



CITRULLIA – QUICK GUIDE

Citrullination identification software

Manual version 1.0
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Citrullia guide

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Installation

To install Citrullia:

1. Go to <https://github.com/Citrullia-SDU/Citrullia>.
2. Download the .MSI or the .EXE-install file.
NB: Users at locations with restrictive security policies on computers such as universities and research facilities may encounter problems with the MSI-installation file. Therefore, the EXE-installation file should be used
3. Follow the installation guide
4. Congratulations. Citrullia has been installed.

To open Citrullia for users mentioned in step 2, it may be necessary to run Citrullia as an administrator.

Requirements

System requirements

- .NET Framework 4.7.2 (Can be downloaded from <https://dotnet.microsoft.com/download/dotnet-framework/net472>. Download the Runtime version and not the Dev pack)
- Windows 10 64-bit (Programmed and tested. It might be working on Windows 7, but it has not been tested).
- Screen with minimum size of 1600x1100. (Dual screens are recommended).

File requirements

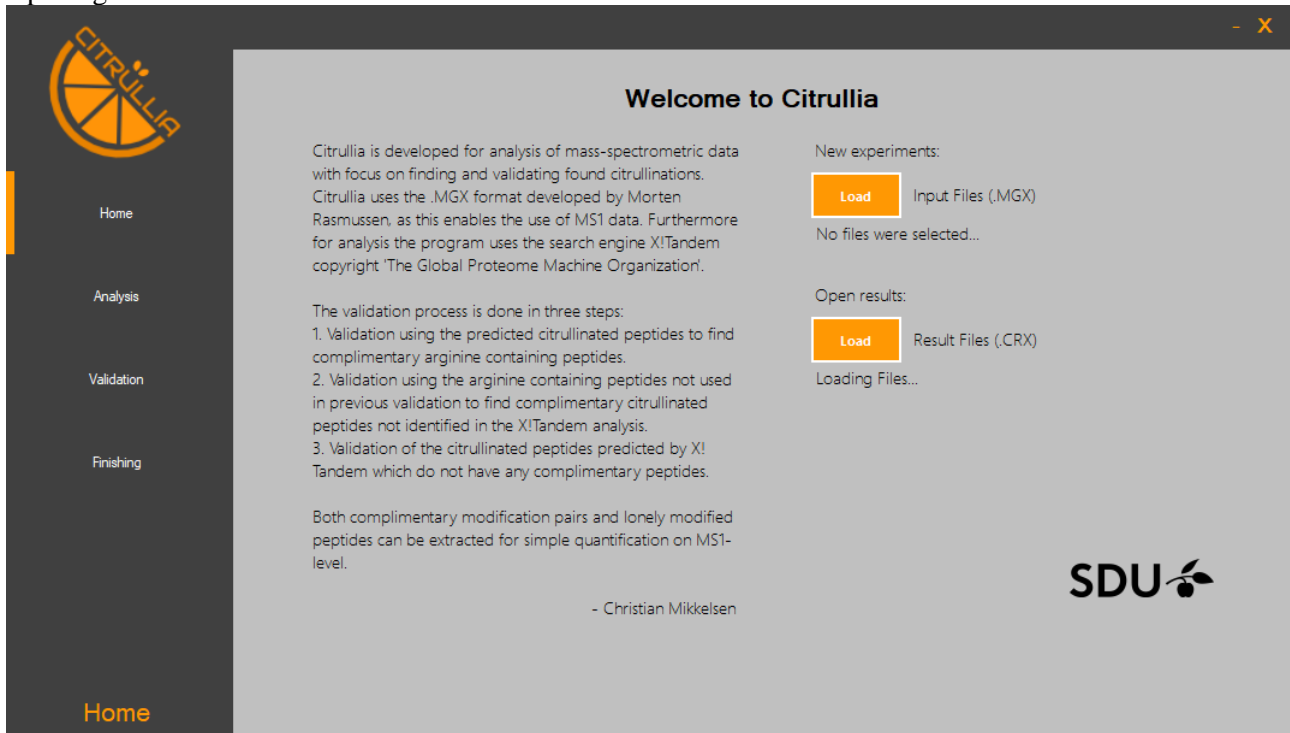
- Input file: The input file must be a Mascot Generic Extended (MGX)-file. This is a custom format that contains additional information such as parent mass and MS1 spectra. To create these files MassAI must be used (<http://www.massai.dk/>).
- Sequence file: A FASTA formatted file containing a list of protein entries with label and protein sequence to be searched. The sequence file can be generated from UniProt etc.

