ISOQuant 1.6
Help pages

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1 Description

1.1 What is ISOQuant?

ISOQuant is an integrated solution for in-depth evaluation of data-independent acquisition based label-free quantitative proteomics.

1.2 About ISOQuant

One of the main bottlenecks in the evaluation of label-free quantitative proteomics experiments is the often cumbersome data export for in-depth data evaluation and analysis. Data-independent, alternate scanning LC-MS peptide fragmentation data can currently only be processed by Waters PLGS software.
PLGS performs absolute quantification only on a run-to-run level, it does not afford absolute quantification of protein isoforms and label-free relative quantification of peptides and proteins based on clustered accurate mass-retention time pairs on a complete experiment basis.
The bioinformatics pipeline ISOQuant directly accesses xml files from the PLGS root folder and browses for relevant data from a label-free Expression Analysis project (quantification analyses, sample descriptions, search results) for fully automated import into a MySQL database. EMRTs are subjected to multidimensional LOWESS-based intensity normalization and annotated by matching exact masses and aligned retention times of detected features with highest scoring peptide identification data from associated workflows. Based on the annotated cluster table, ISOQuant calculates absolute in-sample amounts with an integrated protein isoform quantification method, utilizing average intensities of proteotypic peptides for the partitioning of non-unique peptide intensities between protein isoforms. All data is stored in a local MySQL based database that can be queried directly by experienced users.

1.3 Citing ISOQuant

When you use ISOQuant to produce publication data, please cite the following publication:

Distler, U., Kuharev, J., Navarro, P., Levin, Y., Schild, H., & Tenzer, S. (2013). Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. Nature Methods, advance online publication. doi:10.1038/nmeth.2767

Use the following BibTeX code to import the publication into the reference manager of your choice:

```
@article{distler_2013_drift,
  title={Drift time-specific collision energies enable
        deep-coverage data-independent acquisition proteomics},
  author={Distler, Ute and Kuharev, Jo"rg
    and Navarro, Pedro and Levin, Yishai
    and Schild, Hansjo"rg and Tenzer, Stefan},
  journal={Nature methods},
  year={2013},
  publisher={Nature Publishing Group}
}
```

1.4 Basic Principles

1.4.1 Data import

ISOQuant automatically browses the root folder of PLGS for all project related information (expression analyses, samples, workflow results, etc.). When a project is selected for import, ISOQuant imports all project related information into a MySQL database.

1.4.2 Signal cluster annotation

Signal clusters are annotated by associated peptide identification. For signal clusters with multiple associated peptide identifications, ISOQuant offers the possibility to control the annotation by setting parameter `process.annotation.peptide.sequence.maxCountPerEMRTCluster` to different values:

- If the parameter is set to 1, ISOQuant will only annotate clusters unambiguously associated with a peptide ignoring ambiguous cases.
- In case the parameter is greater than 1, ISOQuant will only annotate clusters associated with equal to or less than maximum peptides by given parameter value using in ambiguous cases the peptide with the highest score sum of peptide scores across all members of the cluster (this way estimating the most confident identification).

1.4.3 Data filtering

ISOQuant provides multiple filters to improve data quality. Several criteria can be set to include or exclude peptides and proteins from absolute quantification. Additionally, peptides to be used for absolute quantification can be selected using by their type. By setting peptide type filters to `true` peptides of selected types are explicitly allowed for TOPx quantification.

1.4.4 Normalization

ISOQuant features multi-dimensional normalization of EMRT intensity data. All options (Intensity-based, Retention time based and Mass based) can be separately switched on/off in the configuration file. You may define your own normalization order by changing the normalization order sequence. By changing the LOWESS-bandwidth you can adjust the normalization process to be more or less sensitive to local systematic errors.

1.4.5 TOPx protein quantification

ISOQuant TOPx quantification is based on the Waters approach to absolute quantification. However, instead of using only peptides identified directly by PLGS in the respective workflow, ISOQuant used annotated EMRT tables for TOP3 quantification. For absolute quantification across protein isoforms, ISOQuant uses a distribution loop.

1. all unique peptides are associated with their parent proteins
2. in each sample, absolute amounts are calculated based on the average intensity of the unique peptides.
3. distribution Loop for peptides matching to more than 1 protein. First, intensities peptides matching to 2 proteins are distributed to their proteins according to the TOP3 intensity calculated in step2, then TOP3 intensity is recalculated. This repeats until all peptide intensities have been redistributed.

1.4.6 Data export

Results of successfully processed data in ISOQuant can be exported to a set of uniform reports:

- `extended protein quantification (Excel)`
  MS Excel file build of multiple spreadsheets containing overview information, such as protein top3-intensities, protein absolute quantification values, workflow to workflow correlation and detailed workflows information
- `single page HTML` or `multi page HTML`
  browsable HTML pages containing detailed quantification results
- `extended peptide quantification (CSV)`
  CSV file containing comprehensive information about EMRT clusters, peptide and proteins
- `simple protein quantification (CSV)`
  CSV file containing top3-intensities for proteins per LC-MS run
- `simple protein sample-average quantification (CSV)`
  CSV file containing average values of top3-intensities for proteins per sample.

We are grateful for other requests regarding data output and would like to ask users for their specific requests. We will try to implement those in future releases.

1.5 Work in progress - future options

We are working on improving overall ISOQuant performance as well as on incorporating the following new options into ISOQuant

- analysis of 2D UPLC
- analysis of SILAC data
- peptide intensity based relative quantification
- protein coverage maps
- pseudo-2D gels based on TOP3 quantification
- possibility to exclude workflows/technical replicates from quantification
- output of a "digestability index"
  (intensity missing cleavage peptides/total peptides)
- PCA analysis
- Summation of intensities of in source fragments with parent peptides for absolute quantification
- Experimental design options
  (e.g combining results from SDS-PAGE lanes)

Feedback and requests for further features are very welcome.

