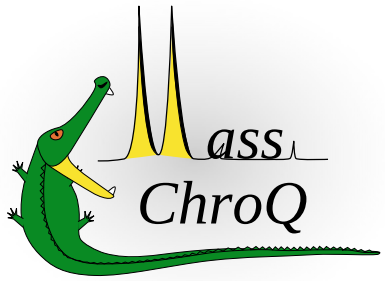

MassChroQ is one of the PAPPSO facility software projects

MassChroQ User Manual

Free and Open Source Mass Chromatogram Quantification Software

MassChroQ 2.4.33

MassChroQ User Manual: Free and Open Source Mass Chromatogram Quantification Software

by Benoît Valot, Edlira Nano, Olivier Langella, and Michel Zivy

January 24, 2025 , 2.4.33

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MassChroQ

http://pappso.inrae.fr/en/bioinfo/masschroq/

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Revision History

Revision 0.7.24	28 May 2021	Filippo Rusconi

- Start actually documenting the software with the Preface.

Revision 0.7.24	21 April 2021	Filippo Rusconi

- Very first setting up of the user manual using the mineXpert2 project as a template.

Dedication

To all the admirable people acting in the *"Free Software Movement"* for a better and more ethical computing world

To all the readers who helped with this manual.

Contents

List of Figures

Preface

1 Software feature offerings and intended audience

This manual is about the *MassChroQ* proteomics quantification software project.

MassChroQ has the following features:

- Load an XML file mainly describing the precursor peptidic ions that were fragmented to produce tandem mass spectra that allowed to identify proteins.
- Compute extracted ion current (XIC) chromatograms for the precursor ions by looking into the mass spectrometry data files;
- Perform under-the-curve calculations to provide intensity values for the precursor ions.

2 Feedback from the users

We are always grateful to any constructive feedback from the users.

The PAPPSO software team might be contacted *via* the following contact page:

http://pappso.inrae.fr/en/travailler_avec_nous/contact/ (search for team members having the "Bioinformatics" specialty mentioned, like Olivier Langella or Filippo Rusconi).

3 Program and Documentation Availability and License

The programs and all the documentation that are shipped along with the *MassChroQ* software suite are available at http://pappso.inrae.fr/en/bioinfo/masschroq/. Most of the time, a new version is published as source, and as binary install packages for *MS-Windows* (64-bit systems only).

For *GNU/Linux*, binary packages are created locally (see http://pappso.inrae.fr/en/bioinfo/masschroq/download/) but are also built in the *Debian*[1] autobuilders and are uploaded to the distribution servers. These packages are available using the system's software management infrastructure (like using the *Debian*'s `apt` command, for example, or the graphical application).

1 http://www.debian.org/

The software and all the documentation are all provided under the Free Software license *GNU General Public License, Version 3, or later, at your option*. For an in-depth study of the *Free Software* philosphy, the reader is kindly urged to visit HTTP://WWW.GNU.ORG/PHILOSOPHY.

1 Generalities

In this chapter, some general concepts around the *MassChroQ* program and the reference to be used to cite the software in publications will be introduced.

1.1 General concepts and terminologies

This section describes the general concepts at the basis of the analysis of quantitative proteomics data that one needs to grok in order to properly assimilate the workings of the *MassChroQ* software.

1.1.1 Spectral Counts or Area Under the Curve?

MassChroQ is a program to perform quantitative mass spectrometry experiments in the context of proteomics projects. Quantitative proteomics projects can be handled in various ways, depending on the data that are used to actually quantify the identified peptides and proteins.

There are two main methods to peform quantitative assessments starting from proteomics data: spectral counting computations and area under the curve computations, as described below.

- *Spectral counts* quantitation: this variant is based on the counting of the mass spectra that have allowed the identification of a given protein. It has the advantage of being a label-free quantitative technique, but it is also too rough to be considered a "robust and precise" technique.
- *Area under the curve* quantitation: this variant is based on the integration of the ion current that can be extracted from the mass data ("extracted ion current", or XIC) for the precursors ions that, upon fragmentation, led to the protein identification. By integrating the XICs for all the peptide precursors that identified a given protein, the software can estimate the abundance of that specific protein. The software thus needs to be able to extract the ion current for all the precursor ions in the MS spectra, when these ions were fragmented and allowed identifying peptides that in turn identified proteins. This is the process that *MassChroQ* implements, which is why it is called *Mass Chromatogram Quantification*, with *Chromatogram* referring to the extracted ion current (XIC) chromatogram used to assign an intensity value to the peptide ion that was fragmented.