NB: Please refer to the full guide for information about the settings.

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Quick guide (For first time users)

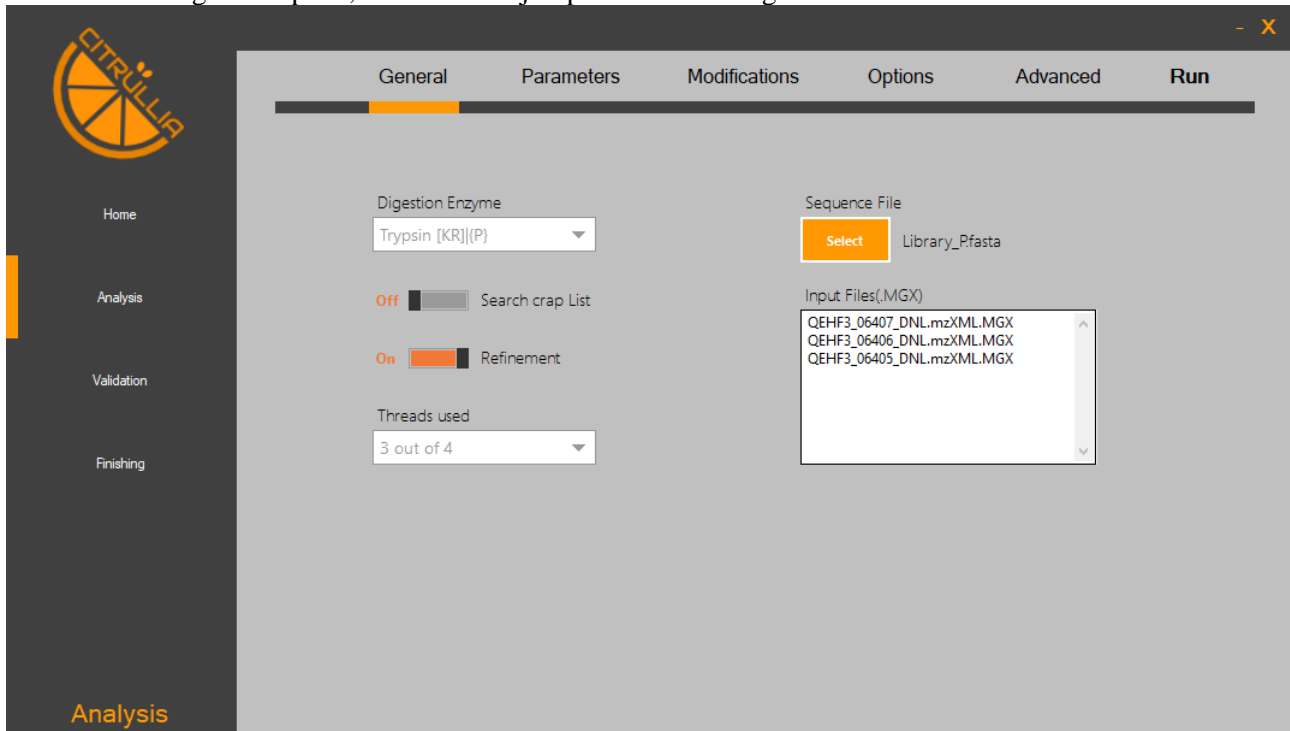
Opening screen when started:



Click on the Load-button under New experiments. A dialog box will pop up prompting for the selection of the files.

Select the MGX-files and click OK. Citrullia will load the files.

