MTXQCvX2 documentation

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Contents

Chapter 1

Welcome

This documentation introduced to you how to use MTXQCvX2 in order to assess the quality of your GC-MS derived data, perform the determination of calibration curves and absolute quantification. It furthermore provides you two normalisation strategies and the calculation of quantities in, e.g., pmol/1e+6 cells or pmol/mg tissue.

MTXQCvX2 does also enable the calculation of stable isotopic incorporation and the evaluation of the underlying data, the mass isotopomer distributions (MIDs).

The tool has been set up to support the in-lab developed workflow for quantitative metabolomics experiments using the in-house developed software Maui for the annotation of data. MTXQCvX2 bridges the gap between quality control and first data post-processing / analysis of GC-MS derived data (MTXQCvX2_part1, MTXQCvX2_part2).

Nevertheless MTXQCvX2 includes a module in order to integrate all kind of data provided in spreadsheet-format, e.g., derived from metmax, extracting required information and creating corresponding files (MTXQCvX2_part4).

Both workflows are introduced in the distinct chapters including their required input parameter (chapter Workflow Maui and Workflow Metmax).

Chapter 2

Content of MTXQCvX documentation

This documentation introduces to you how to use MTXQCvX2 in order to run a first straight-forward data analysis of your metabolomics experiment. Experimental and mathematical concepts have been introduced for the pulsed stable isotope resolved metabolomics (pSIRM) approach published in (Pietzke et al., 2014).

MTXQCvX2 contains a suite of modules is optimized to process GC-MS derived data and processed either in Maui or Chromatof/Metmax. Workflows for both approaches are introduced with step-by-step instructions in chapter @**??**maui) and @**??**metmax).

Subsequently each MTXQCvX2 module (chapter 6 - **??**) is introduced in greater detail including a input / output files and processing parameter.

The configuration of MTXQCvX2 has been split into two categories - (1) a general configuration `config_files` and (2) metabolomics specific parameters `config_mtx`. Latter one is meant to provide flexibility including further substances. How to do so and what files can be customizes is shown in chapter **??**.

Protocols of sample preparation and measurement are summarised in the following chapters **??** and **??**. Workflow-specific processing methods applied Chromatof are introduced separately in the chapter 14 and **??** including all parameter.

The remaining chapter cover a chapter for frequently asked questions (chapter16) and the visualisation of all tables (chapter 17).

Chapter 3

Workflow for Maui-annotation projects

3.1 Read this in case

- you have run a Maui project
- exported all required container (see **??**)
- you have a copy of sequence list and experimental conditions
- you know the extraction procedure

The following article describes briefly how to use MTXQCvX2 in case you used Maui for the annotation of your metabolomics project. It does not matter if you have performed an experiment including stable isotopes or if you just aim for the quantification of a few intermediates.

3.2 Quick view

1. Setup a new R-project and copy MTXQC template files and folders
2. Knit with parameter: `MTXQC_init.Rmd` and create project folder, e.g., `psirm_glucose`
3. Copy input files and rename `ManualQuantTable.tsv` (e18205cz.tsv)
4. Create `annotation.csv` and `sample_extracts.csv` files
5. Define the internal extraction standard
6. Knit with parameter: `MTXQC_ExperimentalSetup.Rmd`
7. Knit with parameter: `MTXQC_part1.Rmd`
8. Knit with parameter: `MTXQC_part2.Rmd`
9. If required, proceed with `MTXQC_part3.Rmd` for ManualValidation

3.3 Input files

Three different kind of export functions have been implemented in Maui. These functions provide the export of the actual data into `.csv` or `.tsv` files that are directly usable as input files for MTXQCvX2. Please refer to section 14.3 how you perform the export and which containers have to be exported using what export function and where to copy them in `psirm_glucose/input/`.

Certain circumstances might wish you to combine *multiple MAUI-projects* into one MTXQC-project. This might be the case when you run the same samples in split and splitless mode on the machine or your

experimental setup has been measured in multiple batches in order to avoid derivatisation effects.

It is highly recommended to combine the input files derived from a different Maui projects beforehand the analysis. In that way you have only to work with a single file `CalculationFileData.csv`[1] containing all data of the your experiment.

The herein described process provides a quick way how to combine the exported files from different Maui projects. The script `combine-sets.R`[2] automatically saves all combined files into the correct `input` folder. Just update the folder and subfolder names. All the rest has been taken care of for you.

1. Create in the MTXQC-project folder (e.g., `psirm_glucose/`) a new folder called `raw-data`
2. Create a subfolder for each Maui-project in `psirm_glucose/raw_data/...`
3. Copy into this folder all your Maui-derived input files altogether
4. Update the parameter of `combine-sets.R`, meaning folder name definitions, file
5. Execute the R script
6. Merged files have been generated and copied into the corresponding folder: `psirm_glucose/input-folder/gc/...` or `psirm_glucose/input-folder/inc/...`
7. Copy the renamed `ManualQuantTable.tsv` files of each Maui project into `psirm_glucose/input/quant/...`

3.4 Annotation-file

The annotation file relate file names with experimental conditions or specify quantification standards in your batch. Two columns - **File and Type** - are obligatory and have to be present in the annotation file. In the case of their absence `MTXQCvX_part1.Rmd` stops processing and shows an error message.

A quick way to generate an annotation file is described below:

1. Copy the first row / header of `quantMassAreaMatrix.csv` file
2. Paste & transpose the content into a new Excel-File into column A
3. Change the first entry: Metabolite -> File
4. Remove the entry QuantMasses at the very end of the column A
5. Add the column Type and specify each file either as **sample** or **addQ1__dilution**[3]
6. Add more columns specifying your experimental conditions, e.g., Cellline and Treatment[4]
7. Save the content as `csv-file` in the `psirm_glucose/input/...`

3.5 Sample__extracts-file

The `sample_extracts.csv` file is required in order to determine automatically absolute quantities in the manner of pmol/1e+6 cells or pmol/mg tissue in the `CalculationFileData.csv`.

This file requires two obligatory columns - **Extract__vol** and **Unit**[5]. Please specify for each experimental condition the amount of extracted cells (count), tissue (mg) or volume of blood/plasme (ul) in the unit shown in the brackets.
The names of the columns of the experimental conditions have to match up with the annotation file. Save the file in the folder `psirm_glucose/input/...`.

If the defined experimental conditions do not match up with the annotation `MTXQCvX2_part1.Rmd` exit data processing. A template file is saved for review and usage at `inst/template_files/...`

[1] stored in `psirm_glucose/output/quant/...`
[2] inst/template_files/...
[3] see for further details additionalQuant
[4] optimal: two-three parameter, max: four parameter. Consider possible combinations, e.g., HCT116-control, HCT116-BPTES
[5] Define: count, mg or ul

3.6 Internal Standard

MTXQCvX2 allows the specification of project-specific internal extraction standards. The only thing you need to do is to define the corresponding compounds as an internal standard in the `conversion_metabolite.csv` file. To do so, add `InternalStandard` to the compound in last column `Standard`.

For an classical pSIRM experiment in the Kempa lab we are using cinnamic acid. The evaluation of this compound has been integrated in Maui. Peak areas of cinnamic acid are exported from a distinct container called `cinAcid`. The exported file has to be renamed to `InternalStandard.csv` though and moved to `psirm_glucose/input/gc/...`.

If you have used a different compound as an internal extraction standard you might need to extract the peak areas of this compound from the Maui export file `quantPeakAreasMatrix.csv` file and save it in the folder `psirm_glucose/input/gc/InternalStandard.csv`, respectively. Prerequisite - you have annotated the compound in Maui.

The report of `MTXQCvX2_part1.Rmd` includes the defined internal standard for each project in a message.

Chapter 4

Workflow for Metmax-extracted projects

4.1 You want to follow this ...

- in case you have measured samples and quantification standards by GC-MS
- performed the annotation of intermediates in ChromaToF or vendor software
- exported all information into `.txt` files
- used metmax to extract peak areas / mass isotopomer distributions (MIDs)

4.2 Introduction

This document describes how to use MTXQCvX2 in combination with metmax[1].

Historically, MTXQCvX2 has been developed and optimized for Maui-derived input files. The `MTXQCvX2-part4.Rmd` functions as a converter of metmax-derived files in order to create suitable input formats for `MTXQCvX-part1.Rmd`.

This module could also be used to convert tables derived from other programs as long as they are stick with the herein described table formats. Mandatory columns are referenced in the text for each kind of input file.

The general workflow of the NMTXQCvX2 project is briefly shown below in quick view. More detailed instructions are summarised in the following paragraphs.

For more detailed explanations about the individual input parameter for each module of MTXQCvX2 please proceed to read the documentation about the individual modules and their knitting parameter. The relation of knitting parameter, input and output files are described in each section.