Note

It should be noted that *MassChroQ* does not know anything about proteins. Instead, it does know about a list of peptidic precursor ions' m/z values, which is all it needs to perform these XIC extractions.

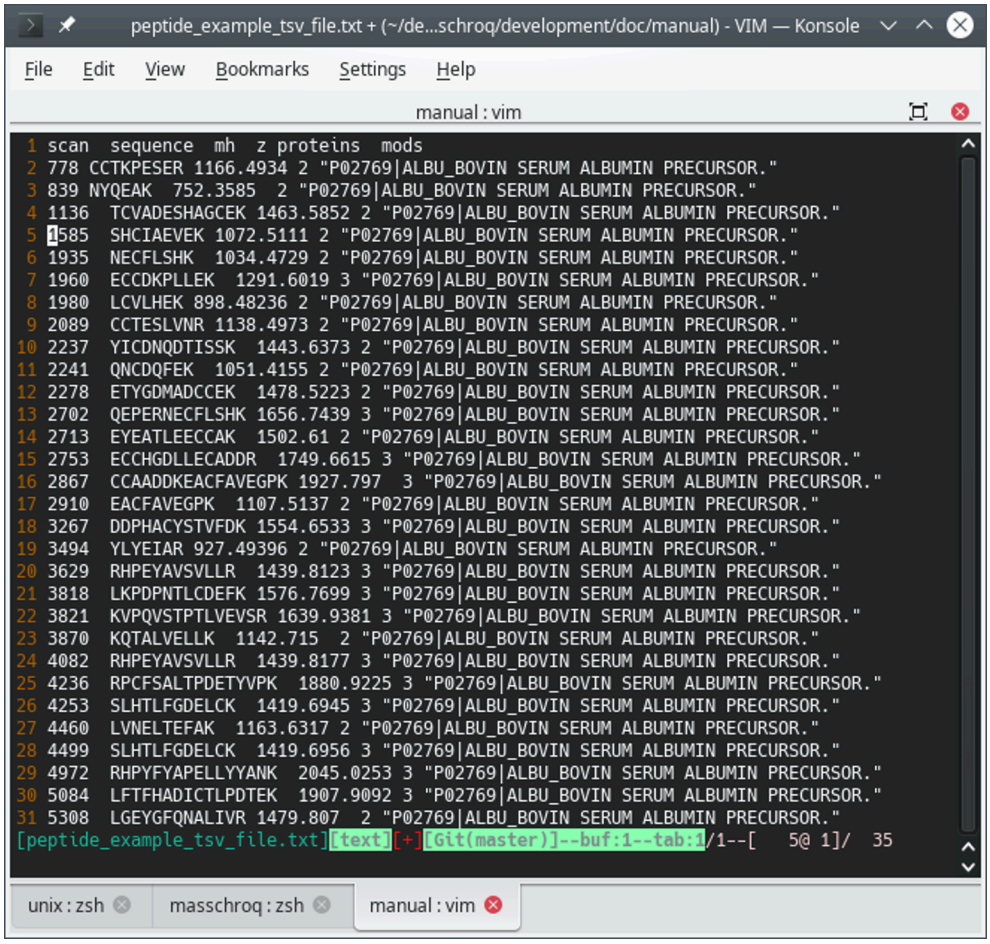
1.1.2 Outline of a *MassChroQ* working session

The very first step is to produce the `masschroqml` file that lists all the peptides that need a XIC extraction. There are two possibilities for this:

- *X!TandemPipeline* can produce that file and has a *MassChroQ* configuration interface that is well documented in the user manual. The `masschroqml` file contains all the data required for the *MassChroQ* operation.
- For scientists not using *X!TandemPipeline* for their proteomics projects, it is possible to craft the `masschroqml` file starting from a rudimentary file and by pointing *MassChroQ* to a `tsv` file (a tab-separated values file) containing the data required by *MassChroQ*. A typical file is shown in Figure 1.1, "The peptide list file in `tsv` format".