After the loading is complete, Citrullia will jump to the following screen:

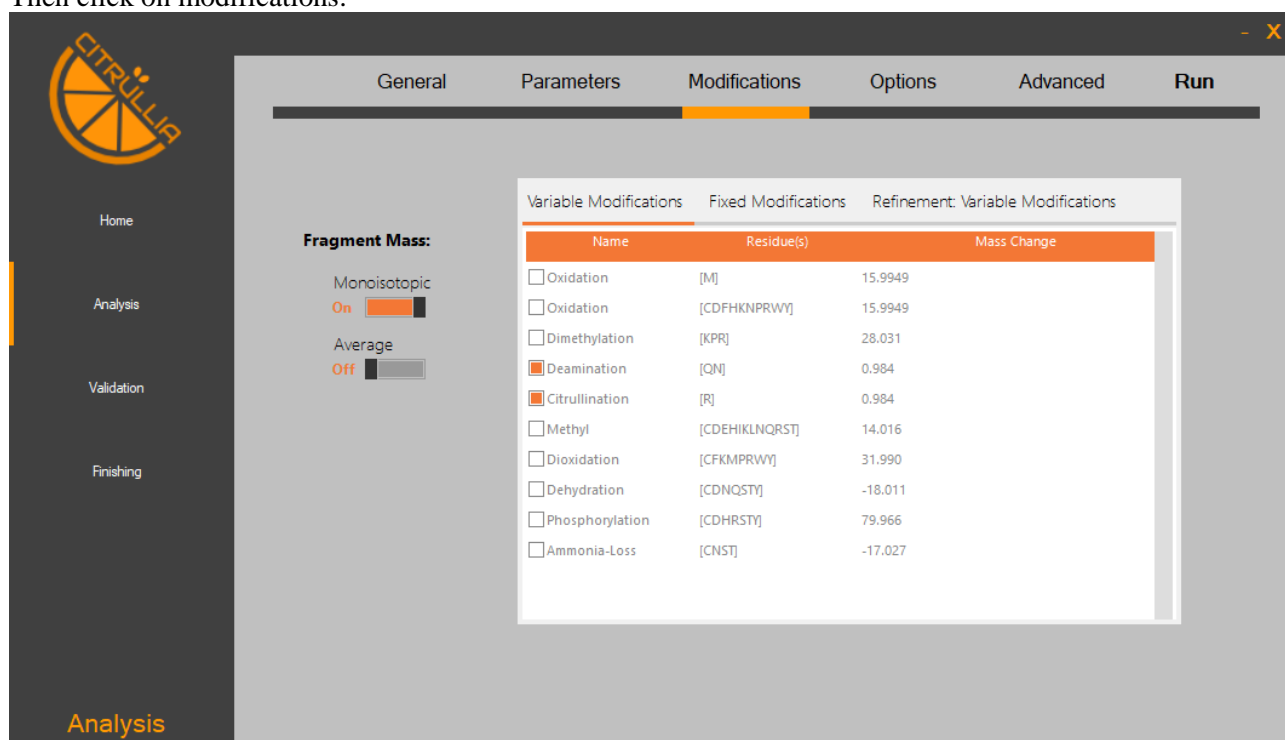


Select the digestion enzyme used to digest the sample

Select the sequence file

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Then click on modifications:



The screenshot shows the Citrullia software interface. On the left is a sidebar with a logo and navigation links: Home, Analysis, Validation, and Finishing. The 'Analysis' link is highlighted. The main window has a top navigation bar with tabs: General, Parameters, Modifications (selected), Options, Advanced, and Run. Below the tabs, there are three sub-tabs: Variable Modifications, Fixed Modifications, and Refinement: Variable Modifications. The 'Variable Modifications' sub-tab is active, displaying a table of modifications. To the left of the table, under 'Fragment Mass:', there are two toggle switches: 'Monoisotopic' (set to 'On') and 'Average' (set to 'Off').

