Mestrelab Research

MNOVA GEARS – QC PROFILING 1.1

USER MANUAL

Document Number

P/N 190 R1.1

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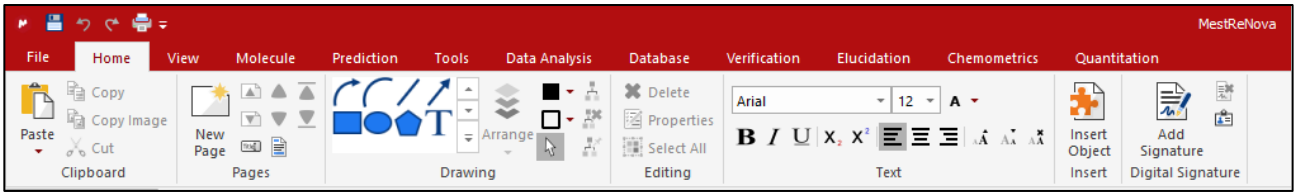
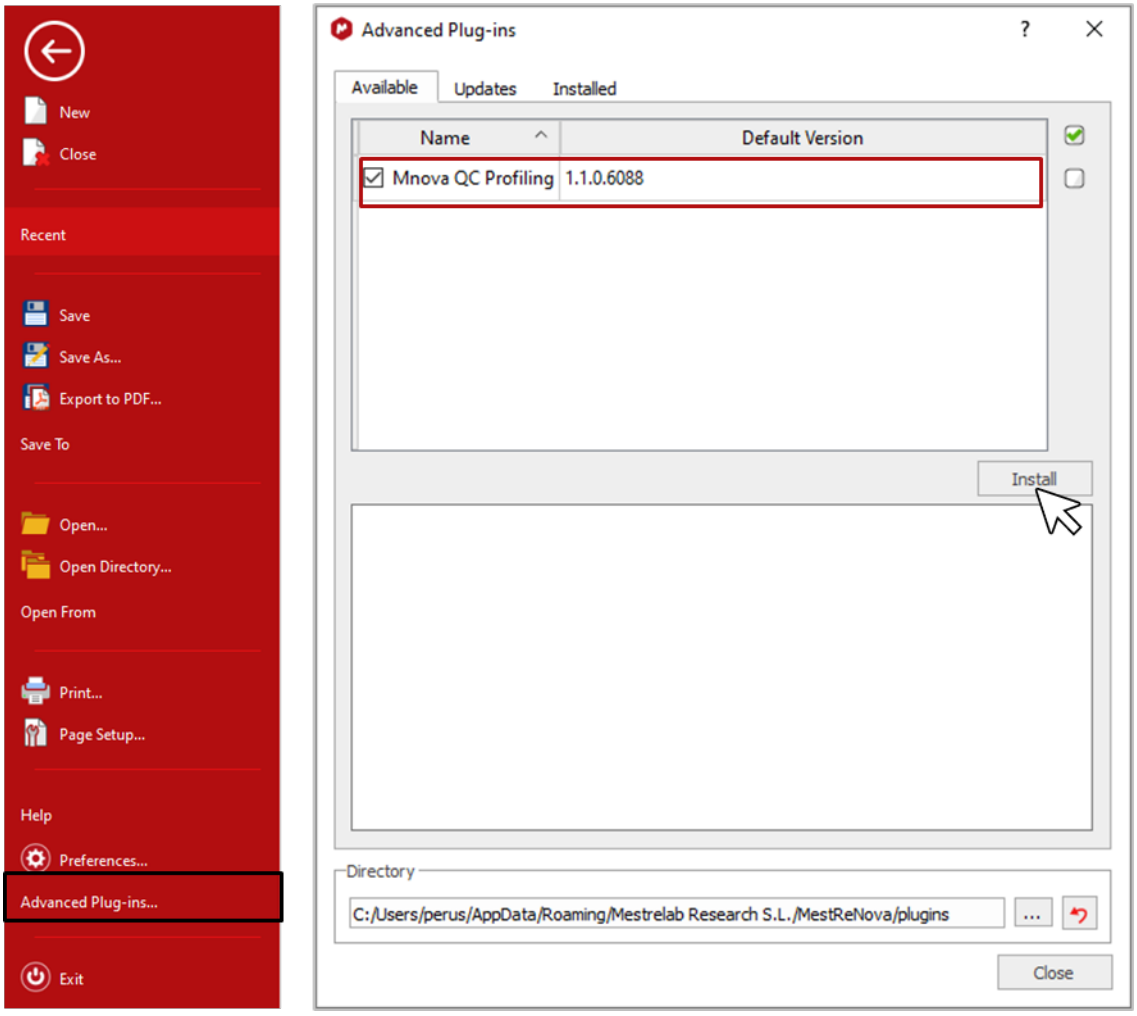
1 INSTALLATION AND LICENSING

The installation process for the QC Profiling plugin is both straightforward and quick. **Mestrelab Research** will provide the user with the appropriate license, and only a few steps are necessary to install the plugin.

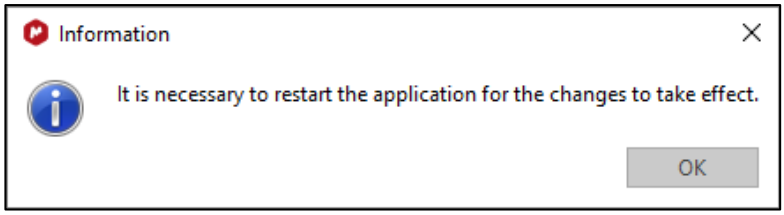
- Go to the **File** tab.



