
	in the second se			3	Database A	Connection to UDS Database
1	1				-	

Validating a Search Result can take some time. While it is not finished, the Search Results are shown greyed with an hourglass over them. The tasks are displayed as running in the "Tasks Log Dialog".

Validation Done

Projects 🕷)
ProjectTest	
Identifications All Imported Replicate 1 Replicate 2 Replicate 3 F067899 Trash	

When the validation is finished, the icon becomes orange and blue. Orange part corresponds to the Identification Summary. Blue is for the Search Result part.

How to filter Protein Sets

See description of Protein Sets Filtering.

The protein sets windows are not updated after filtering Protein Set. You should close and reopen the window



Starting filtering

			_
Identification All Impor All Impor dataset dataset dot ds1 ds3	s ted		
Trash	Search Result	•	
indsri	Identification Summary	•	
	identification builting		
	Properties		
	Add	Þ	
	Merge		
	Validate		
	Filter ProteinSets		
	Change Typical Protein		
	Generate Spectrum matches		
	Comment With SC		
_	Compare with SC		

To filter Protein sets of Identification Summaries:

- Select one or multiple Identification Summaries to filter
- Right Click to display the popup
- Click on "Filter ProteinSets..." menu

Filtering Dialog

ProteinSet Filtering	×
Filter(s)	
Specific Peptides >= 1	×
< Select >	~
< Select >	
Peptides count	Cancel 😱
Peptide sequence count	
Protein Set Score	

you can add multiple filters (Specific Peptides, Peptide count, Peptide sequence count, Protein Set Score) by selecting them in the combobox.

Once the filtering is done, you will have to open a new protein sets window in order to see modification.

Change Typical Protein of Protein Sets

• The protein sets windows are not updated after changing Typical Protein. You should close and reopen the window ()



Open the Dialog



To change the Typical Protein of the Protein Sets of an Identification Summary:

- Select one or multiple Identification Summaries
- Right Click to display the popup
- Click on "Change Typical Protein..." menu

Dialog Parameters

Change Ty	pical Protein				×
0	Specify rules t For each prote	o satisfy for d in set, rule 0 v	noosing typical p vill be tested the	rotei In ru	in, in priority order: le 1
Rule 0 Typical P	rotein Match : anced RegEx	sp*		on	Protein Description $\!$
Rule 1 Typical P	rotein Match : anced RegEx	*		on	Protein Accession 🗸
Rule 2 Typical P	rotein Match : anced RegEx	*		on	Protein Accession 🗸
(*= any s	tring ; ? = any (character)	V Ok	<	🗶 Cancel 😵

You can set the choice for the Typical Protein of Protein Sets by using a match string with wildcards (* or ?) on Protein Accession or Protein Description.

For Advanced users, a fully regular expression could be specified. In this case, check the corresponding option.



Three rules could be specified. They are applied in priority order, i.e. if no protein of a protein set satisfies the first rule, the second one is tested and so on.

Processing

The modification of Typical Proteins can take some time. During the processing, Identification Summaries are displayed grayed with an hourglass and the tasks are displayed in the Tasks Log Window

Combine datasets

Merge can be done on Search Results or on Identification Summaries. You have also to specify which merge mode is to be used (aggregation or union). See description for <u>combining Search Results or Identification Summaries</u>.



To merge a dataset with multiple Search Results:

- Select the parent dataset
- Right Click to display the popup
- Click on "Merge" menu



When the merge is finished, the dataset is displayed with an U or A in the blue part of the icon, indicating that the merge has been done using Union or Aggregation at a Search Result level.

Merge on Search Results



Merge on Identification Summaries



If you merge a dataset containing Identification Summaries. The merge is done on an Identification Summary level. Therefore the dataset is displayed with an U or A in the orange part of the icon.

Data Analyzer

The purpose of the Data Analyzer is to easily do calculations/comparisons on data.

To open the data analyzer, you have two possibilities:

- you can use the dedicated button that you can find in the toolbar of all views. If you use this button, the corresponding data is directly sent to the data analyzer.



Task	Tasks Log 🛛 🔀 🕕 F067897 PSM 🕫 🕕 F067899 PSM 🕬								
		Peptide	Score	MsQuery	Rank	Calc. Mas			
m.	1	ILFQK	21.3 <mark>8</mark>	113	2	647			
T	2	LIFAGK	28. <mark>5</mark> 6	113	1	647			
	3	LLFQK	21.3 <mark>8</mark>	113	2	647			
	4	LVDLIK	25.99	182	1	699			
	5	LVDLLK	25.99	182	1	699			
. .	6	LVDLIK	24.51	185	1	699			
	7	LVDLLK	24.51	185	1	699			
	8	LVDLIK	22.23	187	1	699			
	9	LVDLLK	22.23	187	1	699			
	10	VPFGGVK	31.28	200	1	702			
	11	AAFIER	28. 5 9	223	1	705			
	12	AAFIER	23.53	225	1	705			
	13	LADFLK	24.19	227	1	705			

- you can use the menu "Window > Data Analyzer"



In the Data Analyzer view, you can access all data views, to some functions and graphics. In the following example, we create a graph by adding by Drag & Drop the Spectral Count Data and the corresponding differential analysis function (beta-binomial BBinomial). Then we link them together.





You have to specify the parameters of the Function: right click on the function and select the "settings" menu

In the settings menu, select the two groups of columns on which you want to perform the BBinomial function. When the parameters are set, the calculation is started immediately and an hourglass icon is shown.



When the calculation is finished: the hourglass icon becomes a green tick, and the user can right click and select the "Display" menu to see the result (or click on the "table" icon).



PS	м	F067899 PSM 🛛 🛚	🛃 newSC Protein S	ets 🛛 😵 🚏 Data M	ixer 🛛 🖾 newSC	bbinomial 🛚 🖇	4	Þ	- 6
T		Protein Set	Overview	Basic SC F067897	Basic SC F067899	Basic SC F067898	bbinomial	r,	
	1	BYR1_YEAST		126	124	119	0,722		
	2	Gef2_YEAST		156	147	141	0,509		1
	3	A KPYK1_YEAST		253	279	276	0,527	i i	
	4	G3P3_YEAST		501	573	521	0,771		
	5	PDC1_YEAST		175	198	168	0,757		
	6	La ENO2_YEAST		446	465	483	0,438		
	- 7	BP71_YEAST		128	130	141	0,605		
	8	🔓 HSP72_YEAST		124	114	132	0,75		
	9	BC82_YEAST		124	124	118	0,755		
	10	🔓 FAS1_YEAST		61	78	83	0,219		
								1	

Available Functions

STATISTICS FUNCTIONS

FDR Computation Function

This function is used by ProStar Macro to compute the FDR.

More information: <u>http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_UserManual.pdf</u>

PValue Adjustment Function / calibration Plot

Calibration Plot for Proteomics is described here: <u>https://cran.r-project.org/web/packages/cp4p/index.html</u>

SC Differential Analysis Function

beta binomial function, useful for Spectral Count Quantitations

Xic Differential Analysis Function

This function is used by ProStar Macro. Two tests are available: Welch t-test and Limma t-test.

More information:

http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_UserManual.pdf

Missing values filter Function

This function is used by ProStar Macro to remove rows with too many missing quantitative values.

The available missing values algorithm are:

- Whole Groups: The lines (across all groups) in the quantitative dataset which contain less non-missing value than a user-defined threshold are deleted.
- For every group: The lines for which each condition contains less non-missing value than a user-defined threshold are deleted.
- At least one group: The lines for which at least one condition contains less non-missing value than a user-defined threshold are deleted.

Missing values imputation Function

This function is used by ProStar Macro to impute missing values.



More information: <u>http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_UserManual.pdf</u>

Normalization Function

This function is used by ProStar Macro to normalize quantitative values.

More information on algorithms:

http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_UserManual.pdf

TABLE FUNCTIONS

Join Function

Join data from two tables according to the selected key.

Difference Function

Perform a difference between two joined table data according to a selected key. When a key value is not found in one of the data source tables, the line is displayed as empty. For numerical values a difference is done and for string values, the '<>' symbol is displayed when values are different.

Columns Filter Function

Columns filter, let the user remove unnecessary columns in a matrix. A combobox, with prefix and suffix of the columns allows to select multiple similar columns to filter them rapidly.

Rows Filter Function

Rows filter function lets the user filter some rows of a matrix according to settings on columns.

Log Function

Create a column by calculating the Log (2 or 10) of an existing column.

CSV/TSV Import

This module lets you import data from a CSV or TSV file. Then you can do calculations and display these data directly in Proline Studio.



CSV/TSV Import Settings					
		CSV/TSV Import	-		
CSV/TSV File :	D:\Max	Quant\MaxQuant.csv			
Separator					
🔘 Tab) Co	omma			
Semicolor	n 🔘 Sp	ace			
○ Other					
Preview					
log2(Id)		Protein Set	log2(Raw abundanc 🖽		
	14.1910	B2RQQ1_MOUSE	27.6478 🔺		
	14.1908	B2RXX9_MOUSE	26.9022		
	14.2066	ECHA_MOUSE	28.5793		
	14,1864	NDUS1_MOUSE	27.4212		
	14,1902	AT 242_MOUSE	2/./402		
<			•		
			V OK X Cancel		

The separator is automatically selected according to the csv file. But you can modify it.

The preview zone displays the first lines of the file as it will be loaded.



Expression Builder

Expression Builder Settings			×
	F067897 Expression Builder		
Expression Builder fx = mean(Calc, Mass) fx Functions p = abs p = bog2 p = log10 p = std	Variables Variables	▲ 7 8 9 4 5 6 1 2 3 € 0 . ± () , or not = < > >:	Image: A state of the state
		✓ ОК	X Cancel

The expression builder lets you create an expression with built-in functions or comparators and variables (columns from the linked matrix). In the example, we calculate the mean of a column in the matrix.