Name	Residue(s)	Mass Change
<input type="checkbox"/> Oxidation	[M]	15.9949
<input type="checkbox"/> Oxidation	[CDFHKNPRWY]	15.9949
<input type="checkbox"/> Dimethylation	[KPR]	28.031
<input checked="" type="checkbox"/> Deamination	[QN]	0.984
<input checked="" type="checkbox"/> Citrullination	[R]	0.984
<input type="checkbox"/> Methyl	[CDEHIKLNQRST]	14.016
<input type="checkbox"/> Dioxidation	[CFKMPRWY]	31.990
<input type="checkbox"/> Dehydration	[CDNQSTY]	-18.011
<input type="checkbox"/> Phosphorylation	[CDHRSTY]	79.966
<input type="checkbox"/> Ammonia-Loss	[CNST]	-17.027

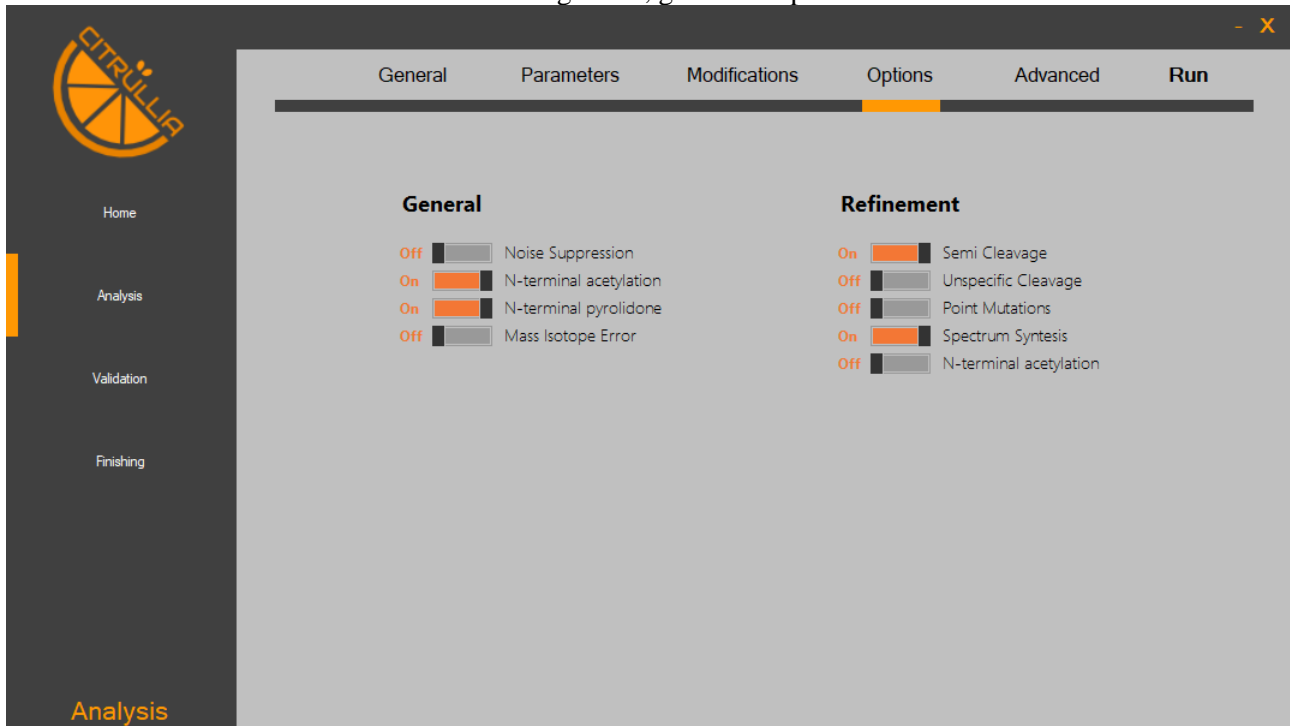
Select Citrullination of Arginine and Deamination of Asparagine and Glutamine by the checking the appropriate box.

The selection of deamination is essential to reduce the number of false positives.

If there are any fixed modifications, click on the Fixed modifications tab and select the modifications.