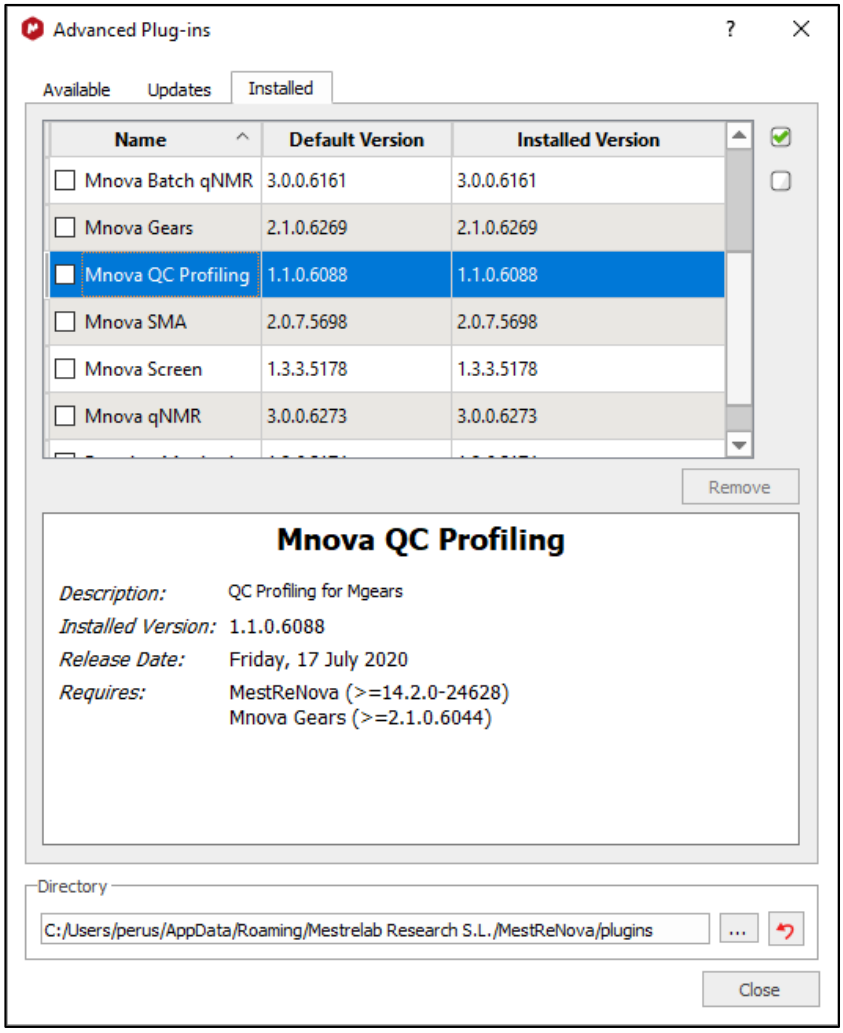
- Click on **Advanced Plug-ins…** and a new window will open. Select **Mnova QC Profiling** and click on **Install**.



- A window will open as in the following figure. Click on **OK**.



- Close **Mnova** and open it again, and repeat the first step: File >> Advanced Plug-ins…
- Check if the **QC Profiling** plugin has been installed correctly.

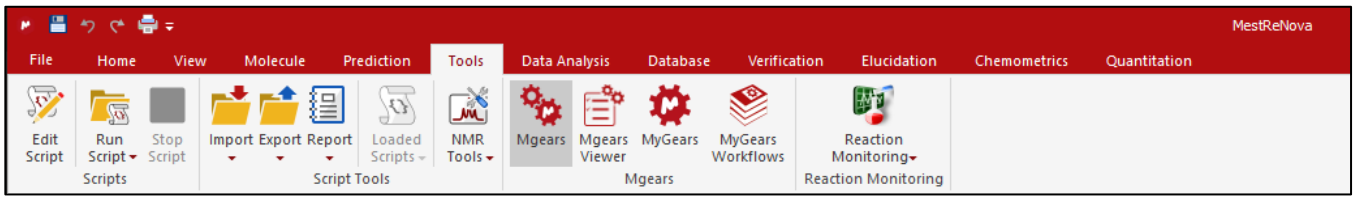


After checking the installation process, you can start to run your evaluations with **QC Profiling!**

2 INTRODUCTION

QC Profiling is an **Mnova Gears plugin** for the automated LC-MS quantitation of DNA labeling reactions. The main application of this plugin is for quality control (QC) of the reactions used to generate DNA Encoded Libraries (DEL) for drug discovery.

Ribbon



3 QC PROFILING

This plugin evaluates the composition of the mixture generated from the reaction between an organic molecule and a biomolecule. A set of LC-MS raw data files and the molecular formulas or monoisotopic masses or the reaction components in each mixture are required as input. These values are provided in a .csv file, which is later copied, and populated with the output of the analysis in the results directory.

- The main requirement is a **.csv** file with data organized in rows and columns, as per the figure.
- The dataset must contain the molecular weights, or the chemical formula of a given compound (yellow columns).
- A column of the .csv file must contain the name of the raw data file (data Id) in order to map it to the csv row (red column).
- Other columns in the .csv file can either be populated with output values or left as in the original file (white columns).

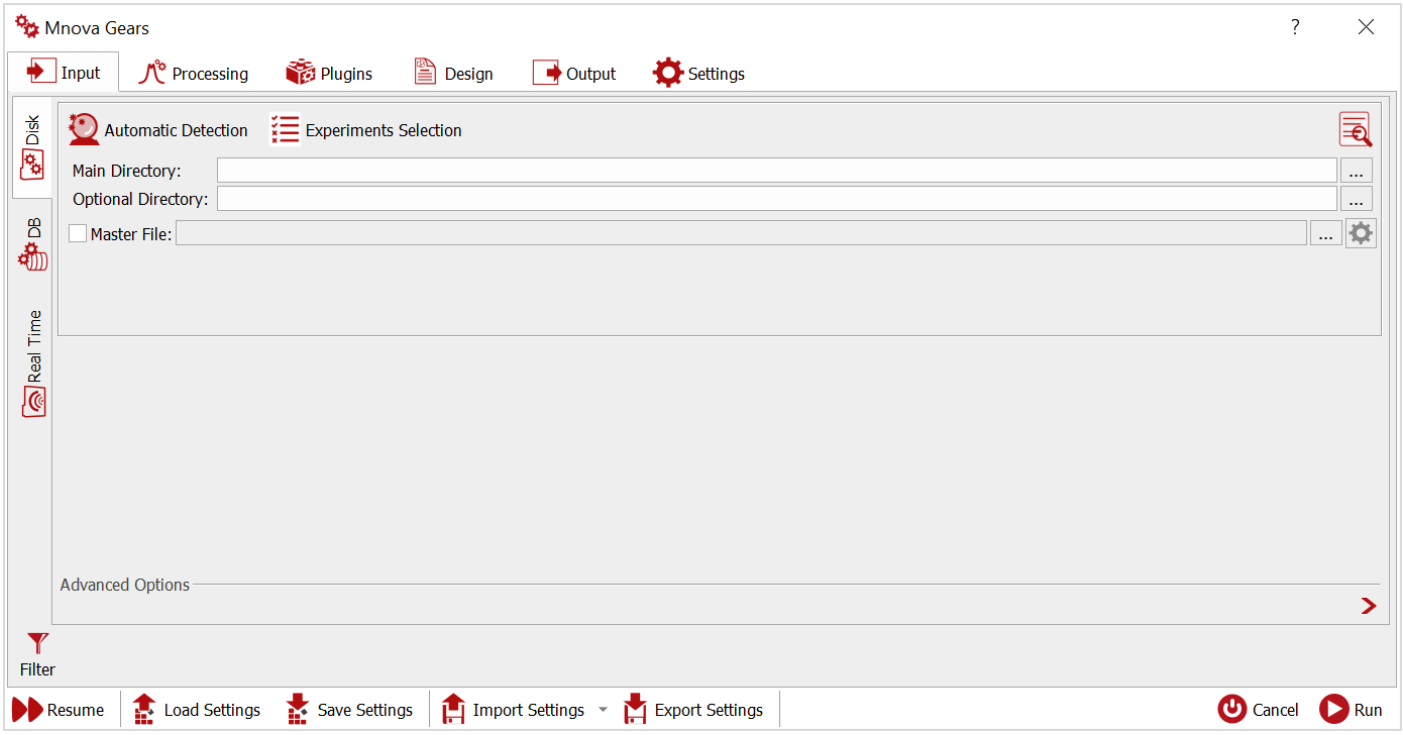
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample A01-2	A01								6622.6	6636.6	6757.9	6487.3	6515.3	6502
2	Sample A02-2	A02								6612.5	6626.5	6737.7	6487.3	6515.3	6502
3	Sample A03-2	A03								6584.4	6598.4	6681.5	6487.3	6515.3	6502
4	Sample A04-1	A04								6598.5	6612.5	6709.7	6487.3	6515.3	6502
5	Sample A05-1	A05								6593.5	6607.5	6699.7	6487.3	6515.3	6502
6	Sample A06-4	A06								6626.5	6640.5	6765.7	6487.3	6515.3	6502
7	Sample A07-4	A07								6585.4	6599.4	6683.5	6487.3	6515.3	6502
8	Sample A08-1	A08								6625.6	6639.6	6763.9	6487.3	6515.3	6502
9	Sample A09-1	A09								6655.6	6669.6	6823.9	6487.3	6515.3	6502
10	Sample A10-1	A10								6636.5	6650.5	6785.7	6487.3	6515.3	6502
11	Sample A11-1	A11								6637.5	6651.5	6787.7	6487.3	6515.3	6502
12	Sample A12-1	A12								6601.4	6615.4	6715.5	6487.3	6515.3	6502
13	Sample B01-2	B01								6599.4	6613.4	6711.5	6487.3	6515.3	6502