Prostar Macro

- 84											
	Refresh Data		Process Graph	Do	calculation	IS					
TO D	ta Analyzer	A					ProS	tar			A.
	bata whoows	100-10fmal 1% Qua						-			îi .
0 e 4	Macros	\sim	tuto6 - XIC 108-1. Oversti Protein Sete	tuto6 - XIC 100-1.	tuto6 - XIC 100-1.	tuto6 - XIC 100-1.	tuto6 - XIC 100-1. Missing Makao Elitor	tuto6 - XIC 100-1.	tuto6 - XIC 100-1. Microing Voluce Imputatio	tuto6 - XIC 100-1.	tuto6 - XIC 100-1. Calibration Rist ALL
-f	C Functions	3		Continue rater	Normo Filler	Loge	missing values riter	normanzadon	missing values imputation		Calibration Proc ALL
E	fx Table		star 🗶 👻	fx 🔻	fx 🔻	fx 🔻	fx 🔻	fx 🔻	fx 🔻	fx 🔻	<u>₩</u> ▼
	-fx Column	s Filter									
	fx Rows F	iter									
	-fX Express	sion Builder E		(4) (5)		(7)	(8) (9)	(10)	tuto6 - XIC 10(11) 📰
	-fx Log10		CSV/TSV Import					C		<u> </u>	Seatter Plot
	-fx Differen	nce	Caerrae amport							1	
	fz Statistics									n n	
	fx Missing	Values Pilter									
	-fx Missing	Values Imputation									(12)
	-fx SC Diffe	erential Analysis	2								FDR Computation 1.0
	-fx xic off	ferential Analysis									
	fx PValue	Adjustment									JX V
1	Graphics	mperation									
	- Box Plot	-									(13).
	m		× [•
8 3	Prostar										
📼 🇯	3 -			-1		- iti	Name		Value		m ¥
9				111	1	T	1 FDB		0.0034415447	07460914395	
1					1	>					
				and the second s	1		(14)				
1 1	8-										
ē	8-	and the second se	- Com		1	- 0					
ā	8-					0					
ē	00					E					
ē	8-				(15)	=					-
		0.2	0.4 0.6	0.8 1.0	(15)						-
M	8	0.2	0.4 0.6	0.8 1.0	(15)		Graphic : Scatter Flot •	X Axis: Limma log Ra	20 v]	Y Asss: [dog10(Jimme PVai	- (a)
M	8 - 0.0 Abun mvi(0.2	0.4 0.6 a PValue dog10	0.8 1.0 D(Limma PValue) Limma	og Ratio Differentia	al Proteins	Graphic : Scatter Hot •	X Avis: Limma log Ra	20 v	Y Axis: Hog10(Jamma PVal	
a M X	0.0 Abun mwl(1.654430 1.57674	0.2 (norm(log2(Abun Lime 6.4338	0.4 0.6	0.8 1.0 0(Limma PValue) Limma 2.6894 2.810	0. 8664 0. 1054	al Proteins	Graphic : Scatter Plot •	X Axis: Limma log Ra	» •]	Y Axis: Hog10(Jamma PVal	un) v m
	Abun mvi(1 -6.4930 2 -5.7674 -4.2942	1 0.2 (norm(log2(Abun Limer -6.4338) -5.7713 -4.2448	0.4 0.6 a PValue -log 10 0.0020 0.0015 0.9006	0.8 1.0 0[Linma PValue] Limma 2.6894 2.8319 1.0428	0 Ratio 0.1664 0.1712 0.0628	al Proteins 73	Graphic : Scatter Plot	X Aves: Limma log Re Selection		Y Axis: dog10(Jamma PVai	-
	0.0 Abun mvij 1 -6.4930 2 -5.7574 3 -4.2942 4 -5.8051	norm(log2(Abun 6.4338 -5.7713 -5.748 -5.768	0.4 0.6 a PValue dog 10 0.0905 0.09806 4.980-9	0.8 1.0 0[Linma PValue] Linma 2.8319 1.0429 3.3492	0 Ratio 0. 3664 0. 4732 0. 6628 0. 2855		Graphic : Scatter Hot •	X Axis: Limma log Ra	8	Y Axis: Hog10(Jamma Pila)	ue) v 55 Ge
	Abun mvel 	0.2	0.4 0.6 a PValue -iog 10 0.0020 0.0015 0.0906 4.990-4 9.152-4 0.008	1 1 0.8 1.0 2(Jinma PValue) Linma 2.6894 2.6894 2.6894 3.038 3.0388 2.3391	0 Ratio 0. 1664 0. 1712 0. 0628 0. 2005 0. 2759 0. 11469		Graphe: Scatter Flot •	X Avis: Limma log Ra	55 · · ·	Y Axis: Hog 10(Janma PVal	13 (2) (2) (2) (2) (2) (2) (2) (2) (2) (2)
	Abun meti 	1 0.2 1000000000000000000000000000000000	a PValue +og 10 0.0020 0.0020 0.0005 0.0005 4.9854 0.0046 0.0046	0.8 1.0 2(Jimma PValue) Limma 2.6894 2.4839 1.0428 3.3982 2.3231 2.779	0g Ratio 0. 1664 -0. 1664 -0. 0628 -0. 2755 -0. 2759 -0. 1449 0. 2528		Graphic: Scatter Hot. •	X Axis: Limma log Re Selection		Y Axis: dog10(Jimma PVai Differential Protein	- (1) - (2) - (2)
	0.0 Abun moi(1 6.4430 2 -5.7674 3 -4.2342 4 -5.8051 6 -5.3666 0 -4.3324 7 -9.047 9 -8.5641	r 0.2 (correllog2(Abun Liner -6.433) -4.2448 -5.7513 -4.2448 -5.7513 -4.2448 -5.6442 -4.3056 -9.8677 -8.8570	1 1 1 0.4 0.6 0.0020 0.0015 0.0906 4.990-4 9.152-4 0.0011 0.0398	0.8 1.0 2.6394 2.6394 1.0428 3.0388 2.3231 2.9779 1.4018	0 Ratio 0. 1664 0. 1712 0. 0628 0. 2355 0. 2355 0. 2355 0. 2355 0. 2359 0. 2359		Graphic : Reatter Hot	X Avis: Limma log Re Selection		Y Axis: diog10(Jamma Pikal	• (u
	0.0 Abun mel 1 -6.4930 2 -5.7674 3 -4.2942 4 -5.8051 6 -6.3466 6 -6.3466 6 -6.3466 9 -8.3466 9 -8.3467 9 -8.0471 9 -8	1 0.2 (rom@g2/Aban Lenn 4.4338 4.7773 4.2448 4.5466 4.6465 4.8675 4.8677 4.8677 4.8677	1	0.8 10 0.8 10 2,6394 2,8359 1,0438 3,0438 2,3251 2,3779 1,408 3,338 2,3251 2,3779 1,408 3,338 2,3251 2,3779	0 g Ratio 0.1664 0.1712 0.0628 0.2759 0.1440 0.2328 0.0549 0.2328 0.2328 0.0549 0.2328 0.0549 0.2400		Graphic: Beatter Flat •	X Axis: Limma log Re Selection		Y Axs: Hog10(Jimma PVal	- (a)
	0.0 Abun mvii 1 -6.4430 2 -5.7874 3 -4.2342 4 -5.8051 6 -5.3054 7 -9.8047 8 -5541 0 -1.7229 1 -6.7229 1 -6.7229	1 0.2 (0.2)	0.4 0.6 0.0000 0.0005 0.0005 0.0005 0.0005 0.0001 0.0000 0.0001 0.0000 0.0001 0.0000 0.0001 0.0000 0.0001 0.0000 0.0000 0.0001 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.00000 0.000000 0.000000 0.00000000	1 1 0.8 10 2.6819 1.04999 1.04999 1.04999 1.04999 1.04999 1.04999 1.04999 1.049	og Ratio 0. 5644 0. 5712 0. 2325 0. 2325 0. 2325 0. 2325 0. 2325 0. 2325 0. 2325 0. 2326 0.		Graphic: Retter Hot +	X Ave: Lumma log Ra		Y Axts: 4g10(Jawa Piel) Differential Protein	• • • •
	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	1 0.2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	400 ± 0.6 0.0000 0.0000 0.0005 0.	0.8 10 2[Lmma PFlabe) Limma 2.4899 1.4498 1.3492 2.3779 1.4018 3.2035 8.8771 2.3080 3.6920	0 Rabi 0.1664 0.1712 0.0684 0.2015 0.2015 0.2019		Graphic: Scatter Flot, e	X Ave: Limma by Re Selection	•	Y Ast: [sq100,ame Peak	
	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0.2 () () () 0.2 () () () () () () () () () () () () ()	0.4 0.6 40.8 40.0 50 50 50 50 50 50 50 50 50 50 50 50 50	1 0.8 1.0 0.8 1.0 20,1mma PYaba) 1.4438 1.4448 1.	4.154 4.154 4.172 4.028 4.2759 4.144 4.2759 4.1542 4.2580 4.2580 4.2188 4.200 4.2		Craybe: Scatter Flot • Craybe: Scatter Flot • Craybe	x Axe: Lumma log Re Selection	6	Y Avis: disg10(amma meal b) b Differential Protein	
	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	A PValue	0.8 10 2,4894 2,4894 2,4894 2,330 1,530 2,2391 2,3799 1,4018 3,2535 8,8771 2,3940 1,5755 1,5755	0 g Rabo 0 g Rabo 0 J Merer Ia 0 J Merer		Graphic : Scatter Hot • Graphic : Scatter Hot • Import 	X Ave: Limma log Ra		Y Auto: ing 100, www. Protein	

1 or 2: Add XIC Data to Data Analyzer from the Protein Set View or by importing data from a csv file.