4.3 Quick view

1. Generate input files: run `MTXQC_part4.Rmd`[2]
2. Setup R-project and copy MTXQC-files
3. Knit with parameter: `MTXQC_init.Rmd`
4. Copy input files into corresponding folders

[1] http://gmd.mpimp-golm.mpg.de/apps/metmax/default.htm
[2] read here the instructions

5. Create annotation.csv and sample_extracts.csv files[3]
6. Update metabolite names in `conversion_metabolite.csv`[4]
7. Define the internal standard and/or alkanes[5]
8. Knit with parameter: `MTXQC_ExperimentalSetup.Rmd`
9. Knit with parameter: `MTXQC_part1.Rmd`
10. Knit with parameter: `MTXQC_part2.Rmd`
11. If required - proceed with `MTXQC_part3.Rmd` for ManualValidation

4.4 Input files

If you need an introduction about how to use metmax - have a look at the separate documentation `Metmax_intro`.

The chapter **??** `MTXQCvX_part4` explains in detail how to use this module to generate suitable input files.

4.5 Annotation-file

The annotation file relate file names with experimental conditions or specify quantification standards in your batch. Two columns - **File and Type** - are obligatory and have to be present in the annotation file. In the case of their absence `MTXQCvX_part1.Rmd` stops processing and shows an error message.

A quick way to generate an annotation file is described below:

1. Copy all file names from a file of your choice
2. Paste & transpose the content into a new Excel-File into column A
3. Call column A -> File
4. Optional: Remove any non-file name entry in this column
5. Add the column Type and specify each file either as **sample**, **Q1_diluation**, ,**addQ1_dilution**[6]
6. Add more columns specifying your experimental conditions, e.g., Cellline and Treatment[7]
7. Save the content as `csv-file` in the `psirm_glucose/input/...`

4.6 Sample_extracts-file

The `sample_extracts.csv` file is required in order to determine automatically absolute quantities in the manner of pmol/1e+6 cells or pmol/mg tissue in the `CalculationFileData.csv`.

This file requires two obligatory columns - **Extract_vol** and **Unit**[8]. Please specify for each experimental condition the amount of extracted cells (count), tissue (mg) or volume of blood/plasme (ul) in the unit shown in the brackets.
The names of the columns of the experimental conditions have to match up with the annotation file. Save the file in the folder `psirm_glucose/input/...`.

If the defined experimental conditions do not match up with the annotation `MTXQCvX2_part1.Rmd` exit data processing. A template file is saved for review and usage at `inst/template_files/...`

[3]Details further down this document
[4]Column: Metabolite_manual
[5]Also in conversion_metabolite.csv; see below paragraph Standards
[6]see for further details additionalQuant
[7]optimal: two-three parameter, max: four parameter. Consider possible combinations, e.g., HCT116-control, HCT116-BPTES
[8]Define: count, mg or ul

4.7 Update metabolite names in conversion_metabolite.csv

The file conversion_metabolite.csv, saved in config_mtx/, serves as a kind of translational table. It defines alternative version of metabolite library names that come in handy to plot data using shorter metabolite names. This file is also used to define settings and standard classifications. Detailed information for each column of the file are shown here: REF

4.7.1 Match your annotation with library names

Prior the analysis you need to match the names of your intermediates with the conversion_metabolite.csv file. You need to update or add the corresponding name for each intermediate in the column **Metabolite_manual**.

General suggestion for naming conventions in ChromaToF: Metabolite_Derivate, e.g., Lactic acid_(2TMS). In case of the presence of main- (MP) and byproducts (BP) use: Metabolite_Derivate_MP/BP, e.g., Glucose_(1MEOX)(5TMS)_MP.

If you have annotated intermediates that are not included so far in this table please follow the instructions how to extend conversion_metabolite.csv.REF

4.7.2 Define your internal standards and alkanes

MTXQCvX2 allows the specification of project-specific internal standards. Corresponding compounds have to be marked as an internal standard in conversion_metabolite.csv by adding the tag **InternalStandard** in the column Standard.

If you check the box - InternalStandard in the parameter selection for MTXQCvX2_part4.Rmd the module searches in your input file for peak areas of the defined standard and extracts the information. It also generates the file InternalStandard.csv and stores it at psirm_glucose/input/gc/....

In the same way alkanes are defined in conversion_metabolite.csv. Each alkane has to be flag tagged with **Alk** in the column Standard. This gives you the opportunity to implement customized mixtures of alkanes in order to determine the retention index. MTXQCvX_part4.Rmd recognises the flag tag and generates Alcane_intensities.csv based on your input file containing peak areas and saves it in psirm_glucose/input/gc/...[9].

The in-lab protocol considers nine alkanes from c10 to c36. Standard annotation includes an hashtag, e.g., #c10. If you use this annotation even Metmax would be able to determine the retention index.

[9] It should be al**k**ane, I know, but Maui doesn't, unfortunately…

Chapter 5

MTXQCvX2_init

MTXQCvX2_init.Rmd - why and how to use it. Advantages of the project folder.

Chapter 6

MTXQCvX_experimentalSetup.Rmd

Chapter 7

MTXQCvX_part1.Rmd

Chapter 8

MTXQCvX_part2.Rmd

Chapter 9

MTXQCvX_part3.Rmd

Chapter 10

MTXQCvX_part4.Rmd - Metmax parser

10.1 This section explains ...

- what MTXQCvX_part4.Rmd does
- how do input files need to look like
- which files are generated
- what the distinct checkboxes mean

This module provides the generation of suitable input files for MTXQCvX2 based on spreadsheet exported information by tools like metmax.

10.2 Input files

10.2.1 Quantification - PeakAreas.csv

In order to perform absolute quantification of

You need a file containing all extracted peak areas for each metabolite and file[1]. The header of metmax-extracted files looks like shown below (see table 1). Please, remember to delete the second header row, representing the column loads for each file before saving as csv-file. Otherwise you end up with weird imported dataframes in R. Quantification masses have to be updated while processing in ChromaToF prior the export of the data e.g., with a reference search[2] or using statistical compare. pSIRM experiments require the definition of pTop5 masses[3] instead of top5 masses in the reference in order to take into account the shift of intensities induced by the application of stable isotopes[4]

name	mass	ri	row.load	file_1	file_2	file_x
Lac	219	1051	0.76	15423	135444	465486
Pyr	174	1042	0.65	56978	46888	4354544
Cit	273	1805	0.99	1326	23321	132121

[1] Tools/Options/Retention analysis, Parameter: Area
[2] See `vignette/ReferenceSearch`
[3] Extended list of quant masses considering isotope incorporation
[4] Mandatory columns: name, mass, files

MTXQCvX_part4 takes care of the formatting and correct column names of the peak areas file and saves it[5]. MTXQCvX_part4 generates also the file PeakDensities-Chroma.csv[6], in case you have selected the option to include sum of area normalisation while knitting this module.

10.2.2 Isotope incorporation - MIDs.csv[7]

In order to determine the incorporation of stable isotopes MTXQCvX2 requires as an input the mass isotopomer distributions (MIDs) for each intermediate and measurement[8]. Fragments for each intermediate have to be pre-defined in metmax at Tools/Options/metabolite masses. They can be imported[9] or manually specified each by each. An example of the metmax output is shown in table 2. The output has to be saved as csv-file, including the deletion of the partial row `column.load`, respectively[10].

name	mass	ri	row.load	file_1	file_2	file_x
Lac	219	1051	0.85	31026	5165829	5829
Lac	220	1051	0.85	3607	662277	277
Lac	221	1051	0.85	1222	111481	81
Lac	222	1051	0.85	188	1003494	10023
Lac	223	1051	0.85	0	33542	342

MTXQCvX_part4 calculates the stable isotope incorporation and exports DataMatrix.csv as well as pSIRM_SpectraData.csv[11]. The mathematics behind are outlined in (Pietzke et al., 2014)

Important: Extracted MIDs have to match with defined mass couples for each metabolite in MTXQCvX2[12]. Please refer for more details to `vignettes/config_mtx-files`.

10.2.3 Derivatisation efficiency - mz73.csv[13]

The extraction of intensities for the ion m/z 73 works analogous to the extraction of MIDs[14]. Mass ranges have to be defined for each intermediate for the mass 73 by defining starting and end mass with 73. MTXQCvX_part4 generates the file MassSum-73.csv[15]. Check `inst\template_files\` for reference. Hopefully soon a new metmax button extracting specific intensities across the batch.

[5] `input/quant/quantMassAreasMatrix.csv`
[6] `input/gc/PeakDensities-Chroma.csv`
[7] Required for `calculation isotope incorporation`
[8] Tools/Options/Isotope concentrator; Parameter: IntensityOfMass
[9] `inst/template_files/MetMax_MIDs.txt`
[10] Mandatory columns: name, mass, files
[11] `input/inc/DataMatrix` & `pSIRM_SpectraData.csv`
[12] `config_mtx/incorpo_calc_masses.csv`
[13] Required for: `sum of area normalisation`
[14] Tools/Options/Isotope concentrator; Parameter: IntensityOfMass
[15] `input/gc/MassSum-73.csv`

Chapter 11

Configuration of MTXQCvX2 - `config_mtx/...`

Herein explained are the customizable tables of the MTXQCvX2 universe.