1.6 Known limitations

- ISOQuant has been tested only with 1 workflow result per technical replicate
- ISOQuant has not beed tested for use of 2D-UPLC data.
- ISOQuant has been tested with expression analyses comprising up to 10 samples with 5 technical replicates each. We observe a nonlinear increase in processing time with number of workflows in an expression analysis and are working on this issue.
- ISOQuant ignores all defined DDA-workflows.

1.7 Known problems

- On some **Windows Vista** or **Window 7** machines ISOQuant can not write its configuration file. In this case you have to execute ISOQuant with administrative privileges or correct file system permissions for ISOQuant installation folder. This is not an ISOQuant issue, sometimes Windows messes up file system permissions by using different and inconsistent user privileges at different time points.
- Importing and Analyzing large projects or runs of high complexity may cause out of memory errors, make sure your PC has enough memory and assign more Heap-Space to Java Virtual Machine for running ISOQuant Application.
- ISOQuant may fail to import and process data if some PLGS project files are broken.
- Running MySQL on Mac OSX machines significantly decreases the performance of ISOQuant . This is a common known problem of MySQL not of ISOQuant. Use Windows or Linux machines and/or install MariaDB instead of MySQL for better performance.

2 Program requirements

ISOQuant will only work properly if the system for running ISOQuant meets following requirements.

- Operating System: Windows, Mac OS X or Linux
- PLGS root folder with projects containing processed MSE/HDMSE/UDMSE data is accessible (tested PLGS versions: 2.3/2.4/2.5/3.0)
- at least 3GB RAM
- Java Runtime Environment version 1.6.0 (or newer) is installed and works properly
- MySQL Server 5.1 (or newer) is installed and running on local machine or network. (tested MySQL versions: 5.1 - 5.5)
- MySQL configuration file options for heap and temporary tables have large values as shown in following listing.

```
max_heap_table_size = 2048M
tmp_table_size = 2048M
```

  In some cases it may be useful also to increase the size of MySQL thread stack by e.g. ~~ thread_stack = 256K ~~

  Depending on your operating system and MySQL-Version the configuration file is named either my.ini or my.cnf and its location may vary.

  Following listing shows an example of MySQL configuration section [mysqld] working for us on MacOSX 10.6.8 Snow Leopard running MySQL Server from XAMPP 1.7.3, the configuration file is located in `/Applications/XAMPP/xamppfiles/etc/my.cnf`

```
[mysqld]
port = 3306
socket = /Applications/XAMPP/xamppfiles/var/mysql/mysql.sock
skip-locking
key_buffer = 128M
max_allowed_packet = 16M
table_cache = 128
sort_buffer_size = 32M
read_buffer_size = 8M
read_rnd_buffer_size = 8M
net_buffer_length = 64K
thread_stack = 256K
myisam_sort_buffer_size = 32M
tmpdir = /Applications/XAMPP/xamppfiles/temp/
max_heap_table_size = 2048M
tmp_table_size = 2048M
sync_frm = 0
skip-sync-frm=OFF
```

  Do not forget to restart MySQL after editing its configuration!

Expert note:
If you get some “out of memory” errors while running ISOQuant please make sure you start the application by giving Java Virtual Machine a chance to have enough memory space by command line options, e.g.

```
java -Xms256m -Xmx2048m -jar ISOQuant.jar
```

this command will start ISOQuant and assign maximum 2GBs (2048m) RAM to it, or increase this value to 3GBs (3072m) or more if needed.

3 Data requirements

3.1 Rawdata type

ISOQuant has been developed for Waters QTOF LC-MSE and Waters Synapt G2/G2-S LC-MSE/HDMSE/UDMSE instrument data. At this time, only 1D-UPLC data is fully supported, 2D UPLC support will be included in later releases.

3.2 Database searches

At this moment, ISOQuant can only process Ion Accounting workflows (MSE/HDMSE-data). Classical DDA-Type experiments are not yet supported.

3.3 Project design

There are two different ways to use ISOQuant either as an extension to PLGS Expression Analysis or completely replacing it.

3.3.1 Expression analysis

You can use your experiment design given by running PLGS Expression analysis. As a prerequisite for this approach, a complete expression analysis of multiple samples and replicates is required. In PLGS, please select autonormalization of samples for generating EMRT and protein tables. Both EMRT and Protein tables have to be created during the expression analysis. Each expression analysis within a PLGS project can be selected during processing in ISOQuant. ISOQuant will create separate databases for storing the data of every single processed expression analysis.

3.3.2 ISOQuant Project Designer

As an alternative to the PLGS Expression analysis, you can use the simple and efficient built-in Project Designer described in section 4.6.

3.4 Peak detection/alignment/clustering

ISOQuant is based on peak detection, alignment and clustering of data performed by PLGS. We are aware of some peak splitting/alignment/clustering issues in PLGS. Therefore, we have have spent a lot of time to develop own methods for these tasks. You can either keep EMRT alignment/clustering results or let ISOQuant do the complete analysis. For details see future publications.

4 GUI and control elements

4.1 Main view

Figure 1 shows the main view of ISOQuant. User interaction is applied by following control elements:

1. List of projects found in PLGS root folder
2. List of projects from ISOQuant database
3. Button choose PLGS root folder
4. Button choose database
5. Button restore project from file
6. Button find projects
7. Button edit configuration
8. Button show help window
9. Button shutdown application