3: Add Prostar Macro by a drag and drop and link XIC Data to the Macro. And do the calculation by clicking on the button Process Graph.

During the process, the Data Analyzer will ask you settings for each function.

4: Filter unnecessary columns from your data if. Settings can be validated with no parameters if you don't need it.

5: Filter is needed only if you want to remove contaminants. Settings can be validated with no parameters if you don't need it.

6: Log is needed to log abundances (Data from Proline). For Data coming from MaxQuant, data is already logged.

7 to 13: follow the settings asked (you can find some help in Prostar documentation, or information in corresponding functions.)

During the process, results will be automatically displayed:

14: FDR Result

15: Calibration Plots

16: Result Table with differential Proteins Table and the corresponding scatter plot. You can select differential proteins in the table, to import them in the scatter plot and create a colored group with them.

If you want to look at other results, right click on a function and select "Display in New Window"

Prostar User Manual:

http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_UserManual.pdf

Prostar Tutorial :

http://bioconductor.org/packages/release/bioc/vignettes/Prostar/inst/doc/Prostar_Tutorial.pdf



Calculator

Calculator lets you write python scripts to manipulate freely viewed data.

1) To open the calculator, click on the calculator icon (not available on all views for the moment)

On the left part of the calculator, you can access all viewed data, double click to add a table or a column to the script.

- 2) Write your python script on the text area
- 3) Execute it by clicking on the green Arrow.

4) When the script has been executed, the results of the calculations (variables, new columns) are available in the "Results" tab. Double click on a new column to add it to the table. Or like in the example, directly add the column to the table programmatically.

Quant. Poptide Peptide Piptide	asks Log	× (F067897	PSM 🕺 🕕 F06	7899 PSM	8	newSC Protein S	ets 🛛 📽 🚏 Data	Mixer 🛛 🖾 newSC	: bbinomial 🛛 🕺 🤇	F067899	4)
Protein Set Overview #Peptide #Quant. *Status #Peptide #Q67897 #9067897 C log(specific:C) 1 PYR1_YEAT 93 80/Ypical 51 15.00 42 2 #F2_YEAT 60 33/Typical 51 156.00 50 3 KPK1_YE. 50 50 50 Typical 46 248.00 5,51 3 Say YEAST 42 37/Typical 34 315.00 50 50 Potton Calculator #### Algorithm to calculate the logarithm of a column ##### # get the Table 3 which corresponds to table newSC Quanti Protein Sets # get the Table 3 which corresponds to table newSC Quanti Protein Sets # anwSc Quanti Protein Sets # get the column 10 of the table t (Specific SC column) # mable() is called to be able to modify data gpecific:SCCol[] # opecific:SCCol[] # anwbe of rows of the column froe in range (0,nb): # anwbe of rows of the column # opecific:SCCol[] # anwbe of rows of the column # opecific:SCCol[] # anwbe of rows of the column # opecific:SCCol[] # opecific:SCCol[] # anwbe of rows of the column # anwbe of rows of the column # opecific:SCCol[] # opecific:SCCol[] # opecific:SCCol[] # op	teins Set	ets (4480))									
1 PYR LYEAST 93 80 Typical 67 125.00 47 2 F2_TEAST 60 53 Typical 51 156.00 50 3 FYR LYE 50 50 Typical 46 248.00 5,51 4 G3P3_YEAST 42 37 Typical 34 215.00 5,75 4 F2_TEAST 42 37 Typical 34 215.00 5,75 5 Python Calculator F### Algorithm to calculate the logarithm of a column #### # get the Table 3 which corresponds to table newSC Quanti Protein Set # antable() is called to be able to modify data geather column 10 of the table t (Specific SC column) # mutable() is called to be able to modify data geath:SCCOI = f101.mutable() 3 <		Prote	in Set	Overview	#Peptide		#Quant. Peptide	 Status F067897 	Peptide Number F067897	Specific SC F067897	log(specificSC)	E
2 BF2_VEAT 60 \$3Typical \$1 156.00 \$0,0 3 KPYK1_YE 50 \$0 Typical 46 248.00 \$,51 4 G23_YEAST 42 37 Typical 34 315.00 \$,57 Python Calculator Image: Calculate the logarithm of a column #### # get the Table 3 which corresponds to table newSC Quant Protein Set t = Table.get(3) # ### Algorithm to calculate the logarithm of a column #### # get the constant column 10 of the table t (Specific SC column) # mutable() is calculate to be able to modify data specificSCCQ unit Protein Sets # mutable() is calculate the column for in range (0,nb): # calculate the log (NAV values for errors) # specificSCCColi() # newSC Coli() # specificSCCColi()	, 1	1 🚺 PY	YR1_YEAST			93	80	Typical	67	125.00	4,8	28
3 KPYK1_YE 50 50 Typical 46 248.00 5,51 4 G3P3_YEAST 42 37Typical 34 315.00 5,75 Python Calculator Tables Tables Python Calculator Tables Python Calculator Tables Python Calculator Python Calculator Tables Python Calculator Python Calculator Python Calculator Python Calculator Python Calculator Pythor Calculator Pythor	2	2 🚺 EF	2_YEAST			60	53	Typical	51	156.00	5,	, ∂ 5 °
4 GSP3 YEAST 42 37 Typical 34 315.00 5,75 Python Calculator Image: Calculate in the image: Calculat	3	з 🚺 КР	PYK1_YE			50	50	Typical	46	248.00	5,5	i13
Python Calculator Data Functions Results Tables #### Algorithm to calculate the logarithm of a column #### # pot 7067899 PSM #get the Table 3 which corresponds to table newSC Quanti Protein Set t = Table.get(3) # pettide B: newSC Quanti Protein Sets B: newSC Quanti Protein Sets #get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets #get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets #get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets #get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets #get the (on the table of the column nb = len(specific SCC col) # newSC Quanti Protein Sets #get the (on the data of the column nb = len(specific SCC col) # calculate the log (VAI values for errors) * = specific SCCol[] * v = specific SCCol[] = float(NaN) else: specific SCCol[] = math.log(v) # set the column name which will be used to the user * add the created column tame ('hog(specific SCC') # add the created column to the table t t.addColumn(specific SCCol)	4	4 🚺 G	3P3_YEAST			42	37	Typical	34	315.00	5,7	753
Data Functions Results Tables #### Algorithm to calculate the logarithm of a column #### get the Table 3 which corresponds to table newSC Quanti Protein Set Tables # get the Table 3 which corresponds to table newSC Quanti Protein Set Tables # get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets # get the constant column 10 of the table t (Specific SC column) # newSC Quanti Protein Sets # newSC binomial # newSC binomial # newSC Column in the len(specific SCCol) # loop on the data of the column the column for in range (0,nb): # calculate the log NaN values for errors) v = specific SCCol[j] if v < 0:	8	🐤 Pyth	non Calcula	itor		-	_	-	-	and the second	×	165 247
	Quan sptide 11 25	Data	Functions s Data Window Fo67899 RewSC 0 Fo67897 rewSC 0 Fo67897 Fo67897	Results WS PFSM Peptides Quanti Protein Sets ewSC Quanti Prote ewSC Quanti Prote pbinomial 7PSM	s ein Sets ides	#### # get t = 1 # get # mu spec # nu for i for i for i el: # set spec # add t.add	# Algorithm to cal the Table 3 whic Table.get(3) the constant col itable() is called to ificSCCol = t[10].r mber of rows of the len(specificSCCol = t[10].r mber of rows of the len(specificSCCol[1]) calculate the log (= specificSCCol[1] = specificSCCol[1] = the column name ificSCCol.setColum d the created colu dColumn(specificSC	Iculate the logarith h corresponds to t b be able to modify mutable() ne column) the column NaN values for en = float('NaN') = math.log(v) = which will be used nnName('Tog(speci umn to the table t CCol)	nm of a column #### table newSC Quanti Pro le t (Specific SC columr / data rors) d to the user (ficSC) ")	ptein Set	3	134 144 129 11 109 96 134 164 173 185 186 177 185 186 177 185 186 177 185 186 177 185 186 177 185 186 177 185 186 177 185 186 177 185 186 177 187 187 187 187 187 187 187 187 188 188 188 188 187 187 187 187 187 187 187 187 187 187 187 187 187 187 187 187
Execution Time: 0:2.29		•		III	•	Exec	ution Time: 0:2.29)				

Examples

Script to calculate a log column

Algorithm to calculate the logarithm of a column

get the Table 3 which corresponds to table newSC Quanti Protein Set t = Table.get(3)

get the constant column 10 of the table t (Specific SC column)
mutable() is called to be able to modify data



specificSCCol = t[10].mutable()

```
# number of rows of the column
nb = len(specificSCCol)
# loop on the data of the column
for i in range (0,nb):
    # calculate the log (NaN values for errors)
    v = specificSCCol[i]
    if v <= 0:
        specificSCCol[i] = float('NaN')
else:
        specificSCCol[i] = math.log(v)
```

set the column name which will be used to the user specificSCCol.setColumnName("log(specificSC)")

add the created column to the table t
t.addColumn(specificSCCol)

Script to perform a difference and a mean between two columns

Algorithm to perform a difference and a mean between two columns

```
t = Table.get(9)
colAbundance1 = t[3]
colAbundance2 = t[5]
# difference between two columns
colDiff = colAbundance1-colAbundance2
# set the name of the column
colDiff.setColumnName("diff")
# mean between two columns
colMean = (colAbundance1+colAbundance2)/2
# set the name of the column
colMean.setColumnName("mean")
# add columns to the table
```

t.addColumn(colDiff) t.addColumn(colMean)

Script to perform a perform a pvalue and a ttd on a XIC quantitation table

```
#### Algorithm to perform a pvalue and a ttd on abundances column of a XIC quantitation
####
t = Table.get(1)
pvalueCol = Stats.pvalue( (t[2], t[3]), (t[4],t[5]) )
ttdCol = Stats.ttd( (t[2], t[3]), (t[4],t[5]) )
pvalueCol.setColumnName("pvalue")
ttdCol.setColumnName("ttd")
t.addColumn(pvalueCol)
t.addColumn(ttdCol)
```



Update Spectrum using Peaklist software

When <u>importing a search result</u>, the software used for the peaklist creation has to be specified. This parameter is mandatory for the <u>XIC quantitation</u> as it is used to find scan number or RT in the spectrum title. Indeed, this information is then used to extract abundances in the *raw files*.