3.1 Mnova Gears Configuration

Mnova Gears presents the user with six tabs. For the purposes of the QC Profiling plugin, we are going to configure four of them:

- Input
- Plugins
- Output
- Settings

To simplify the configuration, this is divided into three steps: **Input File Configuration**, **Output File Configuration** and **QC Profiling Access**.

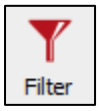


3.1.1 Input Files Configuration

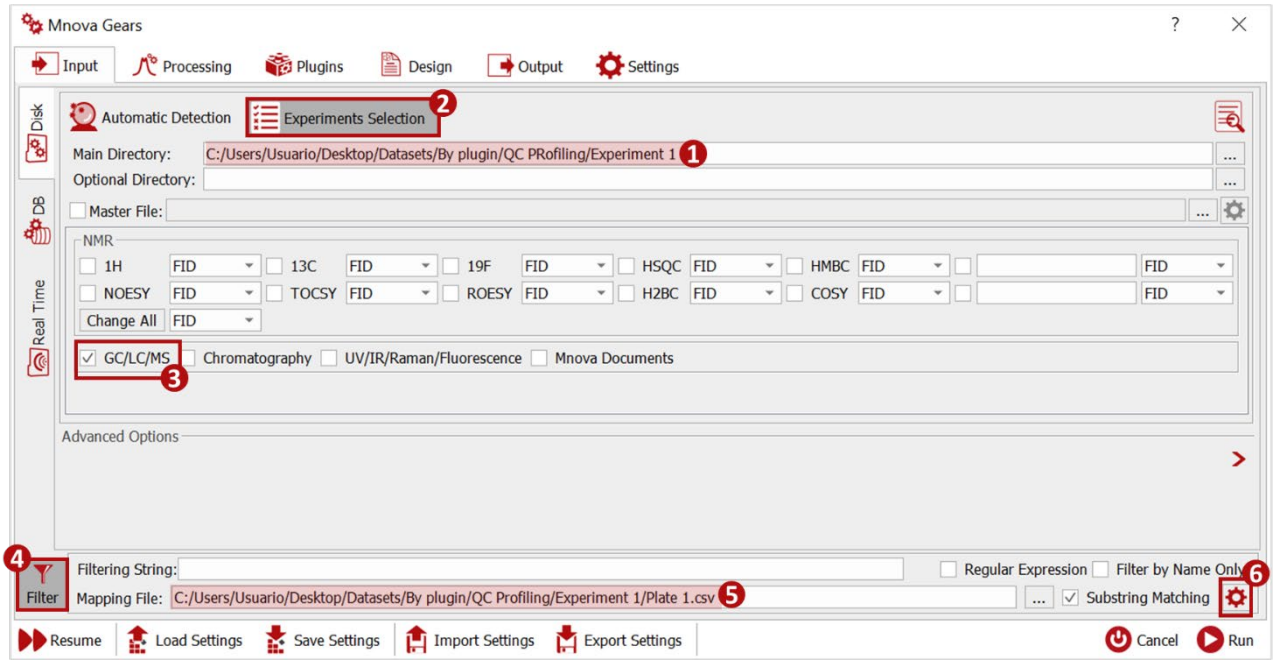
The plugin runs with mass spectrum files provided by laboratory devices. A few important steps must be completed before running the evaluation to check the dataset and its configuration.

- In **Input Tab** to select a **Main Directory** for the dataset of mass spectrum files. This is important to identify the mask for the files for the following step in the **Settings Tab**.
- Click on **Select the techniques and experiments that will be used in the analysis** and select **MS** experiments.

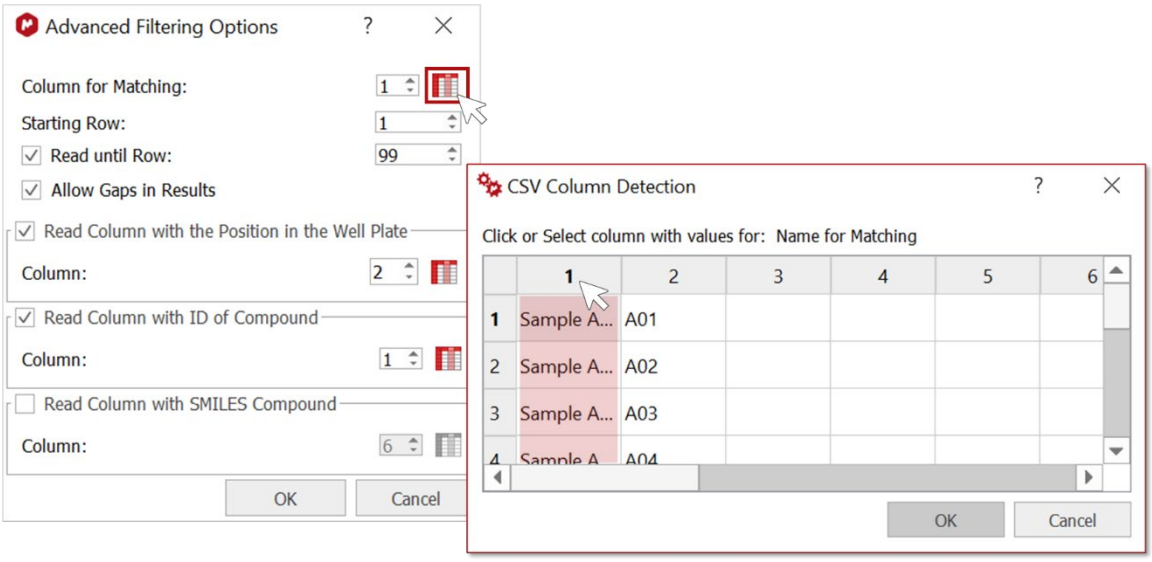
- Click on the **Filter** button and select a directory for the **.csv** file in the **Mapping File** requestor.