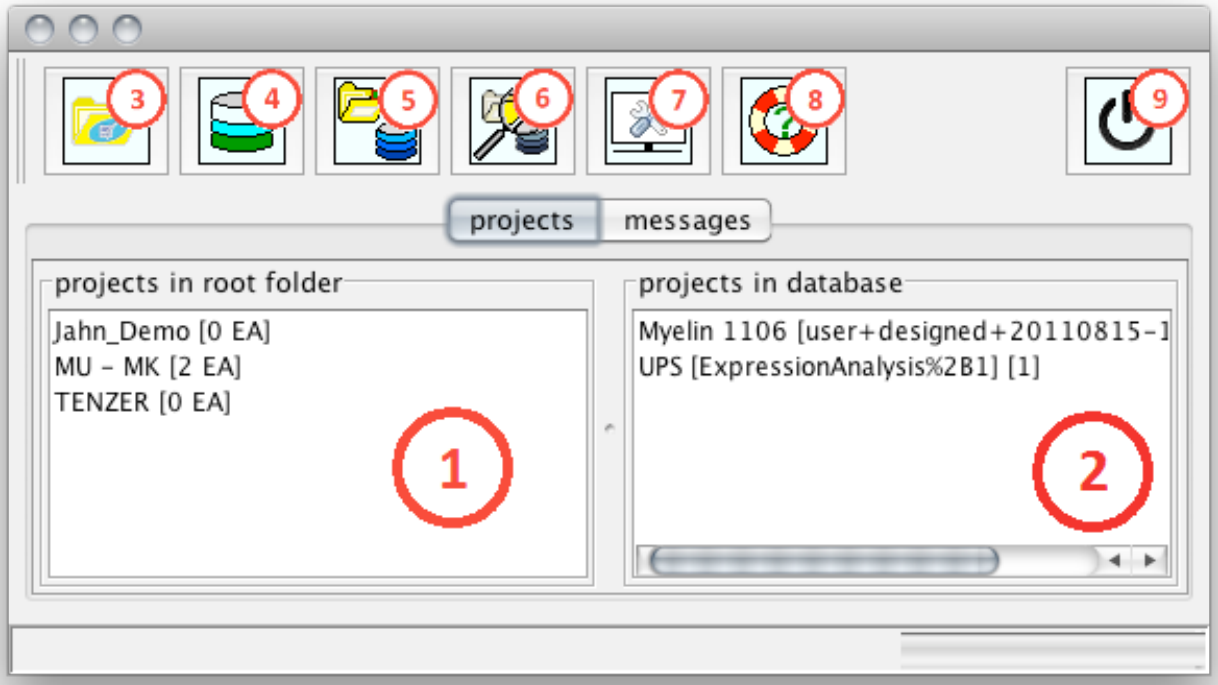

Figure 1: the main view of ISOQuant

4.2 Project Finder

The Project Finder window as shown in figure 2 makes possible to search projects lists for projects by substrings of project titles and regular expressions matching to them. In case your search string matches to one or multiple projects the Project Finder will mark these projects by selecting them in both file system and database projects lists. The Project Finder window can be accessed by clicking the button find projects from the tool bar on the main application window.

Figure 2: Project Finder window

4.3 Context menu for PLGS projects in file system

Advanced options for each project from PLGS root folder are available from a context menu like shown in figure 3:

1. find in database
   finds selected projects in the list of projects from database by comparing their titles and select them if such projects exist.
2. about project
   shows additional information about selected projects.
3. import and process
   allows to select one of predefined processing queues and starts processing selected projects using selected processing queue.

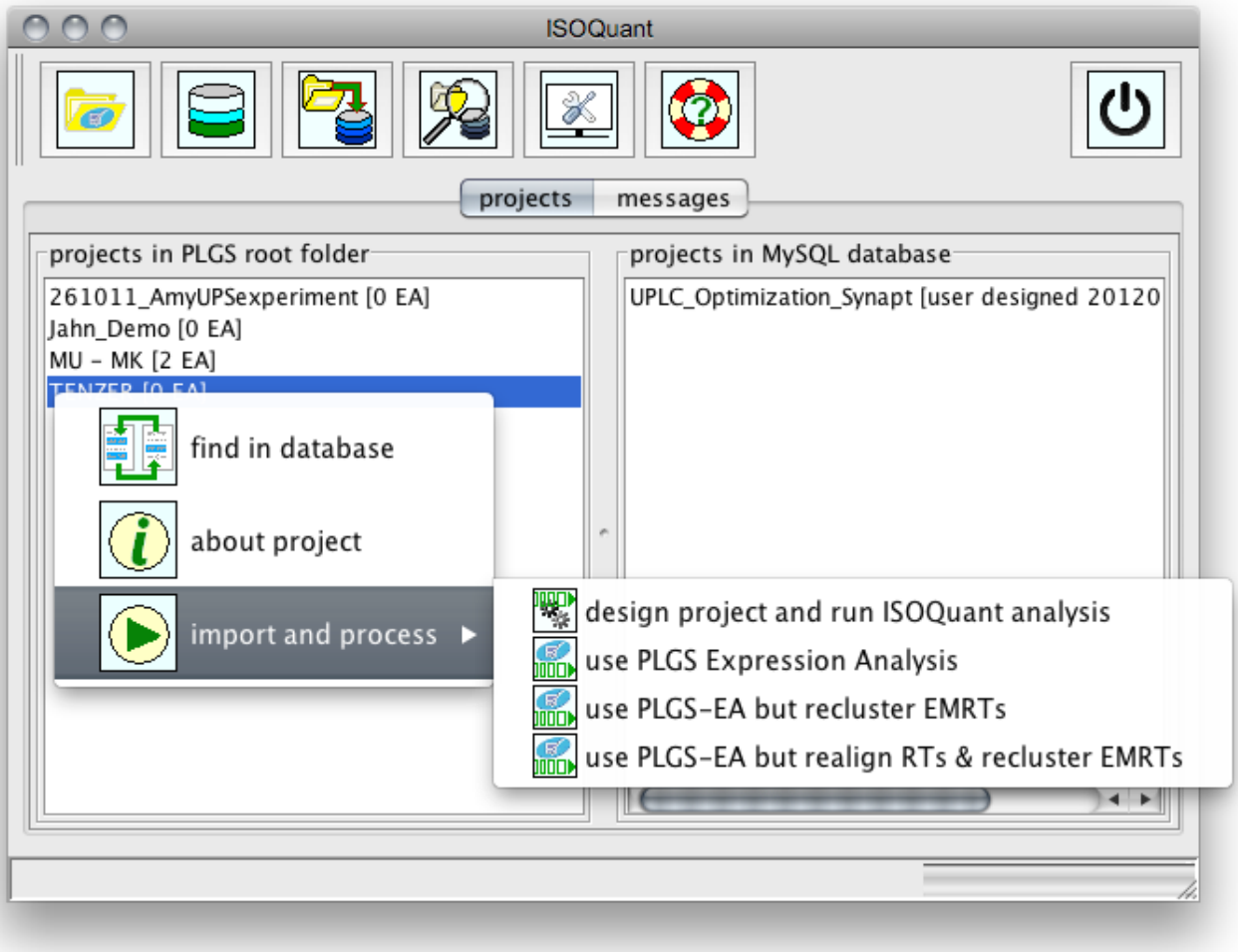

Figure 3: Context menu for PLGS projects in file system

4.4 Context menu for projects in database

Advanced options for each project from database are available from a context menu like shown in figure 4