If an invalid software has been specified when importing, it is possible to change the peaklist software afterwards. This option is only valid for Identification DataSets.

Right click on the identification DataSet, and select "Update Spectrum using Peaklist software"



The following dialog will be displayed allowing the user to select the peaklist software to use.

Peaklist Softwares		
Choose Peaklist Software to u	e to generate (new) s	pectrum parameters.
avtract men ava		<u>^</u>
Mascot Distiller		
mascot.dll		E
MaxQuant		
Proline 1.0		
Protein Pilot 4.5		
Proteome Discoverer		-

Spectral Count

See description of Compare Identification Summaries with Spectral Count.



Generate a Spectral Count

To obtain a spectral count, right click on a Dataset with merged Identification Summaries and select the "Quantify \rightarrow Spectral Counting" menu in the popup. This Dataset is used as the reference Dataset and Protein Set list as well as specifics peptides are defined there.

				Y		id	Category	Criticalit
🏂 Identificatio	ns			0	-	18	Services IMS	High
📔 All Impo	rted			•		17	Services JMS	High
⊨				•	~	16	Services JMS	High
⊡	Display Search Result		'			15	Database A	Medium
	Display Identification Summary		>		•	14	Database A	Medium
						13	Database A	Low
	Add Dataset				۷.	12	Database A	Low
	Add Identification Folder				1	11	Database A	High
□	Conv Search Result				~	10 9	Database A Database A	Medium Medium
					•	8	Database A	Medium
	Paste Search Result					7	Database A	Medium
-	D				•	6	Database A	Medium
	Kename		'			5	Services JMS	Low
F1	Clear				٠	4	Services JMS	Low
🕀 📄 Tes	Delete					3	Services JMS	Medium
🔤 🏦 Tra					×	2	Database A	High
	Import Search Result				×	1	Services JMS	High
	Import MaxQuant Result				-	0	Services JMS	High
	Validate Search Result							
	Merge Datasets		>					
	Filter ProteinSets							
	Change Typical Protein							
	Identify Modification Sites							
	Generate Spectrum Matches							
	Lindata Canatana unian Daaldist - fturr							
	Opdate Spectrum using Peaklist software.							
Quanti	Retrieve Protein Sequences			Task:				
E Tot	Quantify		>		Spe	ectral Co	ounting	
	Export		;		Lab	el Free.		
e re			-		Res	idue La	beling	
tes	Properties				lso	baric La	beling	
🕕 💷 Ouant		T	П	LIN				

In the Spectral Count window, fill the name and description of your Spectral Count and press Next.

	🎭 Spectral Co	unt Wizard	×
	🎢 Step 1:1	Define spectral count name and description.	
l	Spectral Cour	it	
	Name:	Spectral Count agg2	
	Description:	Your Description	
		🚽 Next 🛛 🗶 Cancel	



Then select the Identification Summaries on which you want to perform the Spectral Count and press Next.

spectral Count Wizard
Step 2: Select Identification Summaries.
Select the Identification Summaries for which Spectral Count will be calculated. The common list of protein sets and peptide specificity will be calculated at parent level, from which spectral count has been run.
ds
F083066 F083067 □-••• replicate2 -••• F083068
F083069 F083070
Next X Cancel

Finally select the DataSet where shared peptides spectral count weight should be calculated and press OK.



A Spectral Count is created and added to the Quantitations Panel.


Projects 🕺 MzDB Files	=
Tutoriel	
 Identifications Idl Imported valider puis merger of the merger puis valider of the merger pui	
Quantitations 	

Display a Spectral Count

You can then display a Spectral Count, see Display a Spectral Count

Label Free Quantitation

For description on LC-MS Quantitation you can first read the principles in this page: <u>Label-Free quantitation</u> <u>principles</u>

Create a New Label Free Design

You can create a new label-free design by clicking on the "Quantify \rightarrow Label Free" menu from Quantitation or from a dataset in the Identification tree. In this case, the selected dataset will be used as reference for the quantitation (from where the list of peptides and protein is extracted).



Settle Design

When you start the creation of a new XIC Design, the following dialog is opened:



		🛃 Drag & Drop	
Create Rename Delete OEM OEM	Sample Group 1101_43b.raw 1101_47b.raw 1101_47b.raw MA121101_56b.raw MA121101_45b.raw MA121101_50b.raw MA121101_58b.raw	Identifications F083342 Image: Construction of the second secon	

In the left Area, you can create your design :

- by adding Group and Samples (thanks to mouse right click popup)
- by renaming every items (thanks to F2 or mouse right click popup)
- by rearranging order of groups and samples (thanks to drag & drop)

From the right Area, you can drag and drop desired identifications to the left Area. If you drop in the XIC or in a Group node, Group and Sample nodes will be automatically added. But you can drop directly in a Sample node.

Note : it is recommended that the XIC node is renamed.

When your design is ready, click on Next Button.

Link to Raw Files

The following panel is displayed:



files association					
Group Fr 1 Group Fr 1 Group Fr 1 Group Fr 1 Group 2 Group 2 Group 2	Sample Sample Fr1 Sample Fr1 Sample Fr1 Sample Fr1 Sample Fr1	Sample Analysis F127609 F127603 F12760922 F127614 F127617 F127620	mzDB File 20070918_CL_Orbi2 20070918_CL_Orbi2 20070918_CL_Orbi2 20070919_CL_Orbi2 20070919_CL_Orbi2 20070919_CL_Orbi2	P E	
stomatic MS file ass	es were associated.	С С С С С С С С С С С С С С С С С С С	Drop Zone ur .mzdb files & folder here	rs	VELOS23144.mzdb VELOS23374.mzdb VELOS23376.mzdb VELOS23378.mzdb VELOS23380.mzdb VELOS23664.mzdb VELOS23666_vds.mzdb VELOS23666_vds.mzdb VELOS23666_vds.mzdb VELOS23670.mzdb VELOS23670_vds.mzdb

In order to be able to perform any XiC design, all participating Sample Analysis must be associated with a corresponding raw file. The association is done at the second step of the XIC design by :

- drag & drop the "mzDB File" into the raw corresponding to the correct sample analysis. It must be noted that although it is possible to overwrite an existing association (done in a previous quantitation using the same sample analysis dataset), there is no verification done to ensure compatibility between the mzDb file and the sample analysis dataset.
- using the Drop Zone. This feature can be quite helpful in cases where multiple associations are missing or when the plethora of uploaded .mzdb files intimidates the user from manually searching the files one by one. The feature itself is extremely easy to use as it just requires dragging a set of files or folders containing .mzdb files from the user part. As soon as a drop takes place, all missing connections will be automatically created as long as a matching .mzdb file has been dropped in the Drop zone. It must also be noted that since version 1.5, users have now at their disposal indices about the association source. Furthermore, in order to protect from a possible corruption of an existing association in the database, the latter ones cannot be overwritten.

When all participating Sample Analysis are associated with a raw file, click on Next Button.

Quantitation Parameters

Now you must select the parameters. See <u>Label-free LC-MS quantitation configuration</u> to have more details about the different parameters.

These parameters are not all displayed. You can display a complete set of parameters by clicking on the "Advances Parameters".



Quantitation Wizard		×
Step 3: Specify	quantitation parameters.	
Advanced Parameters		
uantitation Parameters	(3.0)	
Moz tolerance (ppm): 5.	נ	
Cross Assignment —		
Allow cross assignment	Within groups only \checkmark	
RT tolerance (s):	42.0	
Align RT		
Time tolerance (s):	600.0	
🚽 Save 🛛 🕕 Load	🗲 Back 🖌 OK 🎇 Cancel	0

Note: all the parameters are already set with default values, or with previous values if a quantitation has already been run.

Clone a Label Free Design

You can copy a XIC design by selecting the "Clone & Extract Abundances" option from an existing quantitation result. The new XIC is generated using an existing Experimental Design and parameters setting.