Note

It is important that the *mh* column be filled-in with the calculated $[M+H]^+$ ion mass. The charge column (*z* column) represents the actual charge of the precursor peptide ion.



This file documents the peptides that led to the identification of proteins. It needs to be provided to *MassChroQ* in case the identification process has been carried over outside of *X!TandemPipeline*.

FIGURE 1.1: THE PEPTIDE LIST FILE IN `tsv` FORMAT

FIXME: brief description of the steps of the quantification analysis.

- Then do this: FIXME

1.2 Citing the *MassChroQ* software.

Please, cite the software using the following reference:

Benoît Valot, Olivier Langella, Edlira Nano, Michel Zivy. 2011. MassChroQ: A versatile tool for mass spectrometry quantification. *Proteomics*, 11: 3572-3577. https://doi.org/10.1002/pmic.201100120.

1.3 Installation of the software

The installation material is available at http://pappso.inrae.fr/en/bioinfo/masschroq/download/.

1.3.1 Installation on MS Windows

The installation of the software is extremely easy on the MS-Windows platform: the installation program is standard and requires no explanation.

1.3.2 Installation on Debian- and Ubuntu-based systems

The installation on Debian- and Ubuntu-based GNU/Linux platforms is also extremely easy (even more than in the above situations). *MassChroQ* is indeed packaged and released in the official distribution repositories of these distributions and the only command to run to install it is:

`$` [1] **`sudo apt install <package_name>`** RETURN

In the command above, the typical *package_name* is in the form `masschroq` for the program package and `masschroq-doc` for the user manual package.

Once the package has been installed, the program shows up in the *Science* menu. It can also be launched from the shell using the following command:

`$` **`masschroq`** RETURN

1 The prompt character might be *%* in some shells, like *zsh*.

2 Usage

In this chapter, the usage of *MassChroQ* will be introduced.

2.1 Running MassChroQ

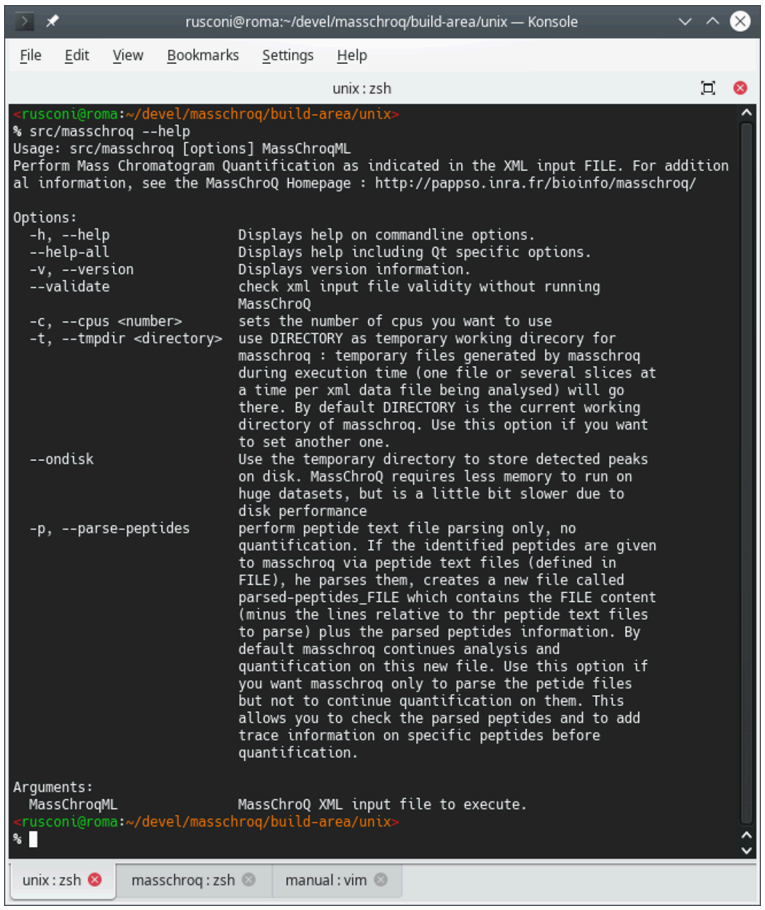
There are more than one ways to run the *MassChroQ* software, that will be described in the following sections.