1. find in file system
   finds selected projects in the list of projects from file system by comparing their titles and select them if such projects exist.
2. show info
   shows additional information about selected projects.
3. rename project
   rename selected projects.
4. reprocess
   reprocess a project starting from user selected processing stage. All needed subsequent processing steps are automatically applied.
5. create report
   generate on of implemented report types.
6. export to file
   export selected projects from database to (backup) files which can be imported by other ISOQuant instances.
7. remove from database
   removes selected projects from database.

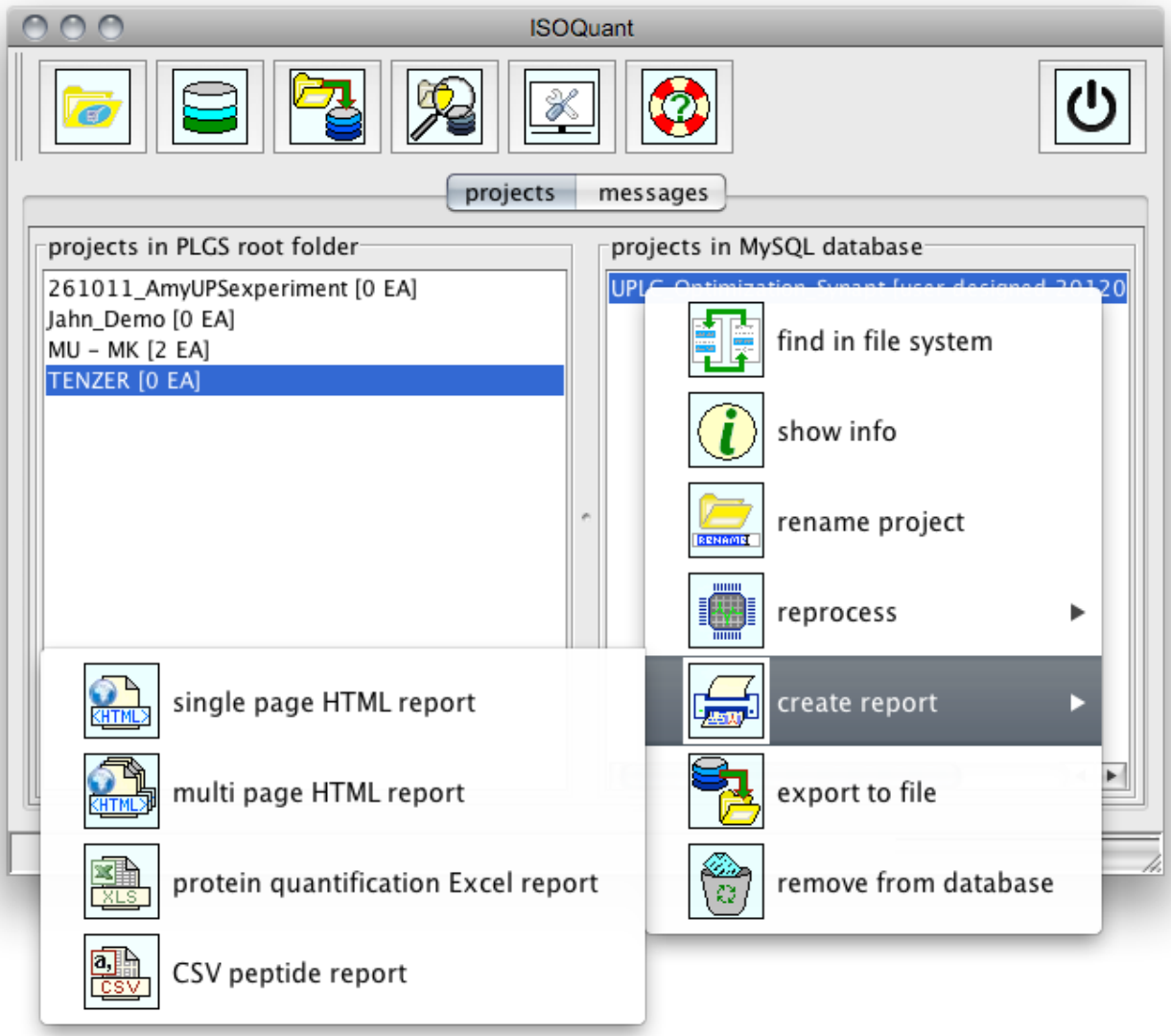

Figure 4: Context menu for already processed projects

4.5 Expression Analysis Selector

In some cases a single PLGS project contains multiple defined Expression Analyses. Some processing queues work with project structures provided by PLGS Expression Analyses. These queues start with the selection of contained expression analyses for each selected project. The selection of expression analyses is done within the Expression Analysis Selector window as shown in figure 5 by activating the checkboxes from the column include for each Expression Analysis to be processed. The Expression Analysis Selector shows each previously selected project in its own tab pane. ISOQuant generates a separate database for each selected Expression Analysis.