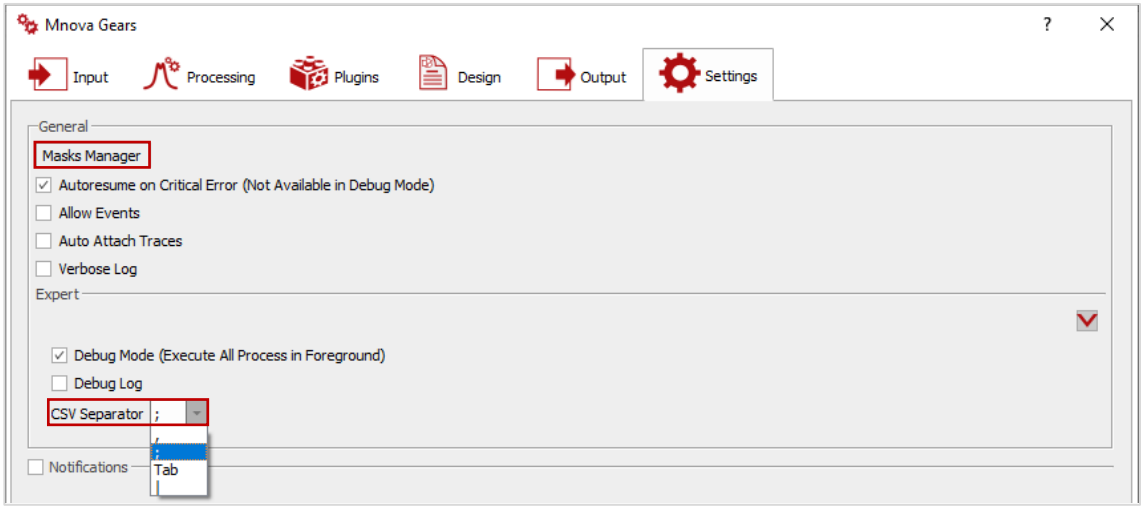
- Click on **Advanced Filter**.

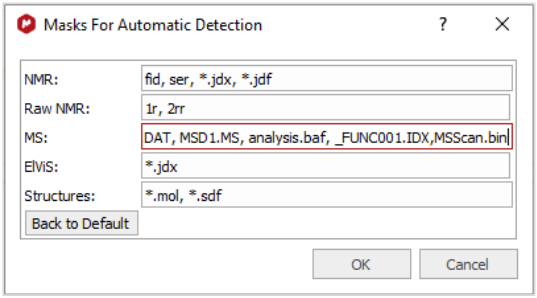


- In the **Advanced Filtering Options** match the **ID column number** with the position of the Data Id in the **.csv** file and select the range of rows for the evaluation.
- Specify the column with **Well plate position** and **SMILES** if available. You can use the **Assistant** tool to view and select the CSV columns.
- It is also possible to check the option **Allow Gaps in Results** to include void results with the lines in the filtering files.

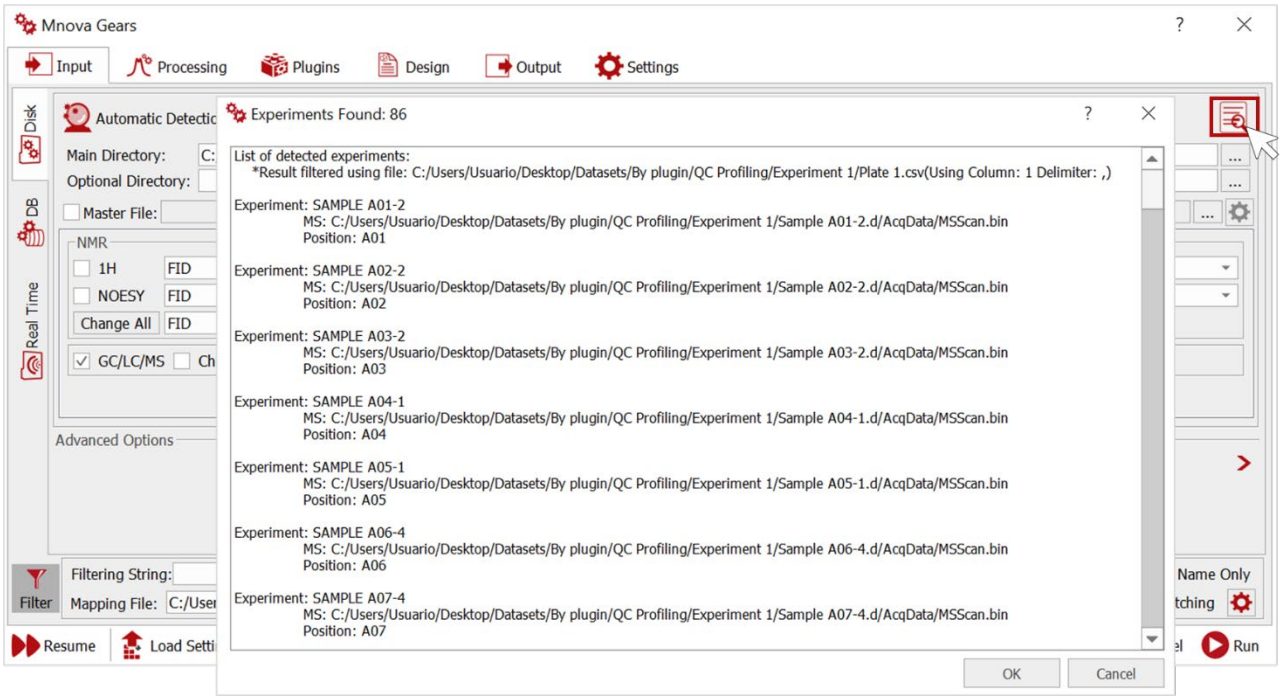


- Go to **Settings Tab** and define the **CSV Separator** (“**;**” is recommended) and check in the **Masks Manager** whether the mask for the **Mass Input Files** is in the list. If not, add it manually.