11.1 `conversion_metabolite.csv`

Column.name	Description	Value
Metabolite_manual	Manual defined metabolite name	#Alanine (2TMS)
Metabolite	Library name of the metabolite	Alanine_(2TMS)_BP_RI:1097_IDENT:B+C
Metabolite_short	Short version of library name of the metabolite	Alanine_(2TMS)
Lettercode	Lettercode version of metabolite name	Ala_2TMS
Q1_value	Checked if quant1:1 value available	x
Mass_Pos	m/z-value corresponding to m_inc	118
SE_sel	Evaluation of the MIDs	x
Q_sel	Evaluation for absolute quantification	x
nopsirm	Exclusively for absolute quantification	
Standards	Defined as standard	InternalStandard, Alk

11.2 `letter_pathway_complete.csv`

Column.name	Description	Value
Letter_Derivate	Derivate definition	Ala
Lettercode	Lettercode name of metabolite	Ala_3TMS
Pathway	Ass.pathway	aa
Pathway.1	Ass. pathway - ordered for heatmap	5-aa
Met_pathway	Ass. pathway - ordered for heatmap incl. Lettercode	5-aa_Ala_3TMS
Subs_class	Substance class	aa
Met_class	Substance class incl. Lettercode	aa_Ala_3TMS

11.3 quant1-values.csv

Column.name	Description	Value
Letter_Derivate	Derivate name of metabolite	3PGA
Quant1_v4	Quantity in (pmol)	43480
Quant1_v3	Quantity in (pmol)	43480

11.4 incorporation_calc.csv & mid_backups.csv

Column.name	Description	Value
Metabolite	Library name of metabolite	Alanine_(2TMS)_BP_RI:1097_IDENT:B+C
Mass_mz	m/z-value	116, 118
LI_MID	Definition of mass level	m0, minc

Column.name	Description	Value
Metabolite	Library name of metabolite	Alanine_ beta-_(3TMS)_MP_RI:1435_IDENT:A+D
Mass.m.z.	m/z value	188
BackupPeakArea	Peak area of Backup MID	4960
BackupMID	MID value for corresponding Mass.m.z.	0.8005

Chapter 12

Protocols - Sample extraction

12.1 Cell extracts

Materials:

- cell culture dishes (10 cm), max. confluency 75%
- washing buffer (Hepes, NaCl, ph 7.4)
- 50% MeOH, ice-cold
- 2 mg/ml cinnamic acid
- chloroform
- 15 ml falcon tubes
- cell lifter

Procedure:

- prepare cell culture dishes accordingly to your experimental conditions
- discard cell culture media
- add quickly 5 ml of washing buffer, discard it
- add very immediately 5 ml ice-cold 50% MeOH suppl. 2 ug/ul cinnamic acid
- detach cells using cell lifter
- collect and transfer cell extract into 15 ml falcon
- store falcons until further processing on ice
- add 1 ml chloroform
- incube for 60 min at cold temperatures (4 C) on rotary or thermo shaker
- centrifuge at max speed for 10 min, cold temperatures
- collect polar and lipid phases into fresh falcons / tubes
- dry under vacuum

In order to generate technical backups:

- resuspend dried extracts in 600 ul 20% MeOH
- shake at cold temperature on thermo shaker for 30 min
- split volumes into equal parts in fresh eppendorf tubes
- dry under vacuum

Suggested cell density: $2 - 3e + 6$ cells / extract.

12.2 Tissue samples

Materials:

- Methanol:Chloroform:Water (MCW) in ratio 5:2:1
- 2 mg/mg cinnamic acid in MeOH
- ddH20
- eppendorf tubes
- tissue lyzer / pulverizer

Procedure:

- snap-freeze tissue samples
- pulverize samples
- aliquote 50 mg of tissue powder
- add 1.5 ml of MCW (suppl. with cinnamic acid final conc. 2 ug/ul)
- shake for 60 min on rotary shaker at cold temperature (4 C)
- add 0.5 ml ddH20 for phase separation
- centrifuge maximum speed, 10 min, cold temperatures
- collect polar and lipid phases in fresh vessels
- dry under vacuum

12.3 Blood samples

Material:

- Methanol:Chloroform:Water (MCW) in ratio 5:2:1
- 2 mg/mg cinnamic acid in MeOH
- ddH20
- eppendorf tubes

Procedure:

- give 20 ul blood / sera directly into 1 ml MCW to avoid lumps
- in case of lumps sonicate samples
- shake samples at 4 C for 800 rpm for 60 min
- add 500 ul ddH20 and vortex shortly
- spin down at 4 C at max speed for 10 min
- aliquote polar phase into 2-3 times 500 ul in 1.5 ml tubes
- aliquote lipid phase 2 times in 100 ul lower in 1.5 ml eppi
- dry in SpeedVac (35 C)

Chapter 13

Protocols - GC-ToF-MS measurement

13.1 Sample derivatisation

Materials:

- Methoxamine (MeOx)
- Pyridine (open under the hood only!)
- MSTFA
- Alkane mix (c10-c36) in Hexane
- chromacol vials and caps (big, small)

Mixtures:

- Solvent 1: 40 mg MeOx in 1 ml Pyridine
- Solvent 2: 10 ul Alkane mix in 1 ml MSTFA

Volumens of both solvents are shown for standard (small vol.) procedures.

Procedure:

- make sure samples are completly dry (1 h speed vac)
- add 20 ul (10 ul) of solvent 1 / sample
- incubate on rotary shaker, 30 C, for 60 min
- add 80 ul (25 ul) of solvent 2 / sample
- incubaate on rotary shaker, 37 C, for 90 min
- centrifuge to spin down insoluble materials
- prepare aliquotes three times 28 ul or two times 15 ul (small glass vials)
- keep on room temperature until measurement (max. 10 days)

13.2 GC-MS measurement

needs to be written

Chapter 14

Data processing - MAUI

14.1 Processing In ChromaToF

Create a new folder in ChromaToF Pegasus Acquired Samples and import your files. The processing of files for Maui-assisted annotation is a two step process. Therefore two data processing methods have to be set up and applied to all files.

14.1.1 Resampling

Resampling is commonly applied and results into a data transformation enabling an improved detection of low abundant peaks and a reduction of noise. (Maybe include an example?)

The processing methods requires to tick `Export of ...`. Subsequently, you are asked to define an output folder and the following paramter:

- Reduction rate: 4
- Beginning to end of the file
- `.peg`-files

14.1.2 Combo-export (`.cdf` & `.csv`)

Re-import the generated `.peg`-files into a subfolder and apply the following data processing method.

Activate the box `asddasd` and define for both file types the following parameter.

`.cdf`-file:

- export directory
-

`.csv`-file:

- export directory
-

14.2 Maui notes

14.3 Maui exports

With initiation of a project folder via `MTXQCvX2_init.Rmd` you created an `input`-folder containing three subfolders: `gc`, `inc`, `quant`. In the following all files that should be exported and copied into these folders are described in detail.

14.3.1 `input/gc/...`

Four input files are exported in order to assess the quality of the GC-MS performance of the run. The menue `Diagnostics` is selectable via right click on your Maui project name. Only the cinnamic acid peak areas are exported via the function `Export Quantification` with right click on the actual container.

Exported Files:

- `Alcane_intensities.csv` - Diagnostics/Export Alcane intensities
- `InternalStandard.csv` - cinAcid container, Export quantification, rename!
- `MassSum-73.csv` - Diagnostics/QC Mass Sum Export; enter: 73 for m/z 73
- `PeakDensities-Chroma.csv` - Diagnostics/ExportPeakDensities

14.3.2 `input/quant/...`

Only one container has to be exported and contains the peak areas of each metabolite and measurement. Keep in mind that you should have uploaded `pTop5 mass list` for the correct determination of peak areas in case of labeling with stable isotopes.

A further note - Maui performs absolute quantification and stores values in the `samplePeakGroups-QMQ` container. These quantities are determined by polynominal regression, and not linear regression.

The file `ManualQuantTable.tsv` is automatically generated by Maui during processing the absolute quantification.