Displays

Newly generated quantitation designs are immediately added to the Quantitation Tree. Through the latter one, and via a popup menu, the end user has the capacity either to view a design's properties as seen to the following screenshot, or to apply a series of actions on it, including among others:

- Display experimental Design parameters, results and abundances : see how to Display a quantitation result
- Delete a quantitation Design, see how to Delete Data
- Rename a quantitation Design, by clicking on "Rename..." in the popup menu.
- Export the quantitation results, see how to Export Data



	Group	Туре	XIC	E.
-1	General Information			*
2		XIC id	3615	
3	Identification Summary	Identification Commonwid	141	
4		Identification Summary Id	141	
8		Date	20 mai 2015	
7	Quantitation Processing Config	Date	201181 2013	
8	Quantitation noccosing comig	aln method name	EXHAUSTIVE	
9		aln_params / ft_mapping_params / moz_tol	5.0	
10		aln_params / ft_mapping_params / moz_tol	PPM	
11		aln_params / ft_mapping_params / time_tol	600.0	
12		aln_params / mass_interval	20000	
13		aln_params / max_iterations	4	
14		aln_params / smoothing_method_name	TIME_WINDOW	
15		aln_params / smoothing_params / min_windo	50	
16		aln_params / smoothing_params / window_o	20	
17		ain_params / smootning_params / window_size	200	
18		dustering_params / intensity_computation		
20		dustering_barans / moz_tol_unit	DDM	
21		clustering params / time_computation	MOST INTENSE	
22		clustering params / time tol	15.0	
23		detect_features	false	
24		detect_peakels	true	
25		extraction_params / moz_tol	5.0	
26		extraction_params / moz_tol_unit	PPM	
27		ft_filter / name	INTENSITY	
28		ft_filter / operator	GT	
29		ft_filter / value	0.0	
30		ft_mapping_params / moz_tol	10.0	
31		ft_mapping_params / moz_toi_unit	120.0	
33		normalization method	MEDIAN INTENSITY	
34		start from validated peptides	true	
35		use_last_peakel_detection	false	
36	Quantitation Method			
37		Name	label free based on the extraction of feature	
38		Туре	label_free	
39		Abundance Unit	feature_intensity	
40	Master Quantitation Channel 1	Master OverFitzFor id	724	
41		Master Quantitation Iu	734	
43		Serialized Properties		
44	Ouantitation Channel 1			
45		Quantitation Channel id	2882	
46		Result File Name	OEMMA121101_43b.raw	
47		Raw File Path	D:\Data\Claire\OEMMA121101_43b.raw	
48		Mzdb Raw File Name	OEMMA121101_43b.mzdb	
49		Identification Summary Id	117	
50	Biological Sample 1			
51		Biological Sample id	2027 Crown de2, 10-male, de2, 1	
52	Ouantitation Chappel 2	biological Sample Name	Group ds3-15ample ds3-1	
54		Quantitation Channel id	2883	
55		Result File Name	OEMMA121101 45b.raw	
56		Raw File Path	\\DSV_D01\Shares\301-Projets BGE\301.1	
57		Mzdb Raw File Name	OEMMA121101_45b.mzdb	
58		Identification Summary Id	123	
59	Biological Sample 2			
60		Biological Sample id	2028	
61		Biological Sample Name	Group ds3-2Sample ds3-2	
				Ŧ



Isobaric labeling Quantitation

For description on isobaric labeling quantitation you can first read the principles in this page: <u>Isobaric labeling</u> <u>quantitation workflow</u>

Create a New Design

You can create a new label-free design by clicking on the "Quantify \rightarrow Label Free" menu from Quantitation or from a dataset in the Identification tree. In this case which is recommended, the selected dataset will be used as **reference** for the quantitation (from where the list of peptides and protein is extracted).

Quantitat	ions	
🛄 💼 Trash	Quantify	Spectral Counting
Add Quantitation Folder		Label Free
		Residue Labeling
		Isobaric Labeling

Settle Design

When you start the creation of a new Design, the following dialog is opened:

🐎 Quantitation Wizard	×
Step 1: Define the experimental design Drag and Drop identification summaries from the right panel to build the experimental design on the left.	
Quantitation Method Name: Image: I	
	Next X Cancel

First select the method name in the drop-down list.

In case of "simple" isobaric quantitation, each run is quantified separately, the different conditions are indeed pulled in the same sample with a specific tag. However, Proline allows you to configure the quantisation of several runs at



once, in the case of fractions for example. Each run will produce a separate quantitation result as if you had run them individually.

Ouantitation Method	
Name: TMT 16plex V Multi Batch Quantitation	
Experimental Design	
Drag&Drop datasets to quantify here :	TMT-Replicats
F 166558 F 166559	F 166559
All Quantifications Quant-1	

But it is also possible to execute a multi-batch quantitation. In this case, a single quantitation will be performed and the result of all the runs will be concatenated as different channels.

Warning: If multi-batch quantitation is selected, at the next step it is mandatory to use "Rescale reporter abundances..."

Quantitation Method Name: TMT 16plex Multi Batch Quantitation	
Experimental Design	TMT-Replicats



Isobaric labeling quantitation parameters

When you click next, a view with isobaric labeling specific parameters is displayed.

🐎 Quantitation Wizard			
Step 1.a: Specify isobaric quantitation method parameters.			
Isobaric tagging parameters			
Reporter ions m/z tolerance (ppm): 25.0			
Reporter ions will be extracted from: $\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			
Rescale reporter abundances to MS1 signal			

- Reporter ions m/z tolerance (ppm): m/z window used to match a peak in MSMS spectra with an isobaric tag mz
 - Reporter ions will be extracted from: specify where intensity values are read
 - **Proline spectrum** : from spectrum stored in Proline database and read in <u>result file</u> when <u>importing</u> <u>search result</u>.
 - mzDB MS2 spectrum : from MS2 spectrum read in mzDB associated with the run.
 - mzDB MS3 spectrum (beta version): from MS3 spectrum read in mzDB associated with the run.
- **Rescale reporter abundance to MS1 signal** (mandatory for multi-batch) : all reporter ions abundance (read in MS2 or MS3 spectra) are rescaled on the MS1 APEX. If this option is selected, you will be prompted to give "label-free parameters" in order to compute MS1 quantitation, and thus MS1 APEX.

The next steps will be:

- Associate mzDB files to sample analysis: see Link raw files
- Label-free parameter, if rescale to MS1 was selected : see <u>Quantitation parameters</u>.

Quantitations Aggregation

It is possible to aggregate data from different label-free or isobaric labeling designs.

First you must select two or more XIC Designs in the Quantitation tree and do a mouse right click to obtain a popup and then select the menu "Aggregate Quantitations".



Quantitatio	ns 1	
⊞ m mash		Aggregate Quantitations
		Compute Post Processing on Abundances
		Export Excel Retrieve Protein Sequences
		Properties

The following dialog is opened:

Se Aggregate Quantitation Wizard	×
 Left Panel: Define the aggregation experimental design The following experimental design was inferred from the quantitation that will be aggregate entities can be modified Change entities order by drag and drop Rename entities by contextual menu (right dick) Create or delete entities from the contextual menu Center Panel: Define quantitation channels mapping Each quantitation channel of the aggregation will correspond to sample analyses of aggreg can be made: Change association by dragging and dropping sample analysis from the right panel Remove association by using contextual menu or toolbar 	ed. Group, samples and channels (replicates) gated quantitations. The following modifications to a cell or from another cell
Experimental Design XIC Aggregation Group Fr1 Channel 1 Channel 2 Experimental Design Mapping	Quant-1 Quant-2 Quant-1 Quant-1 Quant-1 Quant-1 Quant-1 Quant-1 Quant-1 Quant-1 Plance Comp Fr1 Plance F127603 Plance V V V V Cancel

This dialog is divided in three areas:

- **left area**: aggregated experimental design. This design is inferred from the quantitations that will be aggregated.
- **center area** : quantitation channels mapping. Each quantitation channel of the aggregation will correspond to sample analysis of aggregated quantitations.
- right area : XIC Sources



You can completely modify the inferred aggregated experimental design. You can use drag & drop to modify the order of the items and the right mouse popup to create, delete or rename items.



You can modify the quantitation channels mapping by drag and drop from the right area to the center area. You can also remove or reorder the mapping thanks to the right mouse popup or thanks to the floating panel.



When you select a Channel, you can click on the help button to check what the aggregation will exactly do.





Compute Post Processing on abundances

Advanced Protein Sets abundances

Right click on the selected XIC node in the Quantitation tree, and select "Compute Post Processing on Abundances..."



Quanti	tations	
	Display Abundances	>
🗄 - <u>ه</u> Qu	Display Identification Summary	>
i⊞⊷ 📩 sr 1	Display Exp. Design	>
Tra	Add Quantitation Folder	
	Rename	
	Delete	
	Compute Post Processing on Abundances	
	Clone & Extract Abundances	
	Change Typical Protein	
	Identify Modification Sites	
	Generate Spectrum Matches	
	Retrieve Protein Sequences	
	Export	>
	Properties	



Configuration

🐎 Compute Post-Processing on A	bundances		\times
Abundances computing Aggregation	n config.		
Peptides			
Apply Normalization			
Proteins			
Apply Normalization			
Peptides Selection Method	Razor and Specific		~
Discard Miss Cleaved Peptides			
Discard Modified Peptides			
Acetyl (Protein N-term)			
Oxidation (M)			
Carbamidomethyl (C)			
Modified Peptide Filtering Method	Discard all forms		\sim
Discard Pep Ions Sharing Peake	S		
📊 Save 🕕 Load		V OK X Canc	el 🕢 🕢

In the dialog, you can:

- specify peptides to consider for quantitation
- configure abundances computing parameters
- select aggregation mode

For more details, see Post-processing of LC-MS quantitative results

Advanced XIC results

You can see the results by displaying the XIC (Display a XIC) or export them Export Data



General Settings

Since version 1.4 Proline Studio includes a general settings dialog which can be accessed from the top menu bar clicking on "General Settings" as shown in the screenshot below.