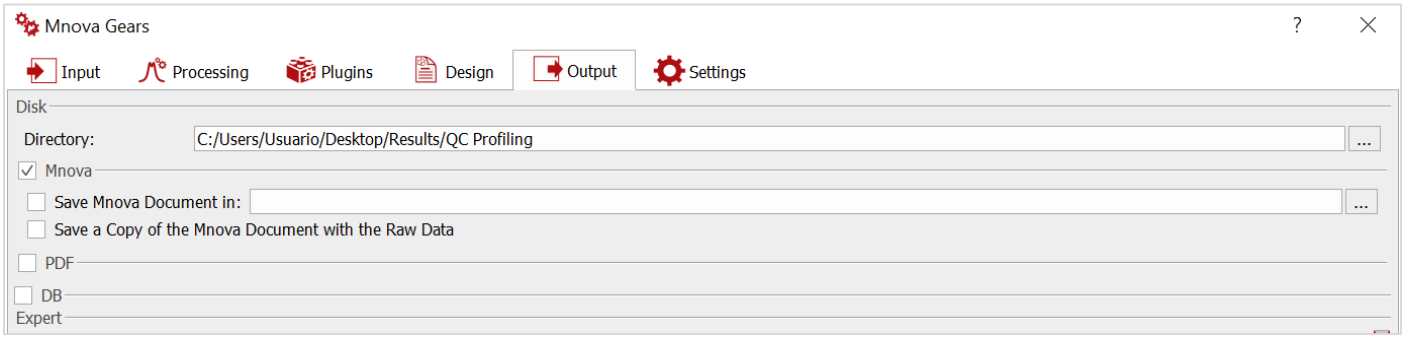
- After configuring the input files, go to the **Input Tab**, click on **Automatic inspection of the selected directory,** and verify the results in the new window (figure) to find errors (i.e., the correct settings for all the parameters defined in the input tab).



3.1.2 *Output File Configuration*

Mnova Gears creates a folder to save the QC Profiling evaluation results. This folder also contains some files generated by each evaluation, as described in point *3.4.1 Output Folder and Files*.

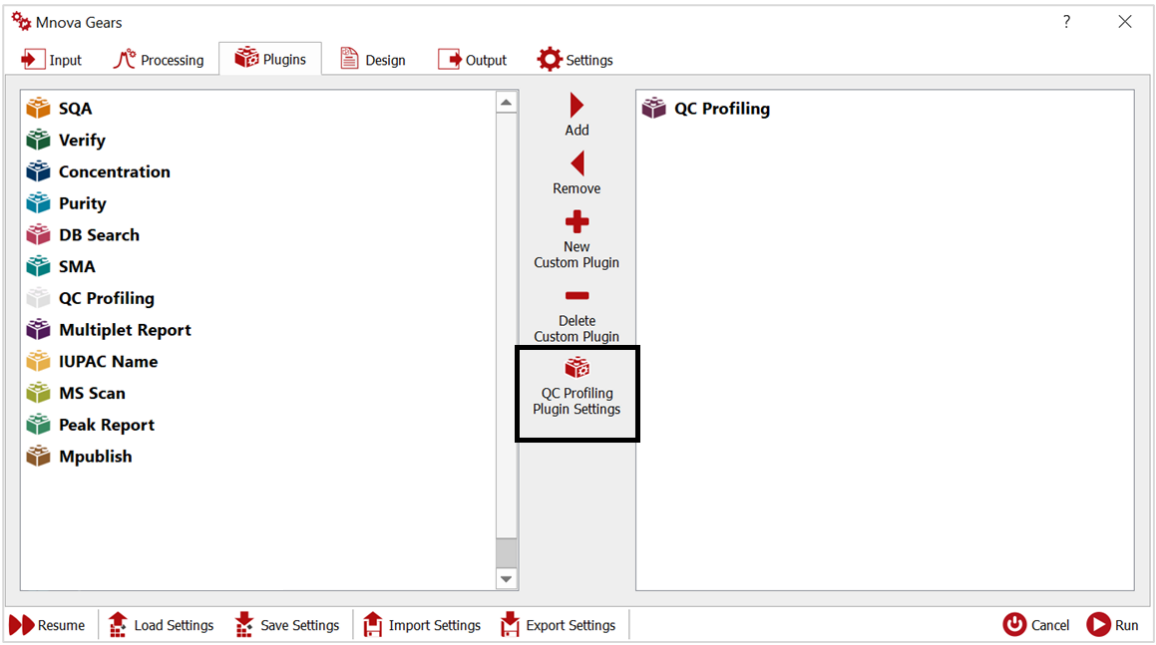
- Select the **Output Directory** in which to save the results files.



3.1.3 *QC Profiling Access*

The access to the Plugin Settings is the same as any other Mnova Gears plugin.

1. Go to the **Plugins Tab**.
2. Select QC Profiling in the plugins list and click **Add**.
3. Select QC Profiling in the added plugins list and click **QC Profiling Settings**.



3.2 QC Profiling Plugin Settings

The settings for this plugin can be entered in three distinct tabs:

- **MS Evaluation**
- **CSV Configuration**
- **Report**

3.2.1 MS Evaluation

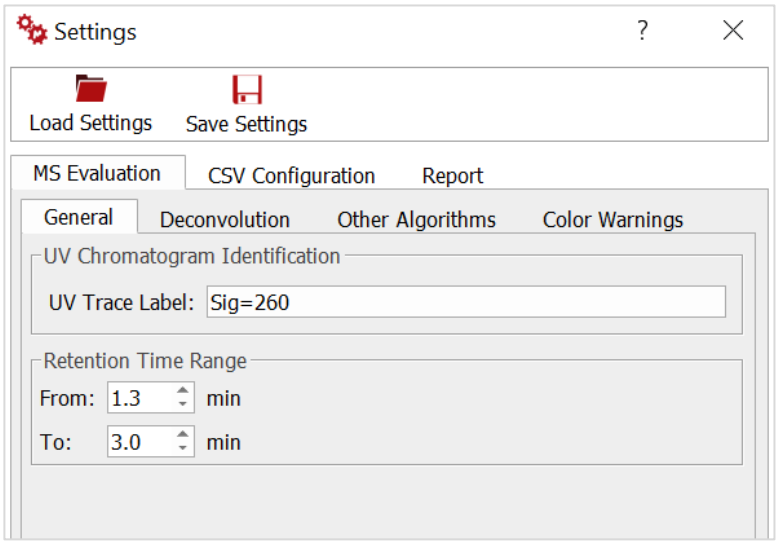
This tab provides access to all the parameters that control the evaluation of the LC-MS data used in this plugin.