Exported Files:

- `ManualQuantTable.tsv` - location: `Maui-project/export/QM-AbsoluteQuantification/...`[1]
- `quantMassAreasMatrix.csv` - Quantification export of the container `samplesPeakGroups`

14.3.3 `input/inc/...`

This exports are only required in case of an experiment including the application of stable isotopes. It´s this the case you should have performed two things:

1. Used the optional upload of `pTop5 mass list`
2. Go through the pSIRM workflow in Maui

Exported Files:

- `DataMatrix.csv` - Export % Label of container `pSIRM-samplesPeakGroups`
- `pSIRM_SpectraData.csv` - pSIRM Spectra Export of container `pSIRM-samplesPeakGroups`[2]

[1] Don´t forget to rename it - e.g., e17123cz
[2] Requires the selection of Natural_MIDs.txt

Chapter 15

Data Processing - Metmax

15.1 Resampling

15.2 1D-basic

15.3 Reference search

15.4 Export for Metmax

15.5 Data extraction with Metmax

15.5.1 Peak areas

15.5.2 MIDs

Chapter 16

Frequently Asked Questions

16.1 What are additional quantification standards

16.2 How do I extend conversion_metabolite.csv

Chapter 17

Appendix - Tables

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

This chapter shows the structure of all input or output csv-files that are referenced throughout the documentation. Please refer to the chapters for more detailed explanations.

17.1 MTXQC base tables

17.1.1 `config_mtx` tables

17.1.1.1 conv_filenames.csv

Column.name	Description	Value
Metabolite_manual	Manual defined metabolite name	#Alanine (2TMS)
Metabolite	Library name of the metabolite	Alanine_(2TMS)_BP_RI:1097_IDENT:B+C
Metabolite_short	Short version of library name of the metabolite	Alanine_(2TMS)
Lettercode	Lettercode version of metabolite name	Ala_2TMS
Q1_value	Checked if quant1:1 value available	x
Mass_Pos	m/z-value corresponding to m_inc	118
SE_sel	Evaluation of the MIDs	x
Q_sel	Evaluation for absolute quantification	x
nopsirm	Exclusively for absolute quantification	
Standards	Defined as standard	InternalStandard, Alk

17.1.1.2 conv_filesnames_manVal.csv

```
## 'data.frame':    3 obs. of  2 variables:
##  $ AssociatedFile: Factor w/ 3 levels "inc","pSIRM_se",..: 3 2 1
##  $ Filename      : Factor w/ 3 levels "DataMatrix_manVal.csv",..: 3 2 1
```

	AssociatedFile	Filename
1	sample_area	quantMassAreasMatrix_manVal.csv
2	pSIRM_se	pSIRM_SpectraData_manVal.csv
3	inc	DataMatrix_manVal.csv
NA	NA	NA
NA.1	NA	NA

17.1.1.3 DEPRICATED FILE: MQ_correction.csv

Not in use anymore since fluffy adventure!

```
## 'data.frame':    7 obs. of  3 variables:
##  $ Metabolite  : Factor w/ 7 levels "Alanine_(2TMS)_BP_RI:1097_IDENT:B+C",..: 1 2 3 6 7 4 5
##  $ Cor_factor  : num  12 12 6.33 11.82 2.41 ...
##  $ Target_value: num  134695 134695 83259 388544 65154 ...
```

Metabolite	Cor_factor	Target_value
Alanine_(2TMS)_BP_RI:1097_I	12.00	134695.25
Alanine_(3TMS)_MP_RI:1367_I	12.00	134695.25
Fructose_(1MEOX)(5TMS)_BP_	6.33	83259.33
Glucose_(1MEOX)(5TMS)_MP_	11.82	388543.52
Glycerol_(3TMS)_MP_RI:1280_	2.41	65153.65
Fructose_(1MEOX)(5TMS)_MP_	6.33	83259.33
Glucose_(1MEOX)(5TMS)_BP_I	11.82	388543.52

17.1.2 `config_files` tables

These tables are supposed to be modified in relation to the individual needs of a project.

17.1.2.1 conversion_metabolite.csv

```
## 'data.frame':    166 obs. of  10 variables:
##  $ Metabolite_manual: Factor w/ 115 levels "","#2OG","#Adenine (2TMS)",..: 1 1 4 1 1 7 5 6 8 9 ...
##  $ Metabolite       : Factor w/ 166 levels "Acetoacetic acid_(1MEOX)(1TMS)_MP_RI:1132_BMD",..: 1 2 4
##  $ Metabolite_short : Factor w/ 161 levels "Acetoacetic acid_(1MEOX)(1TMS)",..: 1 2 4 5 7 6 8 9 10 1
##  $ Lettercode       : Factor w/ 143 levels "2PGA","3PGA",..: 4 5 6 7 13 14 8 9 11 12 ...
##  $ Q1_value         : Factor w/ 2 levels "","x": 1 1 1 1 2 2 2 2 1 1 ...
##  $ Mass_Pos         : int  NA NA NA NA NA NA 118 190 249 NA ...
##  $ SE_sel           : Factor w/ 2 levels "","x": 1 1 1 1 1 1 2 2 2 2 ...
##  $ Q_sel            : Factor w/ 2 levels "","x": 1 1 2 2 1 1 2 2 2 2 ...
##  $ nopsirm          : Factor w/ 2 levels "","x": 2 2 1 2 2 2 1 1 1 2 ...
##  $ Standards        : Factor w/ 3 levels "","Alk","InternalStandard": 1 1 1 1 1 1 1 1 1 1 ...
```

MetaboliteMetabolit	Metabolit	MetaboliteLettercode	Q1_value	Mass_Pos	SE_sel	Q_sel	nopsirm	Standards
	Acetoacet acid_(1M	Acetoacet acid_(1M	Aceto_1M	NA			x	
	Acetoacet acid_(2T	Acetoacet acid_(2T	Aceto_2T	NA			x	
#Adenos (3TMS)	Adenosin (3TMS)_	Adenosin (3TMS)	Adenosin	NA		x		
	Adenosin	Adenosin	Adenosin	NA		x	x	
	Alanine_ beta- _(2TMS)	Alanine_ beta- _(2TMS)	bAla_2T	NA	x		x	

17.1.2.2 letter_pathway_complete.csv

```
## 'data.frame':    100 obs. of  7 variables:
##  $ Letter_Derivate: Factor w/ 69 levels "2HG","2OG","3PGA",..: 1 2 3 4 5 5 6 6 7 8 ...
##  $ Lettercode     : Factor w/ 100 levels "2HG","2OG","3PGA",..: 1 2 3 4 6 5 8 7 9 10 ...
##  $ Pathway        : Factor w/ 9 levels "aa","glut","glyc",..: 2 9 3 4 5 5 1 1 1 1 ...
##  $ Pathway.1      : Factor w/ 9 levels "1-glyc","2-tca",..: 3 2 1 7 9 9 5 5 5 5 ...
##  $ Met_pathway    : Factor w/ 97 levels "1-glyc_DHAP_BP",..: 28 21 17 91 96 96 35 34 36 37 ...
##  $ Subs_class     : Factor w/ 11 levels "aa","amino sugar",..: 6 6 8 3 4 4 1 1 1 1 ...
##  $ Met_class      : Factor w/ 97 levels "aa_Ala_2TMS",..: 55 56 83 43 46 46 2 1 3 4 ...
```

Letter_Derivate	Lettercode	Pathway	Pathway.1	Met_pathway	Subs_class	Met_class
2HG	2HG	glut	3-glut	3-glut_Glut_2h	organic acid	organic acid_Glut_2hydroxy
2OG	2OG	tca	2-tca	2-tca_Glut_2ox	organic acid	organic acid_Glut_2oxo
3PGA	3PGA	glyc	1-glyc	1-glyc_PGA	phosphate	phosphate_PGA
A	A	nucleobase	7-nucleobase	7-nucleobase_A	nucleobase	nucleobase_Adenosine

17.1.2.3 incorp_calc_masses.csv

```
## 'data.frame':    90 obs. of  3 variables:
##  $ Metabolite: Factor w/ 45 levels "Alanine_(2TMS)_BP_RI:1097_IDENT:B+C",..: 1 1 2 2 3 3 4 4 5 5 ...
##  $ Mass_mz   : int  116 118 188 190 245 249 273 275 273 276 ...
##  $ LI_MID    : Factor w/ 2 levels "m0","minc": 1 2 1 2 1 2 1 2 1 2 ...
```

Metabolite	Mass_mz	LI_MID
Alanine_(2TMS)_BP_RI:1097_I	116	m0
Alanine_(2TMS)_BP_RI:1097_I	118	minc
Alanine_(3TMS)_MP_RI:1367_I	188	m0
Alanine_(3TMS)_MP_RI:1367_I	190	minc
Aspartic acid_(2TMS)_BP_RI:1433_IDEI	245	m0

17.1.2.4 quant1_values.csv

```
## 'data.frame':    70 obs. of  3 variables:
##  $ Letter_Derivate: Factor w/ 70 levels "2HG","2OG","3PGA",..: 1 2 3 4 5 6 7 8 9 10 ...
```

```
##  $ Quant1_v4      : int  57270 34220 43480 7400 18710 134700 11480 22710 15030 11220 ...
##  $ Quant1_v3      : int  57270 34220 43480 7400 18710 134700 11480 22710 15030 11220 ...
```

Letter_Derivate	Quant1_v4	Quant1_v3
2HG	57270	57270
2OG	34220	34220
3PGA	43480	43480
A	7400	7400
Adenosine	18710	18710