Change User	Logs	86]				4		•
Change password	User 1	Tasks	Sei	rver Tasks					
General Settings									5
Admin	T		id	Category	Criticality	Task Description	Progress	₽	
Upload .dat File(s) s_BGE\301.6-EDYP-Echange			10	Database Access	Low	Load Data for Datase			1
ojets_BGE\301.6-EDYP-Echan			9	Services JMS	Low	Browse Server File Sy			
ojets_BGE\301.6-EDYP-Echan	_		8	Database Access	Low	Load Data for Datase			
O:\301-Projets_BGE\301.6-EDYP-Echan		*	7	Database Access	Medium	Load Quantitation Da		=	
O:\301-Projets_BGE\301.6-EDYP-Echan			6	Database Access	Medium	Load Identification Da		_	
O:\301-Projets_BGE\301.6-EDYP-Echan			5	Database Access	Medium	Load Projects for Use			
O:\301-Projets_BGE\301.6-EDYP-Echan			4	Services JMS	Low	Browse Server File Sy			
O:\301Projets_BGE\301.6-EDYP-EC		*	3	Services JMS	Medium	Get Server File Syste			
O:\301Projets_BGE\301.6-EDTP-EC O:\301 Projets_BGE\301.6-EDTP-EC			2	Database Access	High	Connection to UDS D		Ŧ	
U: \0:\01+Projets_BGE\01.6-EDTP-EC					III			•	
emote Site	Task:	nesta	amp						
1QEX2_005208_3-3.mzdb 1QEX2_005210_4-1.mzdb □ 1QEX2_005211_4-2 mzdb	As	k Tim	e:						
10Ex2_005212_4-3.mzdb	Sta	art Ti	me:			Start Delay:			
1st_expand.mzdb 	En	d Tim	e:			Duration:			
CAVEN 10635.mzdb	Err	or M	essag	e					η.
(AVEN 105 39 m 700									11

The latter one consists of a constantly but slowly growing number of user preferences regarding various aspects of the utilization of Proline Studio. Based on their context, for the time being, preferences are organized into the following four tabs:

- JMS Settings
- Conversion/Upload Settings
- Table Parameters
- General Application Settings

JMS Settings

JMS Settings tab contains parameters that concern the exchange of messages between your local machine and the JMS Server. It should be made clear that compared to other preferences, preferences that are included in this tab should be treated with caution. Mistreating a communication preference can lead either to communication/connection problems or to users' confusion to whether they are connected to the correct server version.



Proline Studio Settings	Parameters Service Request Queue Name :	ProlineServiceRequestQueue	
			V OK X Cancel

Service Request Queue Name

Parameter can be seen as a name which represents a server address. The parameter's existence is justified by the fact that multiple server versions might run on the same server machine imposing the need to be able to canalize Studio's messages appropriately.

Table Parameters

Table parameters' tab encapsulates a short list of preferences regarding all tables generated throughout Proline Studio. More specifically those preferences control the arrangement of the participating columns as well as their respective width.

Proline Studio Settings	Parameters Columns Arrangement : Column Width :	Smart Column Size
		V OK X Cancel

Columns Arrangement

This field dictates the spatial arrangement of table columns. Three arrangements are possible:

- Fixed Column Size
- Automatic Columns Size
- Smart Columns Size

When "Automatic Columns Size" is used, all columns are width-wise readjusted in a way that they all fit to their container. Given that it is a "fit-to-screen" approach, it lacks scrollbars and does not guarantee the readability of the presented date, especially when the number of columns is high.



File Window Help								
Projects % MzDB Files	Logs	- 88 (🔵 ds 1 Protei	ns %		4		
Project 1	\bullet		Protein	Score	Peptides	Mass	R.	23 100
	1 11	1	FCGB	5505.39	121	572016.69	<u> </u>	
	∇	2	👌 CO3	3372.64	80	187148.06		
	-	3	GFAH	3193.83	65	139096.31		
All Imported		4	👌 A8K5T	3134.43	64	138950.17		
		5	AUC5	2480.67	63	596340.19		
⊞ ·· () dsTest	말	6	🛓 E9PBJ	2480.67	63	596686.62		
🗄 🛞 dsLocal		7	🎍 #C#P	2449.36	58	69366.68		
i i i i i i i i i i i i i i i i i i i		8	ALBU	2449.36	58	69366.68		
Gamme Levure UPS1		9	A7Y9J	2438.92	67	648803.31		
⊕ () ds_test_xic		10	A8K9P	2410.55	57	69267.59		
		11	GEFG_E	2369.66	43	77581.30		
F075556		12	EFG_E	2369.66	43	77581.30		
		13		2309.00	42	77501.30		
test new version		15		2309.00	43	77581.30		
		18	EFG F	2369.66	43	77581.30		
		17	HEVP	2303.00		66052.76		
		18	A K2C1	2202.50	38	66038.73	-	
₩ФРМ		10	a neer m	LLOLIDO		00000170		_
4 11	M						æ	25
Ouantitations	∇							~ ~
SC-de3-1	1 D		L	•••• ••• ••• •	. 2 0 3			-
	X	2 1	Q	•••• ••• ••• •	201	• ••• ••• •••	Ξ	
SC-ds3-2			v	•••• ••• ••• •	201	• • • • • • • • • • • • • • • • • • • •		
SC-OS3		5 8	v		2 0 1			
terre XIC-ds3		6 K	V		. 2 0 1			
🕀 🗄 XIC		7 R	AC		. 2 0 1			
🕀 👷 XIC-Copy		8 R	L		. 3 1 1			
🖶 🔜 XIC-Сору-Сору		9 K	AI(T		. 2 0 1			
🕀 號 XIC-new900		10 R	VV/T		3 0 1			
🗄 🚠 new XIC		11 R	A		. 3 1 1			
E. SIC-new900-Copy		12 R	L		. 2 1 1			
SC-ds3-ds		13 K	G		3 0 3			
		14 K	N		2 0 1			
		15 R	S		. 201			
		16 K	L		3 0 2			_
		17 R	L		2 0 1			+
		18 R	C		2 0 1		Ŧ	
							_	

On the other hand a simpler approach that guarantees readability is to select "Fixed Column Size". In this case all table columns have a fixed width, explicitly dictated by the user using the parameter "Column Width".



rojects 🕷 MzDB Files	Logs	🛛 🕼 ds1 Proteins 🖇	8		4 ▶
💼 Project 1 🚽 🗈 🖉	\square	Protein	Score	F	Pep' 🛱
		1 🔓 FCGBP_HUMAN	1 55	05.39	<u> </u>
		2 🔓 CO3_HUMAN	33	72.64	=
	2	3 🔓 CFAH_HUMAN	31	93.83	
All Imported		4 🗋 A8K5T0_HUMA	N 31	34.43	
		5 🔓 MUC5B_HUMAN	N 24	80.67	
i ∰	92	8 🔓 E9PBJO_HUMAI	N 24	80.67	
🗄 🕢 dsLocal 🔤		7 👗 #C#P02768-1	24	49.36	_
🗄 🕢 ds3		8 🔓 ALBU_HUMAN	24	49.36	
Gamme Levure UPS1		9 🚺 A7Y9J9_HUMA	N 24	38.92	_
		10 A8K9P0_HUMA	N 24	10.55	_
F 083342		11 EFG_ECOBW	23	69.66	_
		12 EFG_ECOLI	23	69.66	_
E083879			.1 23	69.66	- 11
			I 23	69.00	- 11
			23	69.00	- 11
			N 22	82.58	
		III		02.00	•
-	n	Prev. AA	Peptide	Ne	ext / 🖶
Ouantitations	100				
	II	1 R	LPVSLSEGR	L	
	X	1 R 2 R	LPVSLSEGR TPDGSLLVR	L Q	<u>^</u>
		1 R 2 R 3 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR	L Q V	* E
🛃 SC-ds3-1 🛃 SC-ds3-2 🛃 Sc-ds3 ==		1 R 2 R 3 R 4 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR	Q V V	• III
		1 R 2 R 3 R 4 R 5 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR	L Q V V V	*
🛃 SC-ds3-1 🛃 SC-ds3-2 🛃 Sc-ds3 🛃 XIC-ds3 		1 R 2 R 3 R 4 R 5 R 6 K	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR	L Q V V V V	* III
	1 2 2 2 3 4 3 3 3 3 3 4 3 3 3 3 3 3 3 3 3	1 R 2 R 3 R 4 R 5 R 6 K 7 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG	L Q V V V V -	-
Image: SC-ds3-1 Image: SC-ds3-2 Image: SC-ds3 Image: SC-ds3	1 × 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR	L Q V V V - L	•
SC-ds3-1 SC-ds3-2 Sc-ds3 Sc-ds3 XIC-ds3 XIC XIC-Copy XIC-	1 ×	1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR	Q V V V - L T	
SC-ds3-1 SC-ds3-2 SC-ds3-2 Sc-ds3 Sc-ds3 XIC-ds3 XIC-ds3 XIC XIC-Copy XIC-Copy XIC-Copy XIC-Copy XIC-new900 Source XIC	⊥ № ₽	1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 K 9 K 10 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK	L Q V V - L T T	
SC-ds3-1 SC-ds3-2 Sc-ds3 XIC-ds3 XIC XIC-Copy XIC-Copy XIC-copy XIC-new900 XIC-new900 XIC	1	1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR	L Q V V V - L T T A	
SC-ds3-1 SC-ds3-2 Sc-ds3 XIC-ds3 XIC-ds3 XIC-copy XIC-Copy XIC-copy XIC-copy XIC-copy XIC-copy XIC-new900 Rest NIC Rest NIC Rest NIC Rest NIC Rest NIC Rest NIC-new900-Copy		1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R 12 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR NMVLQTTKGLR	L Q V V V - L T T A L	
		1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R 12 K 13 K	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR NMVLQTTKGLR LTYNHGGITGSR	L Q V V - L T T A L G	
SC-ds3-1 SC-ds3-2 Sc-ds3 XIC-ds3 XIC-ds3 XIC-Copy XIC-Copy XIC-Copy XIC-Copy-Copy XIC-new900 Rev MIC XIC-new900-Copy SC-ds3-ds SC-ds3-ds Sc-ds3-ds		1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R 12 R 13 K 14 K	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR NMVLQTTKGLR LTYNHGGITGSR LDSLVAQQLQSK	L Q V V - L T T A L G G	
		1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R 12 R 13 K 14 K 15 R	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR NMVLQTTKGLR LTYNHGGITGSR LDSLVAQQLQSK GEVGFVLVDNQR	Q V V V - L T T A L G N S	
SC-ds3-1 SC-ds3-2 SC-ds3 XIC-ds3 XIC-Copy XIC-Copy-Copy XIC-new900 XIC-new900 XIC-new900 XIC-new900 SC-ds3-ds SC-ds3-ds XIC XIC-xex XIC XIC XIC		1 R 2 R 3 R 4 R 5 R 6 K 7 R 8 R 9 K 10 R 11 R 12 R 13 K 14 K 15 R 16 K	LPVSLSEGR TPDGSLLVR GNPAVSYVR GNPAVSYVR GNPAVSYVR FYPAGDVLR AQDFSPCYG SRLPVSLSEGR AIGYATAADCGR VVAEVQICHGK RVSYVGLVTVR NMVLQTTKGLR LTYNHGGITGSR LDSLVAQQLQSK GEVGFVLVDNQR AISGLTIDGHAVGAK	L Q V V V - L T T T A L G N S L	