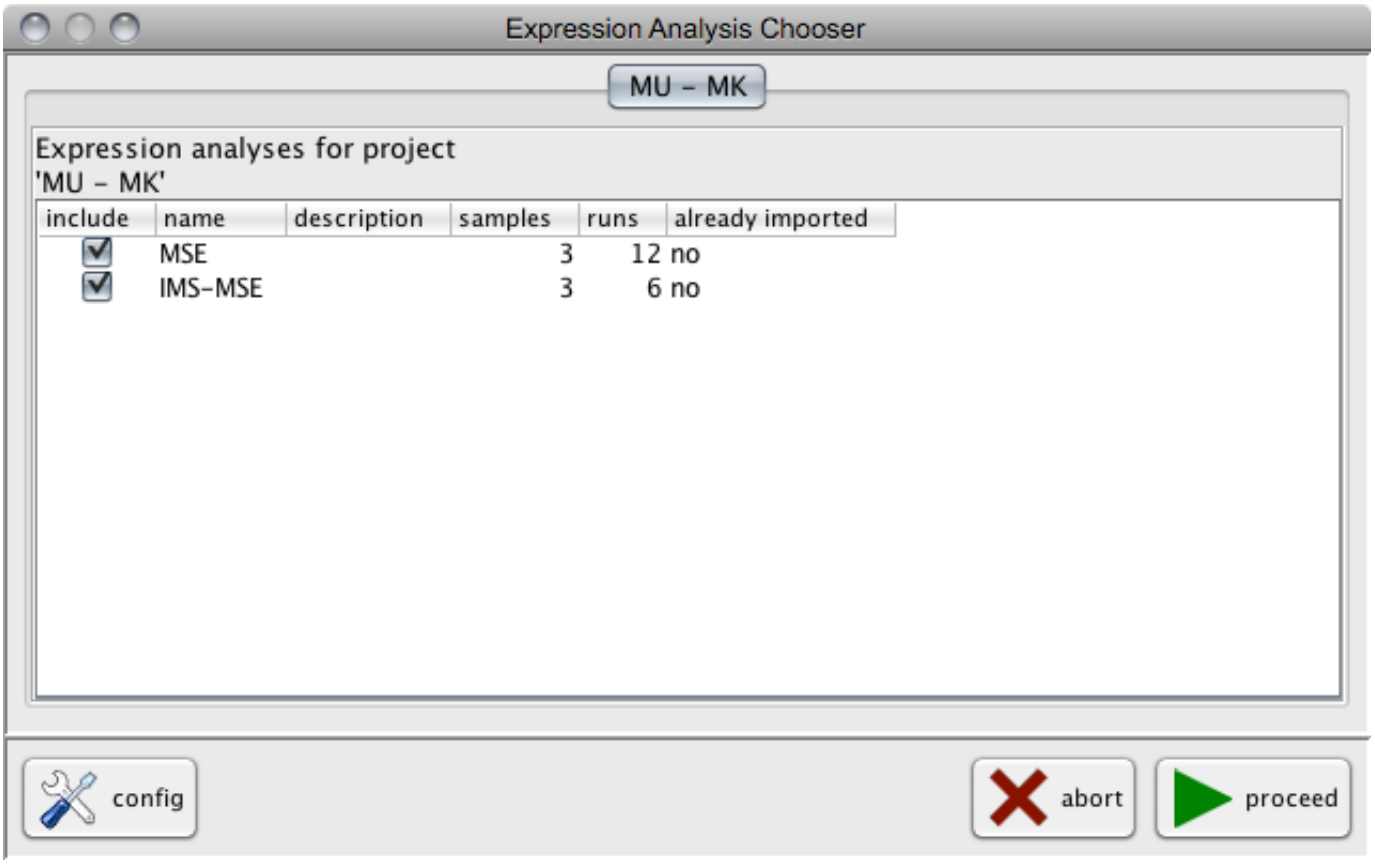

Figure 5: Expression Analysis Selector

4.6 Project Designer

ISOQuant allows to create user defined project structures and then to process these newly structured project data. User defined project structures are created using the Project Designer as shown in figure 6. The Project Designer window shows the PLGS project structure on the left and the user defined structure on the right. A new project structure is created by drag and drop based moving of workflows, samples or groups between left and right structure trees. Additionally to drag and drop actions, right click context menus are available on the right side of Project Designer enabling editing and removing of selected structure elements. On the top of window you can switch between Project Designer panes to restructure each previously selected PLGS project. Processing of designed project can be initiated by clicking the button Ok on the bottom of window or can be aborted by clicking the Cancel button. While processing ISOQuant generates a separate database for each designed project.