The MS Evaluation tab is divided into four further subtabs:

- **General**
- **Deconvolution**
- **Other Algorithms**
- **Color Warnings**

3.2.1.1 General

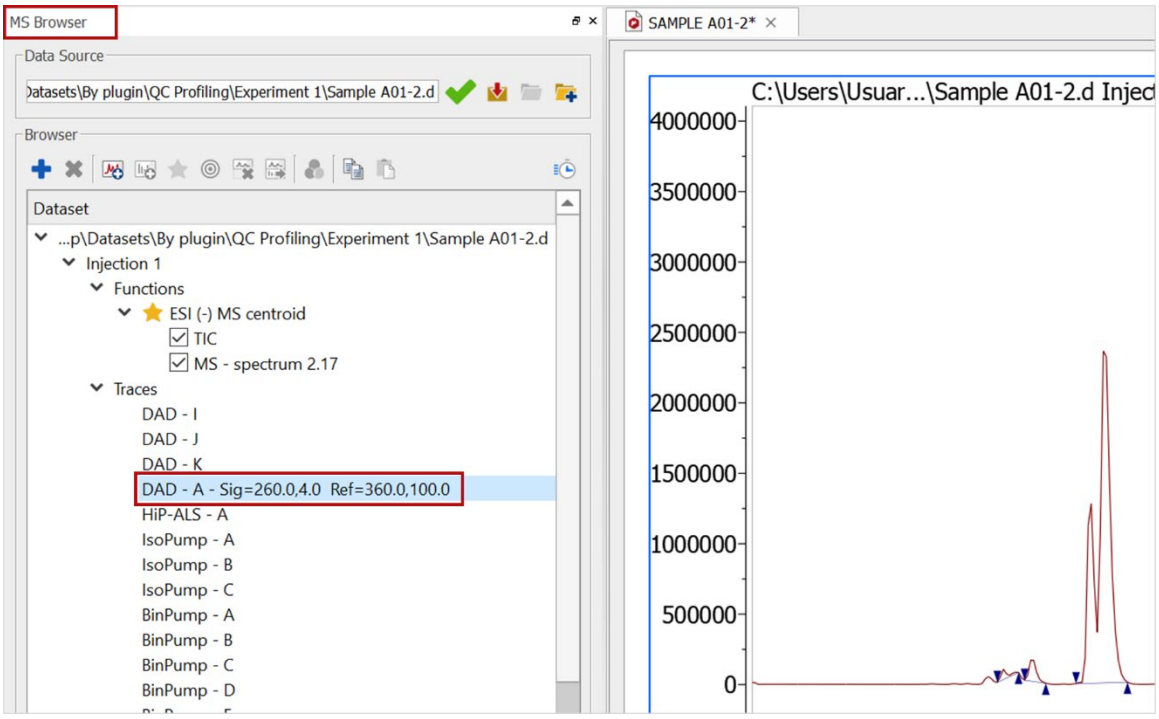
The General tab is used to configure the data that will be used in the evaluation:



UV Chromatogram Identification

This is the algorithm used to assign the main components of the UV peaks, as based on the deconvolution of multiply charged ions.

- **UV Trace Label:** This parameter is used to define the name of the UV trace within the LC-MS dataset that will be used for quantification. To identify the name of the UV trace within the MS Traces, open one file in the dataset prior to the evaluation and choose the name of the desired trace.

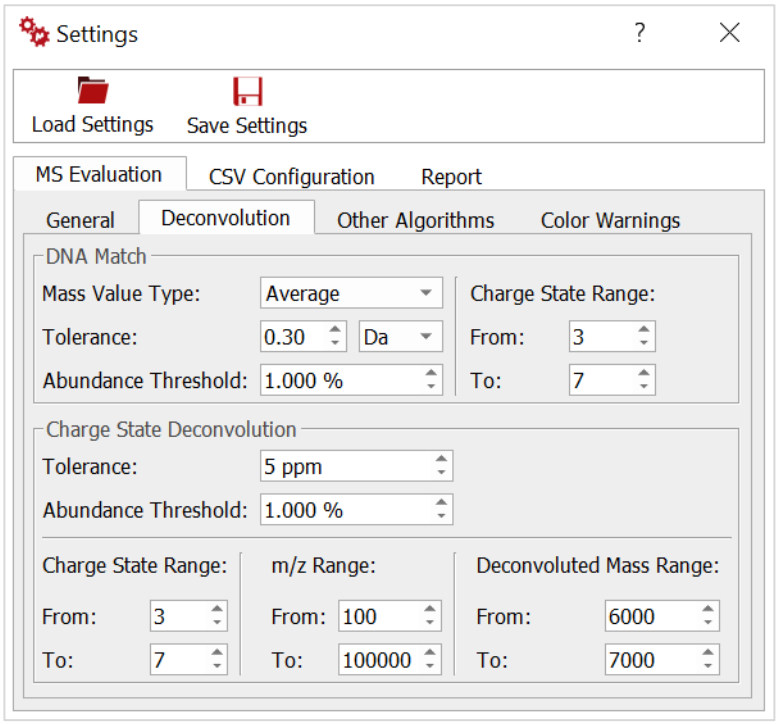


Retention Time Range

- **From, To:** Defines the range of chromatogram retention times that will be used for the evaluation. We recommend choosing a relatively narrow range, focusing on the retention times where the DNA peaks appear, to ensure the best performance of the algorithm for "No Data" detection.

3.2.1.2 Deconvolution

The Deconvolution tab is used to configure algorithms for the evaluation and results display.



DNA Match

This algorithm is used to assign the main components of the UV peaks as based on the deconvolution of multiply charged ions.

- **Mass Value Type:** Average or Monoisotopic.
- **Tolerance:** Precision of the Mass Deconvolution.
- **Abundance Threshold:** Used to filter out noise from MS spectrum.
- **Charge State Range (From, To):** Defines the range of multiply charged ions that will be deconvoluted.

Charge State Deconvolution

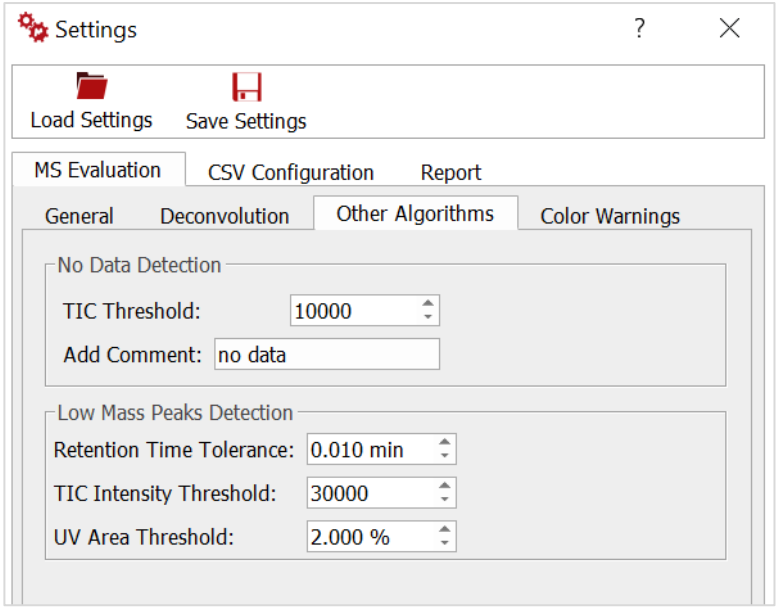
This algorithm deconvolutes multiply charged ions in a MS spectrum to obtain a deconvoluted spectrum. It is used to display the deconvoluted spectra relating to individual UV peaks.

- **Tolerance:** Precision of the Mass Deconvolution.
- **Abundance Threshold:** Used to filter out noise from the MS spectrum.