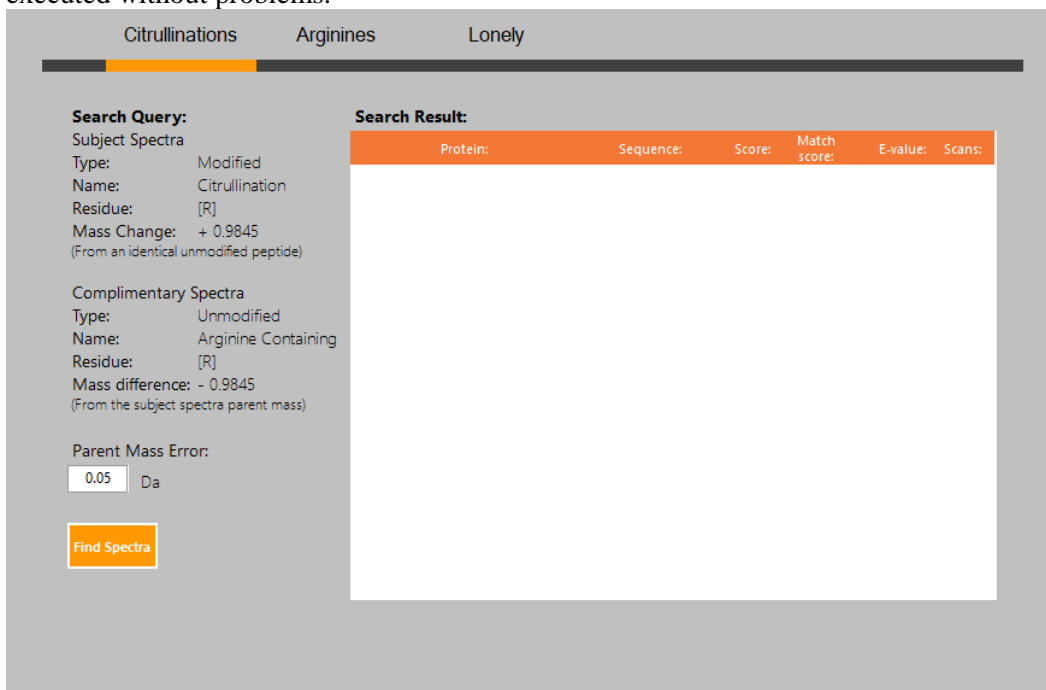
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If X!Tandem should account for missed cleavage sites, go to the Options tab and add them.



Search

Afterwards, click Run. The command window will open and show the program of X!Tandem. This will happen for each selected file. If no dialog box pops up and the following window is shown, X!Tandem executed without problems.



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Click on Find Spectra. Citrullia will try to find citrullinations, which will take a little while. When finished the search result will be populated, and the validation window for the first result will pop up:

The screenshot shows the Citrullia web application interface. On the left is a sidebar with navigation links: Home, Analysis, Validation, and Finishing. The main area is titled 'Citrullinations' and contains a 'Search Query' section with fields for Subject Spectra, Type (Modified), Name (Citrullination), Residue ([R]), and Mass Change (+ 0.9845). Below this is a 'Complimentary Spectra' section with fields for Type (Unmodified), Name (Arginine Containing), Residue ([R]), and Mass difference (- 0.9845). A 'Parent Mass Error' field is set to 0.05 Da. A 'Find Spectra' button is at the bottom. The 'Search Result' section displays a table of search results:

Protein:	Sequence:	Score:	Match score:	E-value:	Scans:
tr[B2RJV3]B2RJV3_PORG3 Uncharacter...	VIVASFPSREEAIA...	8,3	85	5E-05	2
sp[B2RIT0]DPP5_PORG3 Dipeptidyl-pe...	KQITDTESNEVAPA...	5	105	1,1E-05	1
tr[B2RJV3]B2RJV3_PORG3 Uncharacter...	VIVASFPSREEAIA...	4,5	85	0,0031	2
sp[B2RM93]CPG1_PORG3 Gingipain R...	KGTAGFEDTYKR	4,1	80	0,00077	3
sp[B2RIT0]DPP5_PORG3 Dipeptidyl-pe...	KQITDTESNEVAPA...	2,7	105	0,002	1
tr[B2RKH3]B2RKH3_PORG3 Probable d...	NKDEAVITNLD...	0	75	0,0071	1
tr[B2RIM9]B2RIM9_PORG3 Heme-bind...	INVQSLHVIPGR	17,7	90	1,8E-05	1
tr[B2RIM9]B2RIM9_PORG3 Heme-bind...	INVQSLHVIPGR	7,8	90	1,5E-05	1