2.1.1 Running MassChroQ from the Terminal

MassChroQ can be run from the terminal. To get a review of the different options, run the following command at the prompt:

```
$ masschroq --help
```

The output of the command above is described in Figure 2.1, "Printing the help message in the terminal".



This is the help message that prints out in the terminal when the program is run with the `--help` option.

FIGURE 2.1: PRINTING THE HELP MESSAGE IN THE TERMINAL

There are two possibilities, either using the `--parse-peptides` option or not.

- Without the `--parse-peptides` option. In this case, the `masschroqml` file provided as parameter to the command is fully auto-sufficient: it contains both the *MassChroQ* configuration bits, the list of identified proteins and the list of identifying peptide precursor ions that need their ion current extracted (XIC).

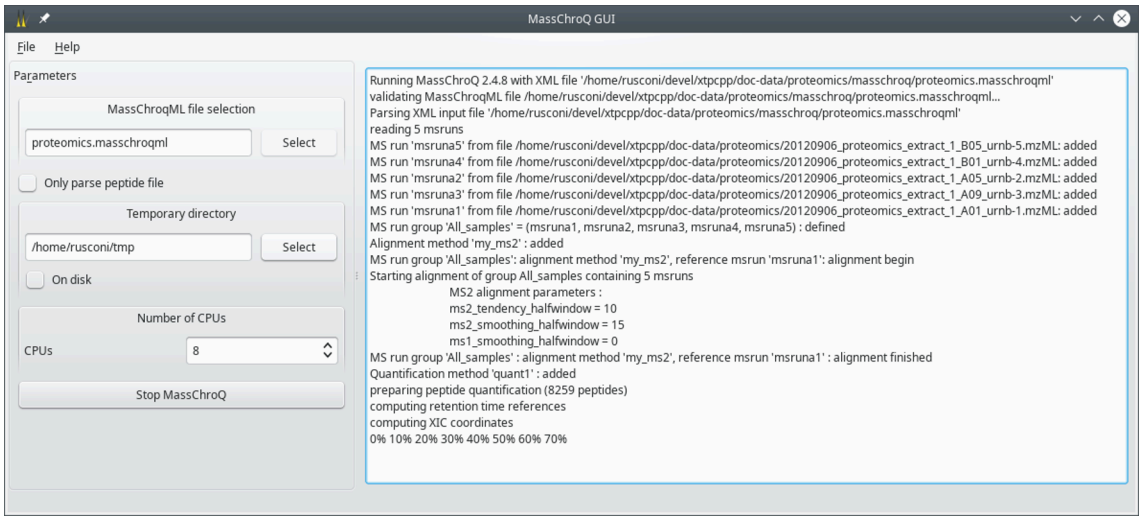
- With the `--parse-peptides` option. In this case, the `masschroqml` file provided as parameter to the command is a partially empty shell: the list of peptide precursor ions is not provided as part of the file. The user must have crafted the peptide list file as described in Figure 1.1, "The peptide list file in `tsv` format". The name of that peptide list file must be provided in the `masschroqml` file provided as parameter to the command.

2.1.2 Running MassChroQ from the Graphical User Interface

MassChroQ can be run from a graphical user interface. To this end, start the following command at the prompt:

```
$ masschroq_gui
```

The window that opens up is shown in Figure 2.2, "The main window".



The main window allows the user to easily configure the *MassChroQ* run. The output of the program in displayed in the text widget on the right hand side of the window.

FIGURE 2.2: THE MAIN WINDOW

The following configuration elements have to be set by the user:

- *MassChroQML file selection*: use the *Select* button to open a file selection dialog window. Choose the `masschroqml` file that specifies all the data required to run *MassChroQ*. This is the *input* file.
- *Only parse peptide file*: check this check box widget if the file set above does not contain the full list of peptide ion precursors that identified the proteins. Checking this widget is analogous to using the `--parse-peptides` option on the command line (see Section 2.1.1, "Running MassChroQ from the Terminal").
- *Temporary directory*: use the *Select* button to open a directory selection dialog window. Choose the directory where *MassChroQ* will write the temporary data it needs to carry over its work.
- *On disk*: check this check box widget to write on disk the detected peaks to alleviate the consumption of random access memory. The program is slower because read/write operations on disk are considerably slower than their equivalent in memory.
- *Number of CPUs*: Enter the number of execution threads that *MassChroQ* is allowed to use when parallelizing its tasks.
- *Start MassChroQ*: click onto this button to start the program. When the program has started the label of this button changes to *Stop MassChroq*.
  When the program is running, the output is printed in the text widget on the right hand side of the window.