17.1.2.5 mid_backups.csv

```
## 'data.frame':    224 obs. of  4 variables:
##  $ Metabolite    : Factor w/ 38 levels "Alanine_ beta-_(3TMS)_MP_RI:1435_IDENT:A+D",..: 1 1 1 1 2 2 1
##  $ Mass.m.z.     : int  188 189 190 191 116 117 118 119 188 189 ...
##  $ BackupPeakArea: int  4960 876 307 53 2616179 323019 99834 19759 4960 876 ...
##  $ BackupMID     : num  0.8005 0.1414 0.0495 0.0086 0.8553 ...
```

Metabolite	Mass.m.z.	BackupPeakArea	BackupMID
Alanine_ beta-_(3TMS)_MP_RI:1435_	188	4960	0.8005000
Alanine_ beta-_(3TMS)_MP_RI:1435_	189	876	0.1414000
Alanine_ beta-_(3TMS)_MP_RI:1435_	190	307	0.0495000
Alanine_ beta-_(3TMS)_MP_RI:1435_	191	53	0.0086000
Alanine_(2TMS)_BP_R	116	2616179	0.8552984
Alanine_(2TMS)_BP_R	117	323019	0.1056035
Alanine_(2TMS)_BP_R	118	99834	0.0326384
Alanine_(2TMS)_BP_R	119	19759	0.0064597
Alanine_(3TMS)_MP_F	188	4960	0.8005000
Alanine_(3TMS)_MP_F	189	876	0.1414000

17.2 Input data

17.2.1 MAUI derived tables

17.2.2 Metmax derived tables

17.3 Output data

17.3.1 Experimental Setup

17.3.2 MTXQCvX2 part1

17.3.2.1 `output/gc/...`

17.3.2.1.1 `HM_GC_values.csv` & `qcmetric_xy.csv`

MTXQC exports a file summarising quality factors for each of the four parameter evaluating the GC performance. A summary representing the values illustrated in the heatmap are shown in table `HM_GC_values.csv`, individual exports for each metric in table `qcmetric_xy.csv`.

```
## 'data.frame':    3 obs. of  3 variables:
##  $ Column.name: Factor w/ 3 levels "Batch_Id","qc_metric",..: 1 2 3
##  $ Description: Factor w/ 3 levels "Batch-Id","Class of QC metric",..: 1 3 2
##  $ Value      : Factor w/ 3 levels "0.937254457",..: 3 1 2
```

Column.name	Description	Value
Batch_Id	Batch-Id	e18274ba
qc_metric	QC metric factor corresponding with 1 - very good and 0 - very low	0.937254457
title	Class of QC metric	alkanes


```
## 'data.frame':    3 obs. of  3 variables:
##  $ Column.name: Factor w/ 3 levels "Batch_Id","qc_metric",..: 1 2 3
##  $ Description: Factor w/ 3 levels "Batch-Id","Class of QC metric",..: 1 3 2
##  $ Value      : Factor w/ 3 levels "0.937254457",..: 3 1 2
```

Column.name	Description	Value
Batch_Id	Batch-Id	e18274ba
qc_metric	QC metric factor corresponding with 1 - very good and 0 - very low	0.937254457
title	Class of QC metric	alkanes

17.3.2.1.2 `IntStandard_normfactors.csv` & `IntStandard_stats.csv`

```
## 'data.frame':    5 obs. of  3 variables:
##  $ Column.name: Factor w/ 5 levels "Batch_Id","File",..: 2 5 1 4 3
##  $ Description: Factor w/ 5 levels "Bacth-Id","Determined normalisation factor",..: 4 5 1 2 3
##  $ Value      : Factor w/ 5 levels "0.837457514",..: 4 2 3 1 5
```

Column.name	Description	Value
File	File name	e18274ba_17.cdf
PeakArea	Peak area of internal extraction standard	89308492
Batch_Id	Bacth-Id	e18274ba
IntStd_fac	Determined normalisation factor	0.837457514
IntStd_eval	Evaluation of normalisation factor in relation to defined range plus/minus one standard deviation	within

```
## 'data.frame':    8 obs. of  3 variables:
##  $ Column.name: Factor w/ 8 levels "Batch_Id","File",..: 2 7 1 4 3 6 5 8
##  $ Description: Factor w/ 8 levels "Batch-Id","Evaluation regarding QC",..: 3 7 1 5 2 6 4 8
##  $ Value      : Factor w/ 8 levels "0.837457514",..: 7 5 6 1 8 4 2 3
```

Column.name	Description	Value
File	File name	e18274ba_17.cdf
PeakArea	Peak area of internal extraction standard	89308492
Batch_Id	Batch-Id	e18274ba
IntStd_fac	Normalisation factor	0.837457514
IntStd_eval	Evaluation regarding QC	within

17.3.2.1.3 `Min_Annotation.csv` & `SumArea_stats.csv`

```
## 'data.frame':    9 obs. of  3 variables:
##  $ Column.name: Factor w/ 9 levels "area_fac","Batch_Id",..: 3 2 6 9 7 4 8 1 5
##  $ Description: Factor w/ 9 levels "Extracted Batch-Id derived from file name",..: 2 1 6 8 9 3 7 4 5
##  $ Value      : Factor w/ 9 levels "1.296568521",..: 9 8 2 6 3 5 4 1 7
```

Column.name	Description	Value
File	File name	e18274ba_17.cdf
Batch_Id	Extracted Batch-Id derived from file name	e18274ba
n_area	Number of peak areas per file	101
sum_area	Sum of all peak areas	44614610885
n_total	Total number of entries (including NA)	107

```
## 'data.frame':    2 obs. of  3 variables:
##  $ Column.name: Factor w/ 2 levels "Batch_Id","n_50": 1 2
##  $ Description: Factor w/ 2 levels "Batch-Id","Number corresponding to fifty percent of the maximum n
##  $ Value      : Factor w/ 2 levels "53.5","e18274ba": 2 1
```

	Column.name	Description	Value
1	Batch_Id	Batch-Id	e18274ba
2	n_50	Number corresponding to fifty percent of the maximum number of annotated peaks per file	53.5
NA	NA	NA	NA
NA.1	NA	NA	NA
NA.2	NA	NA	NA

17.3.2.1.4 `mz73_data.csv`

```
## 'data.frame':    7 obs. of  3 variables:
##  $ Column.name: Factor w/ 7 levels "Batch_Id","File",..: 2 1 3 6 4 7 5
##  $ Description: Factor w/ 7 levels "Batch-ID","File name",..: 2 1 3 6 4 7 5
##  $ Value      : Factor w/ 7 levels "0.002407244",..: 7 6 3 2 4 5 1
```

Column.name	Description	Value
File	File name	e18274ba_17.cdf
Batch_Id	Batch-ID	e18274ba
mean_73	Mean value of the sum of m/z 73 intensities per file	16314646.1
sd_73	Standard deviation of the mean of the sum of m/z 73 intensities per file	143890119.5
n_peaks	Number of intensities used for statistics	600

17.3.2.2 `output/quant/...`

17.3.2.2.1 `calcheck_linearity.csv`

```
## 'data.frame':    21 obs. of  3 variables:
##  $ Column.name: Factor w/ 21 levels "adj_r_squared",..: 9 2 4 17 14 21 10 11 7 16 ...
##  $ Description: Factor w/ 21 levels "","Adjusted Rsquare value of linear regression of the calibrati
##  $ Value      : Factor w/ 18 levels "","-898.3400476",..: 11 12 13 6 7 16 3 10 9 17 ...
```

Column.name	Description	Value
Metabolite	Full library name of the metabolite	Alanine_(3TMS)_MP_RI:1367_IDENT:B+C
Batch_Id	Batch-Id	e18274ba
File	File name	e18274ba_53.cdf
QuantMasses	Defined quantification masses	110.0 133.0 114.0 100.0 188.0 190.0
PeakArea	Sum of peak areas based on defined QuantMasses	12710956

17.3.2.2.2 `CalculationFileData.csv`

This is porbably the most important file that is generated by running `MTXQCvX2_part1.Rmd`. It summarises all quality factors, experimental data and determined quantities of your experiment. This file provides the input for `MTXQCvX2_part2-PostProcessing.Rmd`.

```
## 'data.frame':    43 obs. of  4 variables:
##  $ Column.name: Factor w/ 43 levels "absconc","adj_r_squared",..: 4 6 9 13 36 41 42 12 43 23 ...
##  $ Class      : Factor w/ 9 levels "AnnExp","AnnExtract",..: 1 1 1 1 1 1 1 2 2 3 ...
##  $ Description: Factor w/ 41 levels "Absolute quantity in pmol",..: 3 14 14 16 6 14 40 7 9 18 ...
##  $ Value      : Factor w/ 37 levels "","#Glycerol-3-phosphate (4TMS)",..: 27 24 25 28 1 3 34 18 26 29
```