- **Charge State Range (From, To):** Defines the range of charges that will be deconvoluted.
- **m/z Range (From, To):** Defines the range of m/z that will be deconvoluted.
- **Deconvoluted Mass Range (From, To):** Defines the mass range (Da) that will be deconvoluted.

3.2.1.3 Other Algorithms

This tab completes the previous deconvolution parameters.



No Data Detection

This algorithm detects datasets where no DNA sample is present, which show characteristic noisy chromatograms with very low intensity signal. An associated color warning of such can be displayed.

- **TIC Threshold:** Minimum peak intensity to be detected in the TIC spectrum, below which the sample is considered to contain no data.
- **Add Comment:** Will be written in the Comments column of the output .csv file for the peaks considered to be without data.

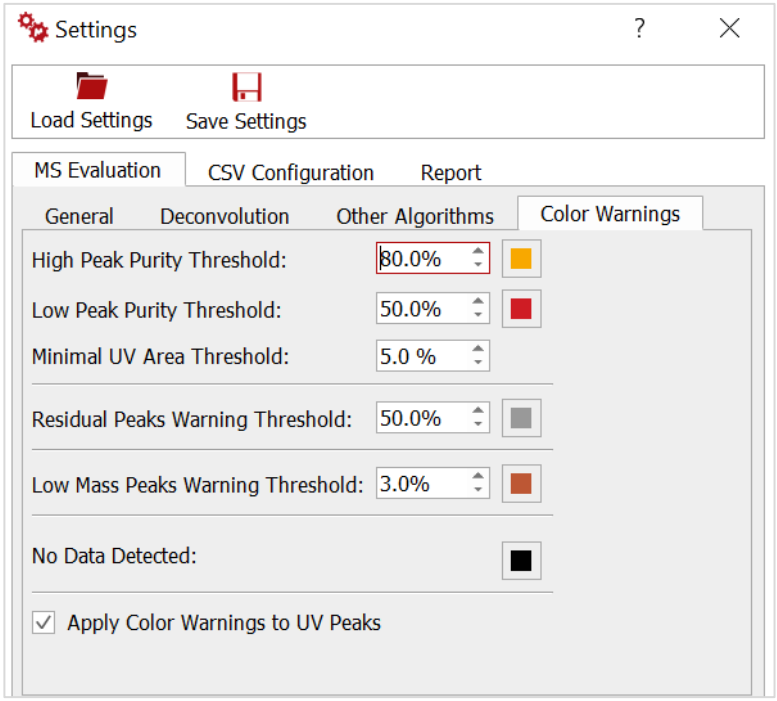
Low Mass Peak Detection

Used to detect UV peaks originating from low molecular weight impurities that must be excluded from the quantification. A color warning related to the presence of these impurities can be displayed.

- **Retention Time Tolerance:** UV peaks with no TIC within this retention time difference are considered to be impurities.
- **TIC Intensity Threshold:** UV peaks with a TIC Intensity at the same retention time below this threshold are defined as impurities.
- **UV Area Threshold:** UV peaks with areas lower than this percentage of the total area are considered to be DNA. This approximation is used to avoid wasting time analyzing very small peaks.

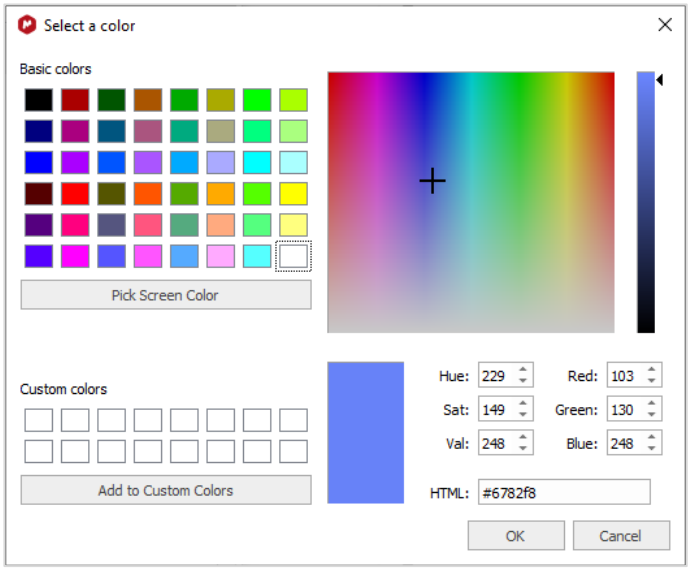
3.2.1.4 Color Warnings

Color codes are used to warn the user about the quality of the results by connecting specific issues arising in the automation with a color warning. The level of the warnings is configured by the user via appropriate thresholds, which allows for a reduction in the number of warnings.



- **High Peak Purity Threshold:** Peaks with an assignment purity, as evaluated via the DNA Match algorithm, that is lower than this value.
- **Low Peak Purity Threshold:** Peaks with an assignment purity lower than this value.
- **Minimal UV Area Threshold:** Peaks with an area below this percentage of total area will not result in associated purity warnings.
- **Residual Peaks Warning Thresholds:** When the total percentage of residual peaks is greater than this value.
- **Low Mass Peaks Warning Threshold:** When low molecular weight impurities are detected.
- **No Data Detected:** When peak intensity is low and indistinguishable from noise. Typically, these peaks are due to solvents or contaminants.
- **Apply Color Warnings to UV Peaks:** Applies the color warning codes above to UV peaks.

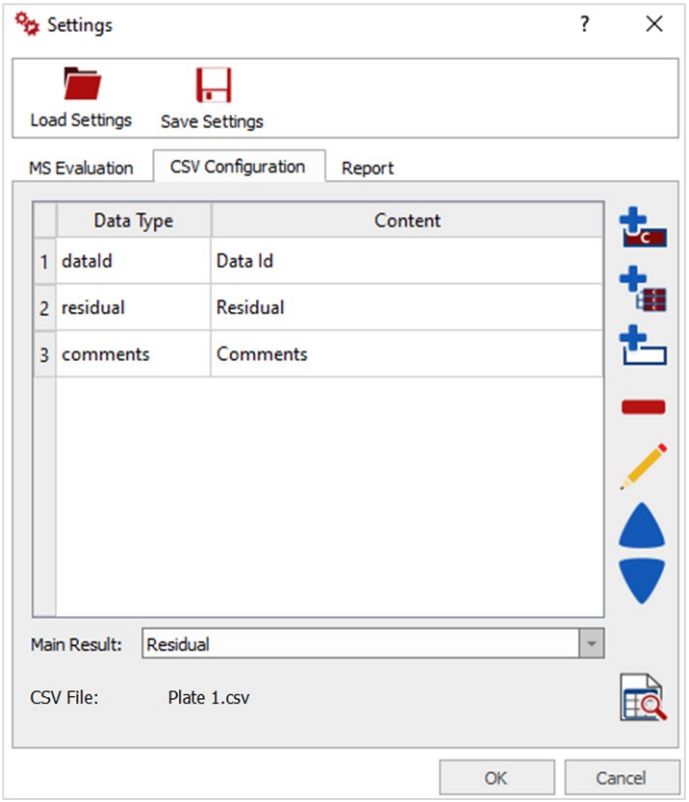
To select or change the color for each of the above, click on the associated colored square to the right of the definition and select your preference in the color window.