2.2 MassChroq Output File

The output of *MassChroQ* is stored in a file named according to the results_<input_file_name>.ods scheme, where the `masschroqml` extension is removed. The file is written inside of the directory that contains the input `masschroqml` file.

The format of the output file is the *Libreoffice* spreadsheet format (*Open Document Spreadsheet*, ODS). That file contains three tabs, described below:

- *MassChroq informations - <id>*: the <id> suffix identifies the quantification set. This tab contains the configuration bits of the *MassChroQ* run.

Note

The <id> suffix is not currently of use, actually. *MassChroQ* was designed to be able to perform multiple quantification runs with different settings on the same set of associated samples. However, this is not currently available in the *X!TandemPipeline* interface.

- *<Sample association group>_proteins*: the <Sample association group> prefix identifies the group in which samples were associated. There can be one or more such groups. If there is only one group, it has the default *All_samples* name. If there are more than one group, then they are named according to the configuration set by the user. This tab contains the *peptide*, *protein* and *protein_description* columns, all self-explanatory.
- *peptides_<id>_<Sample association group>*: the <id> and <Sample association group> suffixes were described above. This tab contains the actual XIC data for each peptide precursor ion that was quantified. There are a large number of columns, described below.

The peptides tab in the results spreadsheet file contains a large number of columns that are described in the paragraphs below. Each row of the spreadsheet is related to a given peptide precursor ion that was fragmented and that allowed identifying a peptide, which in turn contributed to the identification of a protein.

- *quantification*: this is the <id> mentioned above. At the time of writing this identification bit is not significant.
- *group*: this is the name of the group of associated samples.
- *msrun*: automatically-assigned name that is unambiguously attached to the mass spectrometry file.
- *msrunfile*: mass spectrometry data file name for the current sample.
- *mz*: calculated (FIXME) m/z value.
- *rt*: retention time at which the peptide precursor ion was eluted (in seconds). FIXME (the rt for the most intense ion current, that is, the top of the curve or what ? see rtbegin below)
- *maxintensity*: maximum ion current intensity. FIXME
- *area*: are under the XIC chromatogram curve. FIXME
- *peak quality*: assessment on the quality of the peak. The more 'a' characters, the higher the quality. FIXME
- *rt begin*: the retention time at the beginning of the the precursor ion XIC chromatogram peak. FIXME c'est vrai ?
- *rt end*: the retention time at the end of the the precursor ion XIC chromatogram peak. FIXME c'est vrai ?

- *peptide*: the peptide id as set by the dabase search engine.
- *label*: label that might be attached to the peptide in labelling-based quantification. FIXME
- *sequence*: the sequence of the peptide precursor ion.
- *z*: the charge of the peptide percursor ion.
- *mods*: the chemical modifications encountered on the peptide. The format is like *[C 37 H 55 O 10 N 10 S 0]*, which is the net elemental composition of the modifications FIXME !!!!!
- *ninumber*: the isotopologue (0 for monoisotopic, 1 for 1 heavy isotope, 2 for 2 heavy isotopes).
- *nirank*: rank of the peak in the previous column in the isotopic cluster. FIXME
- *niratio*: ratio
- :
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Version 3, 29 June 2007

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Colophon

About the authors. Filippo Rusconi is a senior research scientist at the French national research council (*Centre national de la Recherche scientifique*, CNRS). Filippo has a background in biochemistry and organic chemistry and was trained during his Ph.D. as a bioanalytical chemist. He has extensive knowledge of analytical techniques involved in the study of biopolymers.

Filippo Rusconi is the author of a handbook about mass spectrometry for biochemists (French). The book was published by the French sci/tech publisher Lavoisier (https://www.lavoisier.fr).

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The layout adopted for this book is an adaptation of the SUSE stylesheets. I would like to thank Frank Sundermeyer <fsundermeyer@opensuse.org> and Stefan Knorr <sknorr@suse.de> for being helpful with all my questions.

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