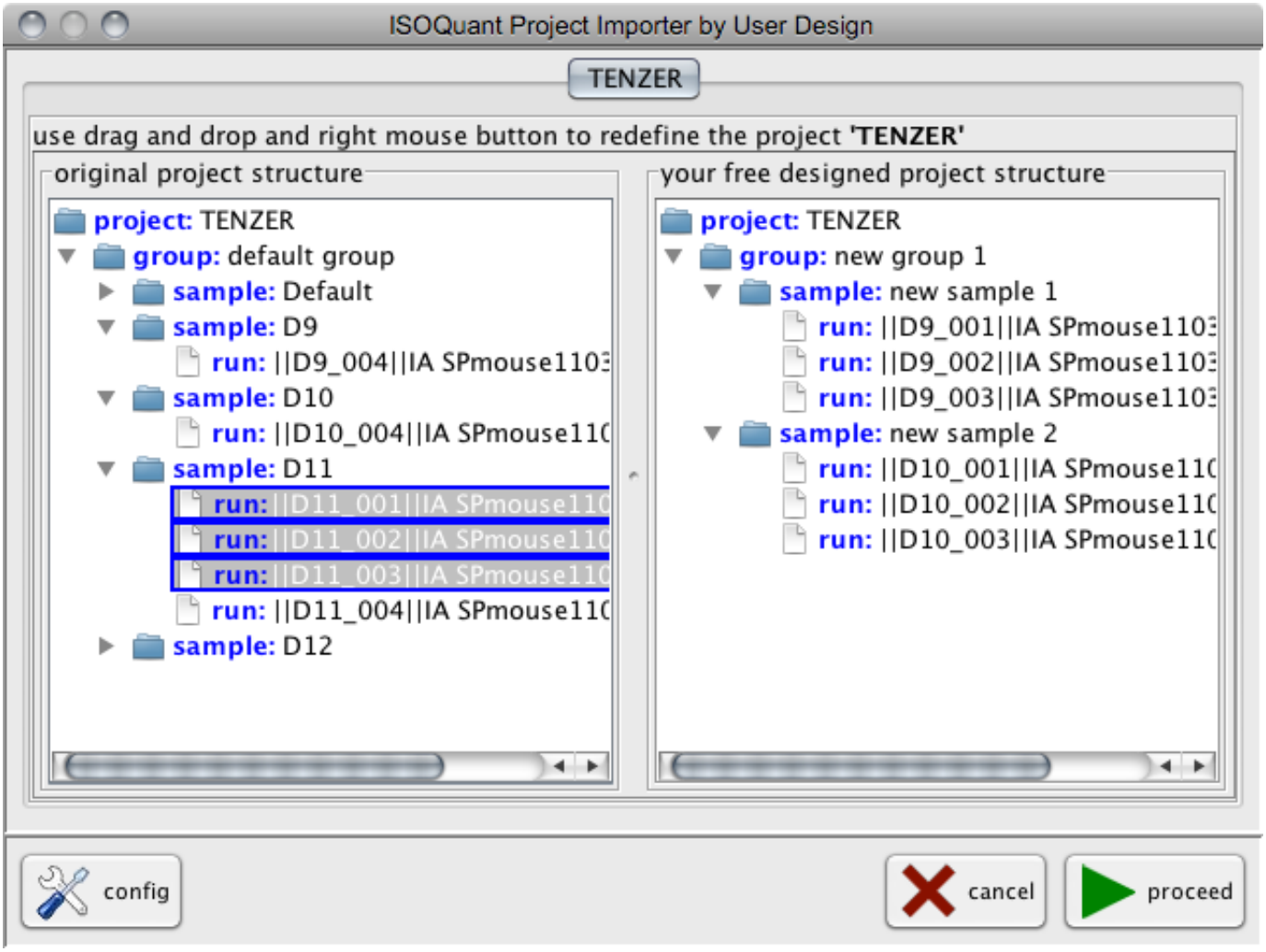

Figure 6: Project Designer

5 ISOQuant configuration

ISOQuant stores parameters for program behavior and data processing algorithms in a single configuration file named `isoquant.ini`. This configuration file is located in the folder you have installed ISOQuant to. For resetting parameters to default values just close ISOQuant then delete or rename the configuration file (or single parameter lines) and start ISOQuant again. If no configuration file can be found on application start a new one will be created using default parameter values.
Do not change the configuration file unless you know what you do!

ISOQuant configuration can be edited from ISOQuant Configuration Editor accessible from graphical user interface. Configuration Editor allows to edit parameters and also export/import configuration settings to/from files.

Two configuration files are provided with ISOQuant installation packages:

- `isoquant_high_confidence.ini` example configuration file for high confidence quantitative analyses
- `isoquant_maxID.ini` example configuration file for discovery proteomics experiments

These files can be imported into ISOQuant from Configuration Editor or manually copied to `isoquant.ini` file.

6 Configuration guide

This chapter lists and describes the main set of available parameters. The number of parameters, their names and the behavior of application caused by parameters are subjects of change because we actively work on improving ISOQuant and underlying methods. Thus the following list of parameters may be incomplete and/or up to date.

6.1 EMRT cluster annotation

6.1.1 Peptide identification filter

User can define minimum reliability criteria for a peptide to be used for ISOQuant processing. *Note:* Only peptides passing this filter will be used for all further analysis steps!

6.1.1.1 Peptide types Peptides identified in first pass of PLGS database search (type: `PEP_FRAG_1`) are generally accepted. Setting one of following parameters to TRUE will configure ISOQuant to accept additional peptide types.

- `process.identification.peptide.acceptType.IN_SOURCE`=false
- `process.identification.peptide.acceptType.MISSING_CLEAVAGE`=false
- `process.identification.peptide.acceptType.NEUTRAL_LOSS_H20`=false
- `process.identification.peptide.acceptType.NEUTRAL_LOSS_NH3`=false
- `process.identification.peptide.acceptType.PEP_FRAG_2`=false
- `process.identification.peptide.acceptType.PTM`=false
- `process.identification.peptide.acceptType.VAR_MOD`=false

Following additional filtering criteria are used as thresholds to select peptides used for further processing steps.

- `process.identification.peptide.minReplicationRate`=2.0
  minimum acceptable peptide replication rate based on absolute number of runs in which every peptide (as sequence-modifier tuple) was identified.
- `process.identification.peptide.minScore`=1.0
  minimum acceptable PLGS peptide identification score.
- `process.identification.peptide.minOverallMaxScore`=1.0
  minimum acceptable value of highest PLGS identification score of a peptide reached in any run of a project. For a peptide detected in multiple runs its maximum reached score has to hit this score to be accepted for annotation. Increasing score will reduce the number of peptides used for EMRT cluster annotation and not necessarily the overall protein quantification quality. Recommended values are between 0.0 and 5.0

- `process.identification.peptide.minSequenceLength`=6
  minimum acceptable peptide sequence length. Recommended value is 6 or more.

6.1.2 Annotation mode

- `process.annotation.useSharedPeptides`=all
  - `all` this is the normal case.
    - `unique` only unique peptides are used for further processing, this option removes all shared peptides from peptides-in-proteins relation instead of protein homology filtering solving the problem of protein inference in a very radical way.
    - `razor` only razor and unique peptides are used for further processing, this option removes all shared peptides from peptides-in-proteins relation after protein homology filtering. Razor and unique peptides are highly reliable for protein quantification because their intensity can be directly assigned to a protein.

6.1.3 Annotation conflict filter

There are cases when multiple peptide identifications map to a single EMRT cluster.

- `process.annotation.peptide.sequence.maxCountPerEMRTCluster`=1
  acceptable number of different peptide sequences (remaining after filtering peptides) allowed to annotate a single cluster. The annotation process will skip ambiguous clusters if this value is set to 1. For values ≥ 2 annotation conflicts are resolved by annotating clusters with the peptide having the highest sum of PLGS identification scores in this cluster.