Column.name	Class	Description	Value
Batch_Id	AnnExp	Batch-Id extracted from file name	e18274ba
CL	AnnExp	Experimental parameter	BE(2)-C
Cond	AnnExp	Experimental parameter	Control
File	AnnExp	File name	e18274ba_25.cdf
Standards	AnnExp	Defined as standard (InternalStandard, Alk)	
Time	AnnExp	Experimental parameter	0
Type	AnnExp	Type of measurement	sample
Extract_vol	AnnExtract	Defined extractes in count, mg or uL defined in Unit	3290000
Unit	AnnExtract	Defined unit for corresponding Extract_vol	count
Lettercode	AnnMet	Lettercode version of metabolite name	Glyc3P

17.3.2.2.3 HeatMap_Quant_pTop5.csv

```
## 'data.frame':    5 obs. of  3 variables:
##  $ Column.name: Factor w/ 5 levels "Batch_Id","Lettercode",..: 2 1 3 4 5
##  $ Description: Factor w/ 5 levels "Batch-Id","Library name of metabolite",..: 4 1 2 3 5
##  $ Value      : Factor w/ 5 levels "0.996053496",..: 2 4 3 5 1
```

Column.name	Description	Value
Lettercode	Short name of metabolite	Cit
Batch_Id	Batch-Id	e18274ba
Metabolite	Library name of metabolite	Citric acid_(4TMS)_MP_RI:1814_IDENT:B+D
Par	Parameter	R2_cal
Val	Value of the parameter for corresponding metabolite	0.996053496

17.3.2.2.4 pTop5_Calibration_Samples_lincheck.csv

```
## 'data.frame':    7 obs. of  3 variables:
##  $ Column.name: Factor w/ 7 levels "Batch_Id","count",..: 4 3 1 5 2 7 6
##  $ Description: Factor w/ 7 levels "Batch-Id","Evaluation of peak area in relation to calibration cu
##  $ Value      : Factor w/ 5 levels "1","3PGA","51",..: 2 5 4 NA 3 3 1
```

Column.name	Description	Value
Lettercode	Short name of metabolite	3PGA

17.3.2.2.5 top5_CalibrationInfo_unique.csv

```
## 'data.frame':    8 obs. of  3 variables:
##  $ Column.name: Factor w/ 8 levels "adj_r_squared",..: 6 5 2 7 1 4 8 3
##  $ Description: Factor w/ 8 levels "Adjsuted Rsquare of calibration curve",..: 6 5 2 7 1 4 8 3
##  $ Value      : Factor w/ 8 levels "0.000194064",..: 6 5 7 8 2 4 1 3
```

Column.name	Description	Value
Metabolite	Library name of metabolite	Citric acid_(4TMS)_MP_RI:1814_IDENT:B+D
Lettercode	Lettercode name of metabolite	Cit
Batch_Id	Batch-Id	e18274ba
Origin	Origin of quant1:1 value	Qstd
adj_r_squared	Adjsuted Rsquare of calibration curve	0.996053496

17.3.2.2.6 top5_QMQcurveInfo.csv

```
## 'data.frame':    15 obs. of  3 variables:
##  $ Column.name: Factor w/ 15 levels "adj_r_squared",..: 8 7 14 10 2 5 3 4 13 11 ...
##  $ Description: Factor w/ 15 levels "Adjusted Rsquare of calibration curve",..: 8 5 13 9 2 6 4 3 12 1
##  $ Value      : Factor w/ 13 levels "0.000194064",..: 9 9 7 11 12 2 6 4 13 10 ...
```

Column.name	Description	Value
Lettercode	Lettercode of metabolite name	Cit
Letter_Derivate	Derivate name	Cit
Quant1_v4	Quant1:1 value in (pmol)	52050
Metabolite	Library name of metabolite	Citric acid_(4TMS)_MP_RI:1814_IDENT:B+D
Batch_Id	Batch-Id	e18274ba
Dilution	Dilution factor	0.2
ChromIntensities	Corresponding peak areas	45074572
Concentration	Concentration in (pmol)	10410
Origin	Origin of quantification standard	Qstd
Metabolite_short	Short name of metabolite	Citric acid 275_(4TMS)
adj_r_squared	Adjusted Rsquare of calibration curve	0.996053496
intercept	Intercept of calibration curve	564.549288
slope	Slope of calibration curve	0.000194064
max	Max. value of calibration curve	52050
min	Min. value of calibration curve	260.25

17.3.2.3 output/inc/...

17.3.2.3.1 HeatMap_Incorporation.csv

```
## 'data.frame':    4 obs. of  3 variables:
##  $ Column.name: Factor w/ 4 levels "Batch_Id","Lettercode",..: 2 1 3 4
##  $ Description: Factor w/ 4 levels "Batch-Id","Lettercode name of metabolite",..: 2 1 3 4
##  $ Value      : Factor w/ 4 levels "0.740740741",..: 2 3 4 1
```

	Column.name	Description	Value
1	Lettercode	Lettercode name of metabolite	3PGA
2	Batch_Id	Batch-Id	e18274ba
3	Par	Parameter	NA_count
4	Val	Value of the parameter shown in heatmap	0.740740741
NA	NA	NA	NA

17.3.2.3.2 SE_calculation_NAscore.csv

```
## 'data.frame':    5 obs. of  3 variables:
##  $ Column.name: Factor w/ 5 levels "Batch_Id","fracr_prop",..: 3 1 5 4 2
##  $ Description: Factor w/ 5 levels "Batch-Id","Class of NA-value",..: 4 1 2 5 3
##  $ Value      : Factor w/ 5 levels "0","0.851851852",..: 4 5 1 3 2
```

Column.name	Description	Value
Lettercode	Lettercode name of metabolite	Cit
Batch_Id	Batch-Id	e18274ba
na_frac_r	Class of NA-value	0
N	Number of MIDs	46
fracr_prop	Fraction of MIDs with x NA-values in relation to total number of evaluated MIDs	0.851851852

17.3.2.3.3 SE_classification.csv

```
## 'data.frame':    9 obs. of  3 variables:
##  $ Column.name: Factor w/ 9 levels "Batch_Id","count_score",..: 4 1 3 8 7 5 6 9 2
##  $ Description: Factor w/ 6 levels "_expl_","Batch-Id",..: 4 2 3 1 1 1 1 6 5
##  $ Value      : Factor w/ 9 levels "1861","2.30897367",..: 4 6 7 3 1 2 9 5 8
```

Column.name	Description	Value
Lettercode	Lettercode name of metabolite	Cit
Batch_Id	Batch-Id	e18274ba
File	File name	e18274ba_26.cdf
sum_spa	_expl_	4297
sum_bpa	_expl_	1861

17.3.2.3.4 SE_validation.csv

```
## 'data.frame':    6 obs. of  3 variables:
##  $ Column.name: Factor w/ 6 levels "Batch_Id","count_score",..: 3 1 2 4 6 5
##  $ Description: Factor w/ 6 levels "Batch-Id","Evaluation of MID",..: 3 1 2 5 4 6
##  $ Value      : Factor w/ 5 levels "1","54","Cit",..: 3 4 5 2 2 1
```

Column.name	Description	Value
Lettercode	Lettercode name of metabolite	Cit
Batch_Id	Batch-Id	e18274ba
count_score	Evaluation of MID	goodQ
N_count	Number of evaluated MIDs	54
sum_files	Number of detected MIDs	54

17.3.2.4 MTXQCvX2 part3

17.3.2.5 MTXQCvX2 part4

Chapter 18

pSIRM experiments

The application of stable isotopes provides a powerful tool to track the activity of metabolic pathways. the time-dependent and atom-specific routing along a metabolic pathway resolved how substrates like glucose or glutamine are used in order to maintain a certain phenotype and energetic homeostatsis.

We developed an approach called pulsed stable isotope resolved metabolomics (pSIRM) enabling the quantitative evaluation of metabolite pool sizes and incorporation of stable isotopes, e.g., $^{13}C_6$-glucose. A thoughtful setup of the experimental design including the applied substrates and carefull experimental handling are prerequisites for a successful pSIRM experiment. Essential aspects are collected in the below paragraphs along with a number of usefull tweaks.

18.1 Experimental design

An *in vitro* pSIRM experiment lasts in total up to three days starting from the cell seeding at day zero. Further along the way up to two media changes should be included until the application of stable isotopes and harvesting the cells maintaining the continouse availability of nutrients and avoiding the accumulation of waste products (Figure **??**fig:psirm)). The media change four hours prior the harvest is set up in order to give cells time to recover from the mechanical stress of the media change. At the time point of harvest cells should be in a perfect happily state regarding metabolic environment and stress.

Choose carefully the *seeding density of you cells* in the first place. High confluency inducing contact inhibition of cell growth has a strong impact on several cellular processes including the uptake of nutrients. Try to aim for petri dishes with a maximum confluency of 75-80 %. A pre-experiment including different cell densities for seeding at a number of experimental conditions helps you to get a feeling for the cell growth in general and an expected output of cells at the time point of the harvest. Later one is useful to plan sample extraction and measurement subsequently.