The less clear option is "Smart Column Size" which serves as a trade off between the aforementioned ones. It tackles with the cases that either we have too many columns to visualize using the "Automatic" approach or too few and the selected default and globally applied width imposes unneeded scrollbars ending in hiding some columns at the same time. In this context, "Smart Column Size" can be seen as a simple rule based on the ratio between the mean column width needed in case of "Automatic Column Size" for a specific table and the globally selected width. For the sake of simplicity we have set a threshold of 0.7 or 70% which on its turn determines which one of the two first modes will be used given a table. If the ratio is smaller than 0.7 then the table in question will be presented in "Fixed Column Size" mode. On contraire if ratio is equal or greater than 0.7, then we consider that using "Automatic Column Size" mode is more appropriate as it balances between a possible slightly smaller than desired width and the possibility of hiding a column using scrollbars.



Column Width

The second preference is more or less self explanatory and corresponds to the globally desired columns' width when fixed rune is applied, either directly or as a result of the smart mode.

General Application Settings

Settings Categories JMS Settings Conversion/Upload Settings Table Parameters General Application Settings	Parameters Default Search Result Name Source :	Hide Getting Started Dialog On Startup Msi Search Filename Export Decorated Use dataset type to create Xic Design by DnD	
			V OK X Cancel

In this tab we can find a diverse set of preferences regarding various tasks encountered in Proline Studio. For the time being those preferences are:

- → Hide gettings Started Dialog
- → Default Search Name Source
- → Export Decorated
- → Use dataset type to create a XiC design by DnD

Default Search Name Source

Unlike the last three, Hide Getting Started Dialog is pretty much self explanatory. The second preference on the other hand, "Default Search Name Source" affects the way identification datasets are named on importation. For this preference we have three possible options:

- → Search Name (E.g Gamme Levure UPS1)
- → Peaklist (E.g OEMMA121101_36.raw)
- → MSI Search Filename (E.g F054967)
- → Mascot Rule (...)

Export Decorated

This parameter affects the .xls and .xlsx files that are produced in the process of export (client side). It could be easily described as a preservation of any existing Rich Text Feature in a table. (Colors, Font Weight etc.)



Use dataset type to create a XiC design by DnD

MsFiles

🗫 General Settings			×
Settings JMS Tables General Plots	Parameters Converter (.exe) : Working Set Entry Label :	: \Local \Programs \HomeTools \raw2mzDl	B_0.9.10_
		✓ ОК	🗙 Cancel 👩

Converter (.exe)

Corresponds to the default raw2mzDB converter. While it is left at the discretion of the user, which version to choose, it must be noted that different versions do tend to work better with specific type raw files. Said that, it is also important to understand that in order for a conversion to be successful within Proline Studio, all system requirements set by the specific raw2mzDB version must be met.

Identify Modification Sites or Clusters

For description on sites and clusters you can first read the principles in this page: <u>Identifying Modification sites and</u> <u>clusters</u>

To identify Modification sites and create Modification clusters, right click on the Identification Summary and select *Identify Modification sites*.



🏂 Identificati	ons orted	
	Display Search Result	>
±	Display Identification Summary	>
	Add Dataset	
🕀 📄 Tes	Add Identification Folder	
± ·· 👚 Tra	Copy Search Result	
	Paste Search Result	
	Rename	>
	Clear	
	Delete	
	Import Search Result	
	Import MaxQuant Result	
	Validate Search Result	
	Merge Datasets	>
	Filter ProteinSets	
	Change Typical Protein	
	Identify Modification Sites	
Quantit	Generate Spectrum Matches	

A dialog is displayed where you can choose Modification of interest and configure the clustering method (see <u>Identifying Modification sites and clusters</u> for more details).

dentify Pt	m sites	
0	Select the list of modifications of interest (other modifications will be ignored during clustering) and set the method's parameters that will be used to clusterize modification si	te
Cluster	ing method	
Rely of use the of the	on localizations inferred from PSMs: this clustering method e site localization inferred from the validated PSMs, regardless reported localization probabilities.	
۲	Group fully cleaved with missed cleaved peptides in same cluster	
0	Separate fully cleaved and missed cleaved peptides in different clusters	
DTMc o	Fisteraet	
	Acetvlation	
	loooacetamide derivative	
	Oxidation or Hydroxylation	
	Phosphorylation	
	V OK 🎇 Cancel	6



Annotate and Edit Modification Clusters

After running Identify Modification for a specific Identification Summary or Quantitation dataset (see above and Identifying Modification sites and clusters for more details), users can annotate and edit these clusters.

In the associated display, few operations are allowed.

- Change the status of the cluster by clicking on the discon. The following dialog appears, allowing user to
 - Validate/Invalidate the cluster
 - Add a confidence level : any number which signification is user specific
 - Add a free description

🐎 Modi	fy Modification Cl	uster Status ×
0	Change the statu * the Status it-s * the Status con * a free descrip	s of selected modification Cluster. Three status properties can be modified : elf : validated or invalidated nfidence : a number indicating the confidence you have in the current status. tion on current duster status.
Modificatio	n Cluster Status:	● Validated 🖒
		🔿 Invalidated 🛛 🖓
Status Con	fidence level:	
		Exact Position Matching
Status Con	fidence description:	
		V OK X Cancel

Edit the selected cluster by clicking on the icon. In this case, the status dialog is displayed in addition with the list of peptides of the cluster. Same status annotation as described above can be done. Users can also remove some peptides from the cluster. The removed peptides will be removed from the whole "PTM Dataset". They will not be added to an existing or new cluster. Note: Peptide used as reference could not be deleted, a single peptide could be deleted at a time.



Þ	dit Cluster 43 [PP6R3_H	HUMAN / IQQFDDGGSI	DEEDIWEEK]				>
6	Edit Cluster						
	Change the statu * the Status it-s * the Status co * a free descrip Change peptides Select peptide to	is of selected modification elf : validated or invalidat infidence : a number indi vtion on current duster s list. remove from duster in th	Cluster. Three status pr ed cating the confidence you tatus. e table and dick on remo	operties can be modified : I have in the current stat ve icon. The pepitde used	us, to represent the clus	ter can not be removed.	
Clus	ter Status						
1odi	ification Cluster Status:	Validated					
		🔿 Invalidated 🛛 🖓	1				
Stat	us Confidence level:						
Stat	us Confidence description	n: Exact Positio	on Matching				\$
Clus	ter Peptides						
ĸ	Id	Peptide	Score	Exp. MoZ	Charge	PTMs	PTMs D.Mass
	30683	IQQFDDGGSDEEDIWEE	41.69	999.4757		4 TMTpro (Any N-term)	688.3806
	25291	IQQFDDGGSDEEDIWEE	107.86	943.4362		3 IMIpro (Any N-term)	688.3806
	<						>
						🖌 ОК	X Cancel

- Merge co-localized clusters by clicking on icon. Users must select clusters that should be merged. These clusters must be co-localized, see <u>Identify Modification Sites or Clusters</u>.
- Save edition by clicking in 🖬 icon. All modifications done on clusters will not be saved in datastore until user explicitly ask for it. When saving annotation, an Annotated Modification Dataset is created and will be accessible through the *Annotated Clusters* menu. This permits users to still access the initial Modification Dataset. Warning : there is only one Annotated Modification Dataset per Modification Dataset. If new annotation is done and saved from initial Modification Dataset, previous Annotated Modification Dataset will be erased

Administration

Some administration views and operations are accessible through the Proline Studio *File > Admin* menu. Edit functions are only permitted to advanced users, who at the same time hold the status of Admin.

User Accounts

The "User Accounts" tab, list all registered users with the group they belong to. Admin users can add new users or modify existing one. Modifications consist in changing user groups or passwords.



lupierris	User	
edyp	User	
erre	User	
J2p	User	
atem	User	
lesse	User	
raut	User	
nartin	User	=
nenetrey	User	
vieter	User	
amus	User	
ardif	User	
estjpm1	User	-

Login :	trauchesse	ec		
Group :	Oser	Admin		
Pasword :	*****			
	(🖌 ОК	X Cancel	0

User account modification dialog

Peaklist Softwares

ser Accounts	Peaklist Softwares	Projects and Databases	Fragmentation Rule Sets		
eaklist Softwa	ares		2		
Peaklist Soft	ware		Version		₽
Data Analysis			5.3		~
Data Analysis			4.0		
Data Analysis			4.1		
extract_msn.	exe				
Mascot Distille	r				
mascot.dll					
MaxQuant					
Proline			1.0		
Protein Pilot					
Proteome Disc	overer				
ProteoWizard			2.0		¥
Spectrum Tit Spectrum Ti	le Parsing Test tle:			P Test]
		+	Add Peaklist Software	😤 View Peaklist Software	



Peaklist Softwares tab permits users to view the list of all available Peaklist Software. A detailed view could be displayed when selecting the "View Peaklist Software" button.