Validation

The validation window:

The screenshot shows the 'Spectra Validation - Citrullinations' window. It displays information for Spectrum ID: 8384. The 'Citrullinated Peptide' section shows ID / Protein: 8384 / B2RJV3, Parent Mass: 1716.935, Charge-state: 2, Sequence: VIVASFPSREEAIAAR, E-value: 5E-05, Retention Time: 1301 sec, File Name: QEHF3_06405_DNL, and CITScore: 8,3. The 'Arginine Peptide' section shows ID / Protein: 7901 / B2RJV3, Parent Mass: 1715.95, Charge-state: 3, Sequence: VIVASFPSREEAIAAR, E-value: 6E-05, Retention Time: 1223 sec, File Name: QEHF3_06407_DNL. Below this is a table of Citrullination ions with columns for b, y, and p values. The 'Arginine Ions' table is also visible. On the right, there are two MS2 spectra: 'Citrullinated' and 'Arginine'. The 'Citrullinated' spectrum shows a base peak at m/z 1301. The 'Arginine' spectrum shows a base peak at m/z 1223. The 'MS1 Scan of Modified Peptide' and 'MS1 Scan of Unmodified Peptide' are also displayed.

In the upper left corner, the information for the citrullination and arginine spectra can be seen.

Bellow it, the ions found are shown for both the citrullinated and the arginine spectra. These can be exported to an Excel spreadsheet by selecting the Export-button.

In the upper right corner, the two MS2 spectra can be seen. The upper spectrum is the citrullinated and the lower is the arginine spectrum. The key to the colours can be seen above the citrullinated spectra.

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Below the MS2 spectra, the scan for the MS1 level are shown. A normal peak is orange, while the precursor is blue and possible isocyanic loss (only in the citrullinated MS1 scan) is marked in cyan.

For all three spectra it is possible to zoom in on the chart by marking it with the mouse. This zoom can be reset by clicking the Reset zoom-button. The spectra can be saved to a TIFF-file by selecting the Save-button.

Moreover, it is possible to move between the complementary spectra by clicking the Next and previous button.

To add or remove the validation pair for quantification, click on the Add or Remove button. The possibility of moving between the spectra is disabled by adding the spectra to quantification.

In the cases, where Citrullia cannot pair an arginine spectrum with a citrullinated spectrum, the citrullinated spectrum can be created as an “Lone Citrullination” result.

The principle is the same for Arginine- and Lone Citrullination-validation. However, the validation window for Lone Citrullination looks different, as it only displays a single spectrum.



If no results are found for any of the validation types, try to go back and check that the settings are as they should be. It is possible that no results are found in the citrullination validation. However, results should be found in the arginine validation.

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Quantification

After finishing the validation, select the finishing tab in the left-hand panel.

Finishing:

Parent Mass Tolerance: ppm.

Retention Time Interval: min.
(Plus-minus the given RT interval)

Enable validation: Off ☐

Create annotation file: Off ☐

Protein:	Sequence:	Score:	Match score:	Cit. XIC:	Arg. XIC:
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Click on start to initiate the quantification:

Finishing:

Parent Mass Tolerance: ppm.

Retention Time Interval: min.
(Plus-minus the given RT interval)

Enable validation: Off ☐

Create annotation file: Off ☐

Protein:	Sequence:	Score:	Match score:	Cit. XIC:	Arg. XIC:
B2RKV3JDP...	HTGDFSVFR	2,8	0	2.629E+11	0

In the quantification step, Citrullia will calculate the extracted ion chromatogram for the spectra and a citrullination percentage.

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From here it is possible to look at the spectra again by selecting the “enable validation”-option. This will open a window like the one in the validation step.

It is also possible to save the quantified annotated spectra to a folder along with a file containing the m/z-values and the ion type for all the spectra (if “Create annotation” is selected).

The quantification data can also be exported to an CSV-file by selecting the Export (.csv)-button.

By selecting the “Save result” you can save the X!Tandem files along the MGX-files. This saves time by bypassing the running of X!Tandem in the future.