For adherent cell cultures only: Include for each experimental condition an additional petri dish that is solely used to determine the cell count at the time point of your harvest. This additional plate ensures a correct determination of absolute quantities and might reduce variation of pool sizes in the statistical analysis[1]. Think carefully about control conditions and include cell culture dishes that are not labeled. These dishes function as a control for your labeling procedure and the natural abundance of isotopes.

[1] Pelleting these cells and snap-freezing might give usefull additional samples for western blotting.

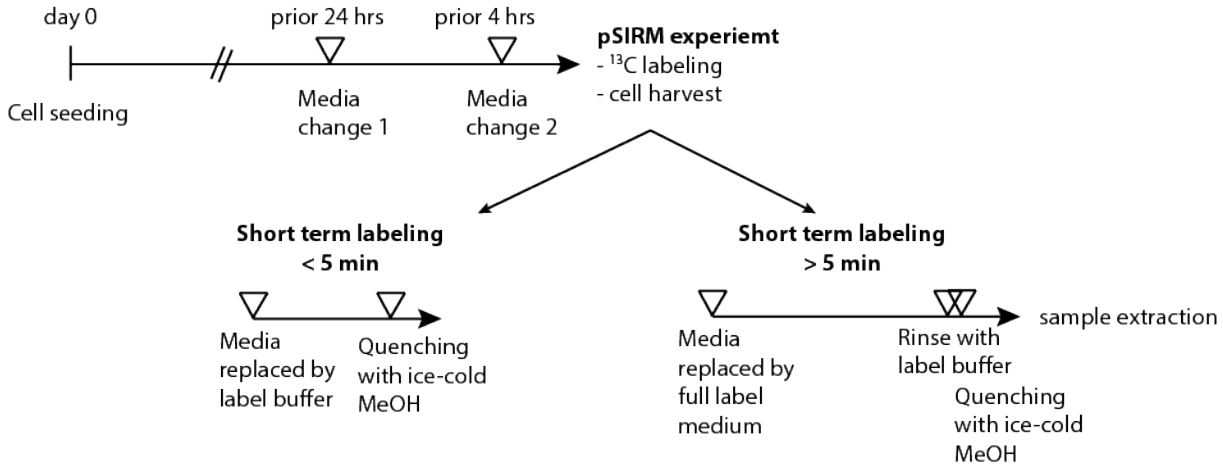

Figure 18.1: Experimental design of a pSIRM experiment distuingishing short and long labeling with stable isotopes.

Table 18.1: Solvent composition of Full label medium (LM) and label buffer (BF) for a pSIRM experiment labeling with 13C-glucose.

Solvent	Base	Carbon.source	Supplem
Full label medium (LM)	DMEM, without glucose, glutamine, pyruvate	13C-Glc (2.5 g/L) 12C-Gln (2 mM)	small mo
Label buffer (LB)	HEPES (5 mM), NaCl (140 mM), pH 7.4	13C-Glc (2.5 g/L) 12C-Gln (2 mM)	

18.2 Experimental procedures

There is a slight difference in the protocols for adherent and suspension cells. Please read instructions and footnotes carefully.

Short[2] and long term labeling procured differ only in the applied solvents during the labeling - either label buffer (LB) only or a combination of full label medium (LM) and label buffer (LB). Latter one is applied in order to remove extracellular metabolites of the media. Both label buffer and media contain the major nutrients / stable isotopes to keep the main substrates at constant supply at all times (Table 18.1). During the application of stable isotopes longer than a few minutes cells might sense the absence of further intermediates provided during standard cell culture procedure and and adjust their metabolic program accordingly.

The quality of your data later heavily relies on the exact handling of the cells and a *consistent timing* throughout the pSIRM experiment. Especially the step removing the LB and quenching the cells should be a matter of a tenth of seconds rather than seconds. It is of great value to perform the cell harvest with a second person.

18.3 Protocol pSIRM

18.3.1 Adherent cell cultures

The herein described protocols are detailed explanations how to perform a pSIRM cell harvest for long term label application. If you want to label for less than 2 minutes omit solely *omit steps 6-8*.

Materials:

[2]I would rather recommend this up to 2 min of labeling

- Cell culture dishes, max. confluency 80 %
- Labeling media (LM) supplemented with substrates (5 ml / dish)[3]
- Label buffer (LB) supplemented with substrates (5 ml / dish)
- Ice-cold 50 % MeOH supplemented with 2 ug/ul cinnamic acid
- 2x 5 ml pipette and tips[4]
- Beaker
- Ice
- 15 ml falcons (chloroform resistant)
- Cell lifter
- Biological waste bin next to your bench

Procedure:

1. Pre-warm LB and LM in the water bath
2. Take a number of petri dishes (condition-wise including all biol. replicates)
3. Discard cell culture media
4. Carefully add *long term labeling* LM OR *short term labeling*: LB
5. Incubate cells on the bench or in an incubator
6. Discard LM (beaker)
7. Add immediatly 5 ml of LB
8. Rotate dish once in order to cover complete surface
9. Meanwhile 2nd person get prepared with 5 ml ice-cold MeOH
10. Discard LB into beaker and *immediatly* 2nd person quenches with ice-cold MeOH
11. Collect cell extracts using cell lifter
12. Transfer cell extracts into 15 ml falcons
13. Store falcons on ice until further processing

Repeat this procedure (step 6-10) for all dishes of a single condition first. Once MeOH is added metabolic processes are interrupted and cell extracts can be collected with the help of cell lifter without rush and subsequently transfered to 15 ml falcon and stored on ice until further processing (see chapter Cell extraction methanolic extracts).

Determine the cell count using your additional petri dishes for each condition.

18.3.2 Supension cell cultures

Materials:

- Cell culture flasks
- Labeling media (LM) supplemented with substrates
- 5 ml pipette and tips[5]
- 1 ml pipette
- Beaker
- paper tissues
- Liquid nitrogen
- 15 ml falcons
- 1.5 ml eppendorf tubes
- Biological waste bin next to your bench

Procedure:

1. Pre-warm LM in the water bath
2. Determine the cell count of your cell suspension(s)

[3]Not required for short term labeling
[4]Highly recommended, makes labeling and harvest super quick
[5]Highly recommended, makes labeling and harvest super quick

3. Take aliquots of $10 - 15e + 6$ cells and transfer into 15 ml falcon
4. Spin down cells very gently 300 g, 2 min at room temperature
5. Discard media into beaker
6. Resuspend cells gently in three-times 1 ml[6]
7. Incubate and keep warm
8. Fractionate cell supension in three eppendorf tubes (3x 1 ml each)
9. Spin down quickly in top-bench centrifuge[7]
10. Discard media blandtly on paper tissues
11. Snap-freeze immediatly in liquid nitrogen
12. Store cells until further processing (see chapter Cell extraction suspension cells)

The important step here to be quick is the alquotation of the cell suspension and subsequent spin down in the table centrifuge. Suspension cells are rather small, nevertheless $3e + 6$ cells per extract are a good starting point for GC-MS measurements.

18.4 Hints & notes

- The only way to be reproducible and fast is to team up with a second person.
- Keep timing consistently through the experiment.
- Keep substrate concentrations constant throughout the experiment in all solutions.
- Supplement one stable isotopic labeled substrate with all remaining substrates in non-labeled form.
- Think about nutrient levels in your cell culture and your experimental conditions. Maybe you want to change things to physiological levels.
- Add additional plate to each condition in order to have material for western blotting and others.
- Check carefully the confluency of your dishes and determine seeding densities for different conditions.
- In case of small molecule inhibitors: Try to avoid to solve them in DMSO - strong impact on chromatography.

[6] To generate three replicates
[7] Most of the times 30 s are already enough

Chapter 19

GC-MS settings

In the following paragraphs details of GC-MS settings are described in detail. The herein described settings have been optimized for cell extracts measured in split-mode 1:5 on the instrument Pegasus 4D-C GC-ToF-MS in 1D mode equiped with an autosampler Gerstel MPS.

19.0.1 Autosampler settings

The table **??** summarises the defined parameter for method settings of the Gerstel MPS.

The injector itself is a temperature-regulated system providing the advantage of a focused injection of the sample. Gradient

19.0.2 Gas chromatography

The most important settings of the GC method are listed below. A graphical representation of the gradient in the oven is shown in figure 19.2 and corresponding values in table 19.1.