6.1.4 Homology / isoform and FPR filtering

- `process.annotation.protein.resolveHomology`=true
  Should proteins be filtered for (peptide sequence based) homology/isoform. Only one of detected homologue proteins will be reported.
- `process.annotation.peptide.maxFPR`=0.01
  maximum accepted false discovery rate level for peptides. (value 0.01 means maximum 1% FPR)

6.2 Data preprocessing

- `process.peptide.deplete.PEP_FRAG_2`=false
  should PEP_FRAG_2 peptides be completely removed from database.
- `process.peptide.deplete.CURATED_0`=false
  should CURATED=0 peptides be completely removed from database. If **true**, low-quality peptide IDs are removed.

6.3 EMRT table creation

- `process.emrt.minIntensity`=1000
  peaks having intensities below this limit are assumed to be noise and will not appear in EMRT table

- `process.emrt.minMass`=500
  peaks having masses below this limit are assumed to be noise and will not appear in EMRT table

6.4 Retention time alignment

- `process.emrt.rt.alignment.match.maxDeltaMass.ppm`=10.0
  maximum accepted mass difference between two signals to be assumed as matching for retention time alignment.
- `process.emrt.rt.alignment.match.maxDeltaDriftTime`=2.0
  maximum accepted drift time (ion mobility) difference between two signals to be assumed as matching for retention time alignment. This value is ignored for non-ion-mobility projects. Large value, e.g. 200 will disable the effect of ion mobility on the time alignment.
- `process.emrt.rt.alignment.minIntensity`=1000
  only peaks with intensity over this threshold value are considered for the retention time alignment procedure
- `process.emrt.rt.alignment.minMass`=800.0
  only peaks with mass over this threshold value are considered for the retention time alignment procedure
- `process.emrt.rt.alignment.normalizeReferenceTime`=false
  if true, resulting reference times are adjusted to median distortions at every time point.
- `process.emrt.rt.alignment.maxProcesses`=4
  the maximum number of concurrent retention time alignment processes. We recommend values between 1 and the number of available CPU cores. Default value is set to ½ of the number of CPU cores

- `process.emrt.rt.alignment.maxProcessForkingDepth`=4
  the maximum multithreading depth for each retention time alignment process

6.5 EMRT clustering

- `process.emrt.clustering.distance.unit.mass.ppm`=6.0
  minimum mass based distance between clusters. This is an instrument dependent parameter, e.g. 6 ppm is a good value for Waters Synapt G2/G2-S and 10-12 ppm for Waters Q-TOF Premier and Synapt G1
- `process.emrt.clustering.distance.unit.time.min`=0.2
  minimum retention time based distance between clusters. This is a LC gradient length and peak width dependent parameter, good values are observed to be between 0.06 and 0.2, we recommend to try 0.08, 0.12, 0.16, 0.2; please report which values would work for your setup at which gradient length
- `process.emrt.clustering.distance.unit.drift.bin`=2.0
  menimum drift time based distance between clusters. This is an instrument and also project setup dependent parameter, e.g. for pure IMS projects containing G2 or G2S data, we recommend a value of 2.0. This value is ignored for non-ion-mobility projects. Large value, e.g. 200 will disable the effect of ion mobility on the EMRT clustering.
- `process.emrt.clustering.dbscan.minNeighborCount`=2
  the minimum cluster size (except of noise) and also the minimum required number of peaks inside the reachability radius for cluster expansion. This is a DBSCAN specific parameter and should be increased for big projects. The value of this parameter also depends on used clustering distance units.
- `process.emrt.clustering.maxProcesses=8`
  the maximum number of concurrent clustering processes. For best performance is reached by setting this value to the number of available CPU cores. Default value is set by the estimated number of available CPU cores.

6.6 Peak intensity normalization

- `process.normalization.minIntensity`=3000
  systematic errors of peptides with intensities below this limit are ignored during normalization process.

- `process.normalization.lowess.bandwidth`=0.3
  bandwidth parameter for non-linear regression method (LOWESS) used for exploring systematic errors during normalization process. Recommended values are between 0.3 and 0.6

- `process.normalization.orderSequence`=XPIR
  The processing order sequence of dynamic multi-dimensional normalization. The processing order sequence is defined as a word build from following characters: **X, E, P, W, I, R, M**. The occurrence of a letter either defines the next dimension for EMRT normalization or changes the normalization mode or discards previously calculated values:

  X reset emrt intensities to original values E
  equalize emrt intensities by adjusting sums of intensities for each run P
  activate IN-PROJECT normalization mode S
  activate IN-SAMPLE normalization mode W
  activate Workflow/Run-Value based normalization mode.
  The run to be the normalization reference is automatically set by choosing the run having most emrts. I
  normalize emrt intensities using log-intensity dimension R
  normalize emrt intensities using retention time dimension M
  normalize emrt intensities using mass dimension

  The order sequence is processed from left to right, e.g. the recommended order sequence `XPIR` stands for clean in-project normalization using intensity domain followed by normalization using retention time domain.

6.7 Protein quantification

6.7.1 Peptide filtering

Peptides for protein quantification may be filtered by their type and minimum reached score of a peptide per EMRT cluster. `PEP_FRAG_1` peptides are always accepted. User may decide to accept additional peptide types for quantification. Allowing additional types may result in higher number of quantified proteins but also may affect the quality of quantification. *Note:* This peptide filtering step can not recover peptides not passed the peptide identification filter.