Peaklist Software				
	Name :	Data Analysis		
	Version :	4.1		
Spectrum Title Parsin	g Rules			
Raw File Identifier :			< Select Predefined >	· · · · · · · · · · · · · · · · · · ·
First Cycle :			< Select Predefined >	
Last Cycle			< Select Predefined >	
First Scan :	Cmpd. +MS\d. +, \d+\. \d+	min #(\d+)	< Select Predefined >	
.ast Scan :	Cmpd. +MS\d. +, \d+\. \d+	min #(\d+)	< Select Predefined >	
First Time :	Cmpd. +MS\d. +, (\d+\. \d-	+) min	< Select Predefined >	
Last Time :	Cmpd. +MS\d. +, (\d+\. \d-	+) min	< Select Predefined >	
Spectrum Title Parsin	a Test			
Spectrum Title:	-			🗗 Test
			V OK	X Cancel

In both windows, It is possible to test a spectrum title to verify which data are extracted for a given Peaklist Software.

Jser Accounts	Peaklist Softwares	Projects and Databases	Fragmentation Rule Sets				833.993
Peaklist Softwa	ires				_		491.287
Peaklist Softw	vare		Version		Spe	ectrum Ti	itle Par 🗙
extract msn.e	Accounts Peaklist Softwares Projects and Databas ist Softwares klist Software act_msn.exe to Distiller cot.dll Quant ne ein Pilot eoWizard eoWizard eoWizard ectrum Title Parsing Test ectrum Title: 348: Scan 3408 (rt=10, 1026) [D:/Dat					Raw File	a Identifier :
Mascot Distiller	Accounts Peaklist Softwares Projects and Database dist Softwares aklist Software ract_msn.exe scot.dll xQuant line tein Plot teome Discoverer teoWizard teoWizard teoWizard teoWizard teoWizard teoWizard teoWizard teoWizard teoWizard teoWizard				Einet Cu	cla :	
mascot.dll						THECY	cie.
MaxQuant						Last Cy	cle :
Proline	log Peaklist Softwares Projects and Databases Fragmentation Rule Sets ares ware Version exe Spectrum Title Par. Raw File Ident First Cycle : Last Cycle : Last Cycle : Strate Cycle : Last Cycle : First Scan :340 Last Time :10.1 3.0 Last Time :10.2 Last Cycle : Close Close Close Close Close Close	an •3408					
Protein Pilot				restresuit		11130.50	
Proteome Disc	overer					Last Sc	an :3408
ProteoWizard			2.0			First Tir	ne :10.1026
ProteoWizard			2.1				
ProteoWizard			3.0		1	Last Tin	ne :10.1026
Spectrum Mill	Peaklist Softwares Projects and Databases Fragmentation Rule Sets 643.5 ares 833.9 9491.2 ware Version Spectrum Title Par exe 1.0 Test result scoverer 1.0 Test result d 2.0 First Cycle : d 2.1 Last Scan :3408 d 3.0 Last Time :10.102 itle Parsing Test X Close 478.3 r title to test Add Peaklist Software Modify Peaklist Software						
Spectrum Tit	le Parsing Test						💢 Close
Spectrum Tit	tle: 348: Scan 3408	8 (rt=10.1026) [D:/Data/N	ISData/All/HF1_010328.ra	w] 🕞 ا	est		478.313
							499.786
		- A	dd Peaklist Software	Modify Peaklist Sof	twar	-	
Enter	title to test			Prodity r caldist sor	contain (Calc. Mass
				Close	e		

Admin users also have the possibility to add new Peaklist Software definition (name, version and extraction rules using regular expression) or to modify existing definitions by changing the name or version. Rules are not modifiable since some already parsed data may not be consistent with the new definition. New rules definition may be done by creation of a new peaklist software.



Peaklist Software	
	Name :
	Version :
Spectrum Title Parsing	Rules
Raw File Identifier :	< Select Predefined >
First Cycle :	< Select Predefined >
Last Cycle	< Select Predefined >
First Scan :	< Select Predefined >
Last Scan :	< Select Predefined >
First Time :	< Select Predefined >
Last Time :	< Select Predefined >
Spectrum Title Parsing	Test
Spectrum Title:	ि Test
	V OK X Cancel

Projects and Databases

This tab lists all projects defined on the Proline Suite.

User	Accou	nts Peaklist Software	Projects and Databases Fra	gmentation Rule Se	ts								
Proje	ects												
7		Id Project	Description	Size (MB)	Owner	Raw Files Cou	unt	Databases		Dataset Date	P	roperties	Ę.
		2 Vero_Dev(2)	Base de test "prod" p.	7409	dupierris		5	lcms_db_project	2, m	11 févr. 2021			-
		246 VDS_2901201	9 d	NaN	dupierris		0	lcms_db_project	246	27 sept. 2019	{	is_active":false}	
		172 VDS_201706	Test New Prj (RC 1.5	19782	dupierris		10	lcms_db_project	_172	16 déc. 2021			
		29 TachesDeVert	Analyses ponctuelles .	NaN	tardif		0	lcms_db_project	29,	16 nov. 2015	{	is_active":false,	"e
		153 REYNOIRD	Analyses pour Nicolas	NaN	tardif		20	lcms_db_project	153	21 févr. 2017	{	is_active":false,	"a ∨ .
	<												>
		Creation Date	Identifier	Raw File Name	R	aw File Directory	Project	Ids	Projec	ts Count	Propert	ies	₽
r in the second		6 févr. 2015	OVEMB150205_21.raw	OVEMB150205_21	.raw <r< td=""><td>mzdb_path>/VDS_TEMP</td><td>172</td><td></td><td></td><td></td><td>1</td><td></td><td>~</td></r<>	mzdb_path>/VDS_TEMP	172				1		~
-		6 févr. 2015	OVEMB150205_23.raw	OVEMB150205_23	.raw <r< td=""><td>mzdb_path>/VDS_TEMP</td><td>172</td><td></td><td></td><td></td><td>1</td><td></td><td></td></r<>	mzdb_path>/VDS_TEMP	172				1		
		6 févr. 2015	OVEMB150205_25	OVEMB150205_25	.mzdb <r< td=""><td>mzdb_path>/VDS_TEMP</td><td>172</td><td></td><td></td><td></td><td>1</td><td></td><td></td></r<>	mzdb_path>/VDS_TEMP	172				1		
		6 févr. 2015	OVEMB150205_27	OVEMB150205_27	.mzdb <r< td=""><td>mzdb_path>/VDS_TEMP</td><td>172</td><td></td><td></td><td></td><td>1</td><td></td><td></td></r<>	mzdb_path>/VDS_TEMP	172				1		
		3 oct. 2015	QEx2_006341	QEx2_006341.mzd	lb <r< td=""><td>mzdb_path></td><td>66,172</td><td></td><td></td><td></td><td>2 {"mzdb_</td><td>file_path":"mz</td><td></td></r<>	mzdb_path>	66,172				2 {"mzdb_	file_path":"mz	
		3 oct. 2015	QEx2_006343	QEx2_006343.mzd	lb <r< td=""><td>mzdb_path></td><td>66,172</td><td></td><td></td><td></td><td>2 {"mzdb_</td><td>file_path":"mz</td><td>. /</td></r<>	mzdb_path>	66,172				2 {"mzdb_	file_path":"mz	. /
		3 oct. 2015	QEx2 006345	QEx2 006345.mzc	lb <r< td=""><td>mzdb path></td><td>66,172</td><td></td><td></td><td></td><td>2 {"mzdb</td><td>file path":"mz</td><td>. 🗵</td></r<>	mzdb path>	66,172				2 {"mzdb	file path":"mz	. 🗵
												Class	6

Upper view: List of projects with associated generic information (owner, description) and more administration information such as the name of the databases containing data, size of these databases, last dataset creation... The satus column, first one, indicates if the project is active (green), inactive (blue) or archived (grey)

Lower view: for selected project, list of the mzdb files used for quantitation. This is useful to do some cleanup on disk when archiving projects. The status indicated if this mzdb file is used in active projects only (green), in active and archived projects (yellow) or in archived projects only (grey).

Fragmentation Rule Sets

This tab show all fragmentation rule sets defined in Proline Suite and the rules they use. The display is similar to the one defined by Mascot [®]



singly charged			corr more (ESI-QUAD (ESI-QUAD	ESI-TRAP (ETD-TRAP (FTMS-ECD (LTQ-ORBIT	117
1 11 1	x	x	x	x	x	x	x	x		
doubly charg	x	x	x	x	x	x	x	x		
doubly charg										
immonium	x	x								
a	x	x								
a-NH3	x									
a-H2O	x									T
b	x	x	х	х	x	x				
b-NH3	x	x	x	x	x	x				T
b-H2O	x	x	x	x	x	x				
с							x	x		T
x										
У	x	x	x	x	x	x	x	x		T
y-NH3	x		x	x	x	x				
y-H2O	x		x	x	x	x				T
z		x								
yb	x	x								T
ya	x	x								
y or y++ mu										T
y or y++ mu										
z+1							x	x		T
d										
v										T
w										
z+2							x	x		T
	1			1					1	_
1				_						
`										

This information is used for <u>generating spectrum matches</u>. Custom fragmentation rule set can be added if needed by "admin" user.