Flow path:

1. Inlet: Front
2. Capillary: GC Oven 50 m, 250 u int. diameter, 0.25 u film thickness, RTX-5 phase
3. Capillarty: Detector 0.21 m, 250 u int. diameter, 0.25 u film thickness, RTX-5 phase
4. Detector: TOF

19.0.3 Mass spectrometer settings

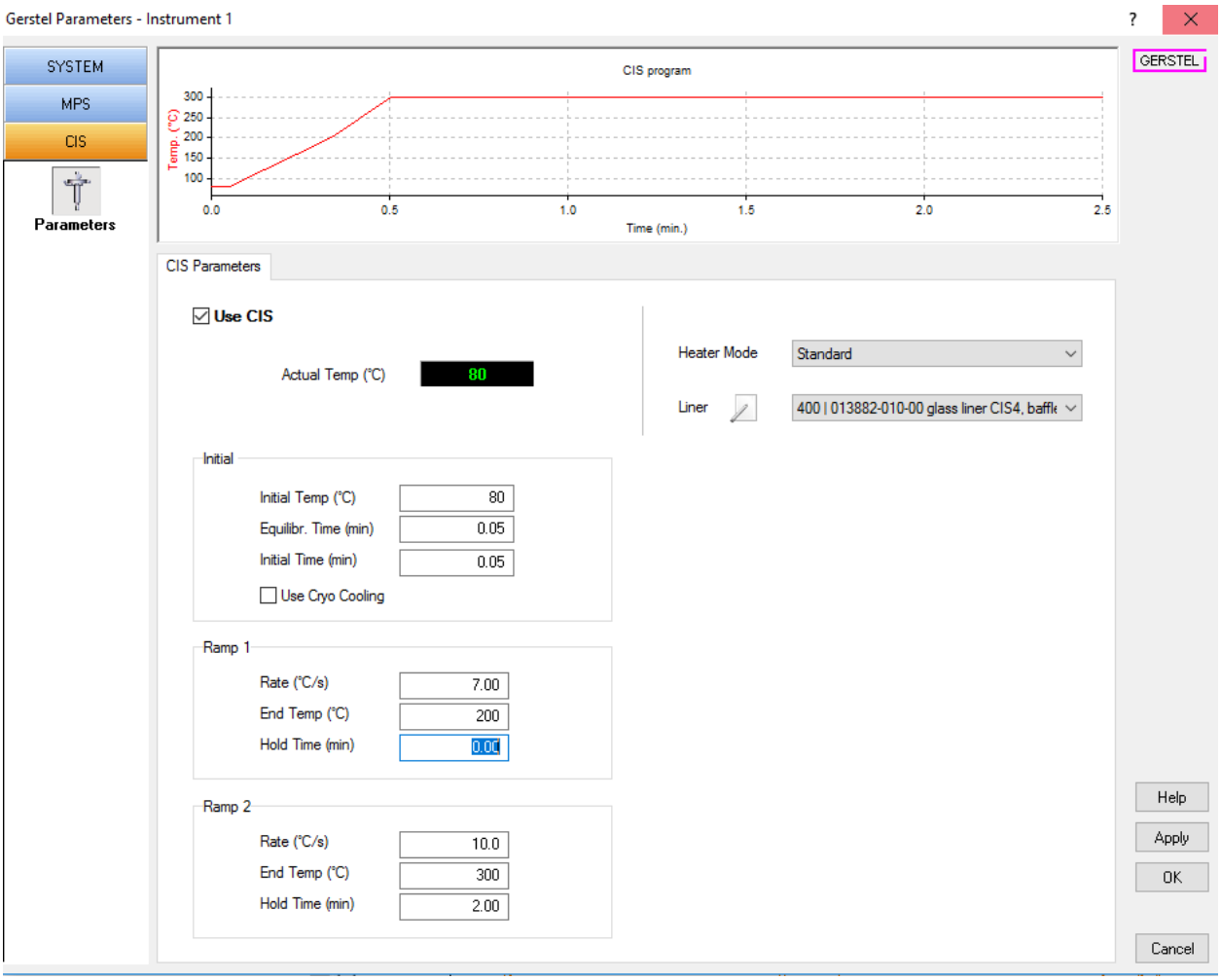

Figure 19.1: Settings temperature-regulated injection for Gerstel MPS

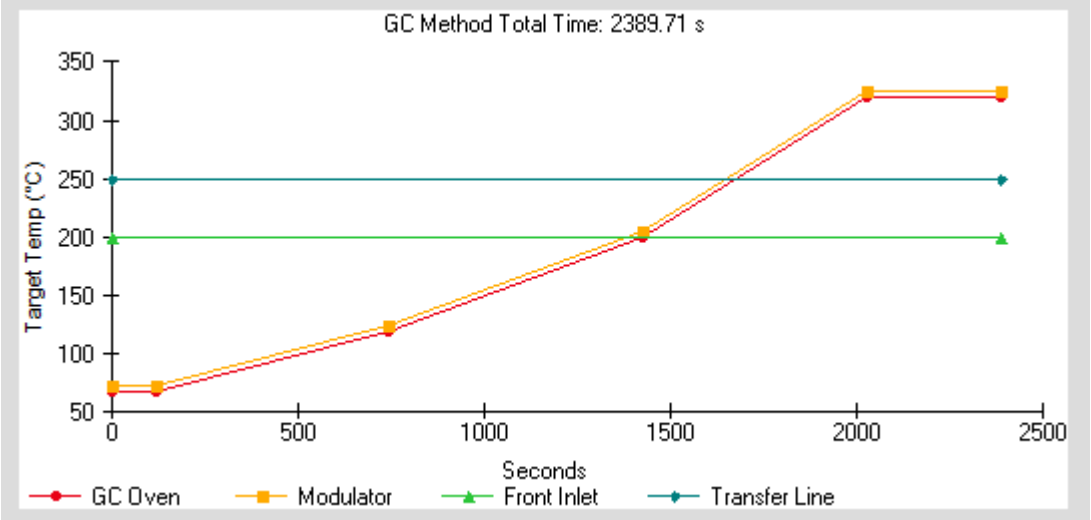

Figure 19.2: GC gradient - graphical representation. Rate in (°C/min), Target temperature in (°C), Duratin in (min).

Table 19.1: GC gradient profile - Rate shown in (K/min), Target Temp in (C), Duration in (min)

Rate	Target_Temp	Duration
Initial	68	2
5	120	0
7	200	0
12	320	6

Chapter 20

Solutions and Materials

20.1 Solutions

20.1.1 Label buffer

Materials:

- ddH2O (500 ml)
- 140 mM NaCl (4.1 g)
- 5 mM Hepes (0.569 g)
- pH calibration 7.4

Procedure:

- Weigh the correct amounts of Hepes and NaCl
- Resolve in a glas bottle with 450 ml of water
- Stir carefully
- Check and adjust pH
- Adjust volumes to 500 ml

20.1.2 MCW

Materials:

- Methanol
- Chloroform
- ddH2O
- Cinnamic acid stock in MeOH (2 mg/ml): final conc. 2 ug/ml

Procedure:

- Mix the solvents in the ratio of volumes - Methanol:Chlorofom:Water – 5:2:1
- Supplement cinnamic acid stock 1:1000
- Store at -25°C

20.1.3 Alkane-Mix

Materials:

Table 20.1: List of chemicals including company names and catalog number.

Chemical	Catalog.Nb	Company	Storage
Chloroform	132950-1L	Sigma	RT
Cinnamic acid	C80857 5g	Aldrich	RT
Hexane for HPLC > 97% (GC)	34859-1L	Sigma	RT
Methanol	1060351000	Merck	RT
Methoxyamine hydrochloride	226904-5G	Aldrich	RT
MSTFA - Methyltrimethylsilyltrifluoracetamide	701270201	Macherey-Nagel	4°C
Pyridine	270970-100ML	Sigma	RT

- Hexane
- Alkanes: c10, c12, c15, c17, c19, c22, c28, c32, c36
- Thermo mixer
- Glass vials and caps

Procedure:

- Prepare stock solutions in hexane:
- c10 - c17 (liquid): 25 ul/ml
- c19 - c32: 20 mg/ml
- c36: two-times 15 mg/1.5 ml
- Warm up alkane stocks in thermo mixer 40°C
- Prepare a text mixture in equal amounts, e.g., 50 ul each, but use twice the volume of c36
- Mix test mixture with MSTFA: 10 ul / 1 ml MSTFA
- Check alkane profile by GC-MS
- If required: adjust volumes and re-test or create larger volume of zour mixture for aliquots
- Store aiquots in glass vials, close well and store at 4°C
- For usage: gently warm up glass vials at 30 C on thermo mixer for 10 min and vortex before adding it to the MSTFA

Adjust the volumes of the alkane stocks in order to create a curve shaped distribution of all alkanes in the chromatogram: lower intensities for c10 and c32-36, slowly increasing intensities for the alkanes in between.

20.2 Materials

Bibliography

Pietzke, M., Zasada, C., Mudrich, S., and Kempa, S. (2014). Decoding the dynamics of cellular metabolism and the action of 3-bromopyruvate and 2-deoxyglucose using pulsed stable isotope-resolved metabolomics. *Cancer & metabolism*, 2(1):9.