- `process.quantification.peptide.acceptType.IN_SOURCE`=false
- `process.quantification.peptide.acceptType.MISSING_CLEAVAGE`=false
- `process.quantification.peptide.acceptType.NEUTRAL_LOSS_H2O`=false
- `process.quantification.peptide.acceptType.NEUTRAL_LOSS_NH3`=false
- `process.quantification.peptide.acceptType.PEP_FRAG_2`=false
- `process.quantification.peptide.acceptType.PTM`=false
- `process.quantification.peptide.acceptType.VAR_MOD`=false
- `process.quantification.peptide.minMaxScorePerCluster`=5.0

6.7.2 Protein quantification setting

- `process.quantification.absolute.standard.entry`=ENO1_YEAST
  entry of protein used as quantification standard
- `process.quantification.absolute.standard.fmol`=50.0
  amount of quantification standard protein
- `process.quantification.absolute.standard.used`=true
  is a quantification standard protein used at all?
- `process.quantification.topx.degree`=3
  maximum number of peptides for quantifying single proteins
- `process.quantification.maxProteinFPR`=0.01
  maximum accepted false discovery rate level for reported proteins. (value 0.01 means 1% FPR level)
- `process.quantification.minPeptidesPerProtein`=1
  a protein is reported only if it can be quantified by using as minimum this number of peptides

6.8 Application behavior

6.8.1 User interface

- setup.ui.captureConsoleMessages=true
  show Java console messages inside ISOQuant message panel
- setup.ui.location.left=560
  ISOQuant window location, pixels from left
- setup.ui.location.top=360
  ISOQuant window location, pixels from top
- setup.ui.size.height=480
  ISOQuant window height
- setup.ui.size.width=800
  ISOQuant window width
- setup.ui.promptForExit=true
  ask user on closing window
- setup.ui.iconScaleFactor=1.0
  scale original icon sizes by this factor, may be useful on unusually small or large screens.

6.8.2 Data source

- setup.db.autoLoad=false
  should application connect database on start
- setup.db.host=localhost
  the MySQL database host name or ip address. Default tcp port number for MySQL servers is 3306. If your MySQL server is running using an other tcp port number, its host name has to be expanded by adding ':' and the correct port number, e.g. localhost:3307 for MySQL server running on local machine and listening at tcp port 3307
- setup.db.user=root
  MySQL user name
- setup.db.pass=
  MySQL users password
- setup.plgs.root.showEACount=true
  should number of PLGS expression analyses be determined and shown
- setup.plgs.root.showFSSize=false
  should file system size of a projects folder be determined and shown
- setup.plgs.root.dir=/Volumes/RAID0/PLGS2.5/root
  path of last selected PLGS root folder
- setup.plgs.root.autoLoad=false
  should application read last used root folder on start

6.8.3 Report

- setup.report.dir=/Volumes/RAID0/reports
  path of last selected report output folder.
- **set of user defined parameters for all CSV output formats:**
  - setup.report.csv.columnSeparator=','
    column separator string (enclosed in ' or "), usually either ',' or ';'
  - setup.report.csv.decimalPoint='.'
    decimal point string (enclosed in ' or "), usually either '.' or ','
  - setup.report.csv.textQuote='"'
    string for quoting text blocks, usually
- **set of user defined parameters for mzIdentML output:**
  - setup.report.mzidentml.DBNCBITaxID=
  - setup.report.mzidentml.DBOrganismScientificName=
  - setup.report.mzidentml.DBversion=
  - setup.report.mzidentml.researcherFirstName=John
  - setup.report.mzidentml.researcherLastName=Doe
  - setup.report.mzidentml.researcherOrganization=Uni-Mainz
- **set of user defined parameters for Excel output:**
  - setup.report.xls.showAbsQuantFMOLUG=true
    create an extra sheet for absolute protein quantity in femtomoles per microgram.
  - setup.report.xls.showAbsQuantFMOL=true
    create an extra sheet for absolute protein quantity in femtomoles.
  - setup.report.xls.showAbsQuantNG=true
    create an extra sheet for absolute protein quantity in nanograms.
  - setup.report.xls.showAbsQuantPPM=true
    create an extra sheet for absolute protein quantity in parts per million.
  - setup.report.xls.showAllProteins=false
    create an extra sheet for some PLGS based proteins details.
  - setup.report.xls.showRTAlignment=false
    create an extra sheet for retention alignment results.

Generated excel sheets are limited to maximum 65536 rows when using old XLS (Excel 97/2000/2003) format due to its technical limitations. In case of doubt, please use XLSX format for creating ISOQuant reports.

7 End-user license agreement

7.1 External components

ISOQuant relies on external components (re)distributed under different conditions. By using ISOQuant you need to agree to terms and conditions of third party libraries and software included in ISOQuant or needed for running ISOQuant.

ISOQuant uses following external Java libraries:

Library	Version	License Type	Purpose
JDOM	1.1.3	BSD License	XML handling
Tagsoup	1.2.1	Apache v2.0	XML parsing
MySQL Connector/J	5.1.13	GPLv2 with FOSS Exception	database communication
JSiX	1.0	BSD License	Java extensions
POI	3.8	Apache v2.0	spreadsheet file creation
DOM4J	1.6.1	BSD License	POI dependency
StAX	1.0.1	Apache v2.0	POI dependency

Binary versions of these libraries are repackaged into and redistributed with ISOQuant software package according to their license conditions. Please find original licenses as part of ISOQuant package.

Furthermore, ISOQuant relies on external environmental software being not a part or component of ISOQuant but needed to run it:

- Operating System
- Java Virtual Machine
- MySQL 5.1 compatible database engine (e.g. MySQL Server: `http://www.mysql.com/` or MariaDB: `http://mariadb.org/`)
- Waters ProteinLynx Global Server

Please pay attention to terms and conditions arising from any software usage in any way related to ISOQuant.

7.2 ISOQuant license agreement

ISOQuant binaries and source code are available under conditions of BSD license as follows:

ISOQuant - integrated solution for LC-MS based label-free protein quantification

7.3 Disclaimer in plain language

- ISOQuant is an academically developed freely available software implemented using only freely available technologies
- By using ISOQuant you automatically agree to terms and conditions of third party libraries and software included in ISOQuant or needed for running ISOQuant
- We provide ISOQuant as is, without any kind of warranty
- You are using ISOQuant at your own risk, we are not responsible for any kind of damage, errors or data loss produced in any way related to ISOQuant.

8 About developers

ISOQuant is developed by

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