Note: *selecting white as the color is equivalent to switching off the warning.*

3.2.2 CSV Configuration

Content descriptions of the columns in the input .csv file being used as a template by the application.



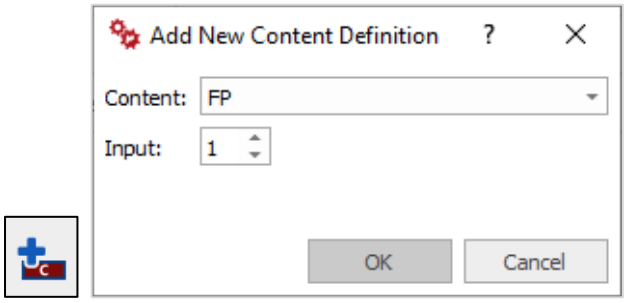
General Features:

- Each row in the table describes the content input or output of a column in the .csv file and represents a column header from the raw csv file.
- The **dataId**, **residual** and **comments** rows are always present and cannot be deleted from the table but can be moved to match data as appropriate.
- Practically unlimited input and output columns can be configured by the user, which currently can be one of three **Data Types:**
  - **Input**: A numeric column in the .csv file describing the molecular weight of a given component.
  - **Contents Group**: The total composition of a group of input components will be saved as output in the .csv file; for example, adducts from the same component.
  - **Contents Group Comment**: A comment describing how the total composition of a group of input components is distributed among the various peaks of the sample. This column is optional.
- Input and output row from the same component can be highlighted in color for visualization purposes.
- Rows with no data declaration will be ignored in, but not deleted from the .csv file.
- The Main Result menu below the table allows the choice of which component in the mixture will be considered the main result in the output review options.

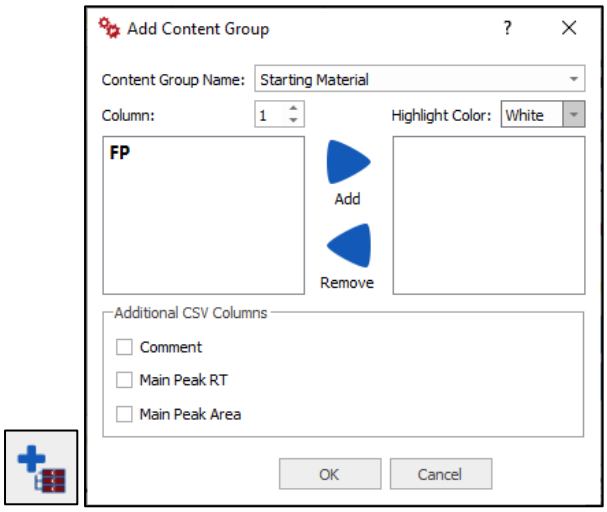
CSV Configuration Tools

There are many tools to create, edit, move, and remove rows:

- **Add a content**



- **Add a group of contents**



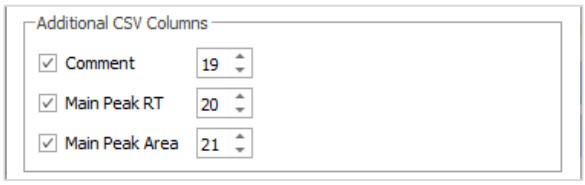
Content Group Name: Final, Final Product, or Starting Material

Column: is the number for the input/output of the Content Group.

Highlight color: white, yellow, lime, cyan, beige, red and orange are available for colored Content Groups.

Additional CSV Columns:

- **Comment:** summarizes the different peaks contained in these groups.
- **Main Peak RT:** shows the retention time of the main peak assigned to this group.
- **Main Peak Area:** shows the UV area of the main peak assigned to this group.

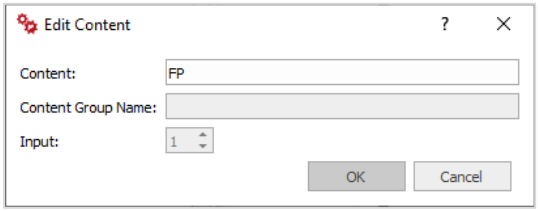


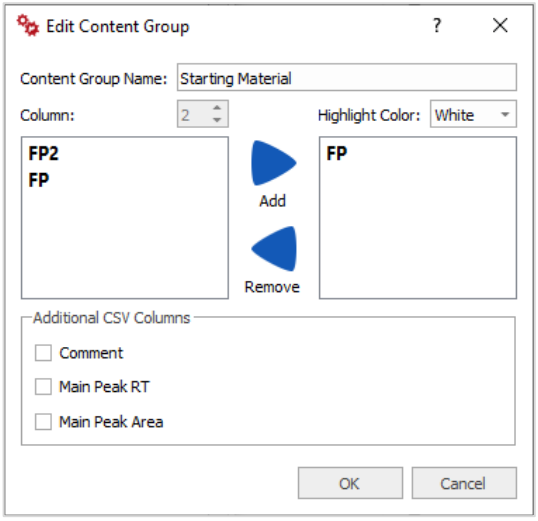
Note: *We recommend that all the Input content columns be created before grouping them.*

- **Insert empty row**

- **Remove selected row:** delete a row in the table. Notice that some rows cannot be deleted (dataId, residual, and comments).

- **Edit selected row:** edit any added row in the table**.** Note that some rows cannot be edited (dataId, residual, and comments).





- **Move selected row up/down:** move any row up or down in the table.

- **Magnifying Glass button:** inspect the CSV definition of the first lines of the .csv file. A dialog with the overview of the first two lines will appear, showing the content of this file relating to the definitions given by the user.

Note: *the following chapter gives a step-by-step explanation of how to create a table.*

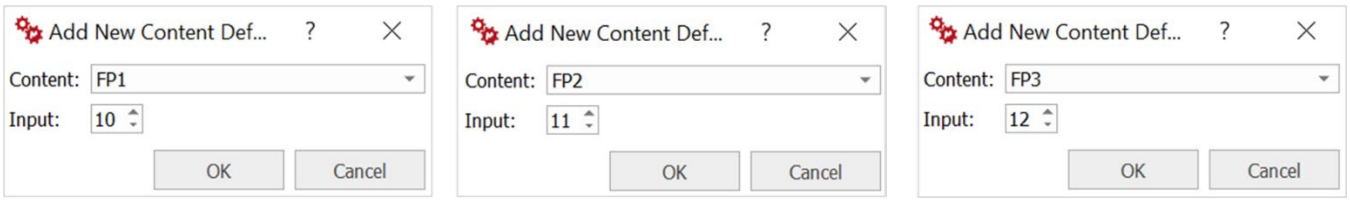
3.2.3 How to create a table

After configuring Mnova Gears and QC Profiling Settings, the user can create a table that maintains the CSV file structure, as per the following example:

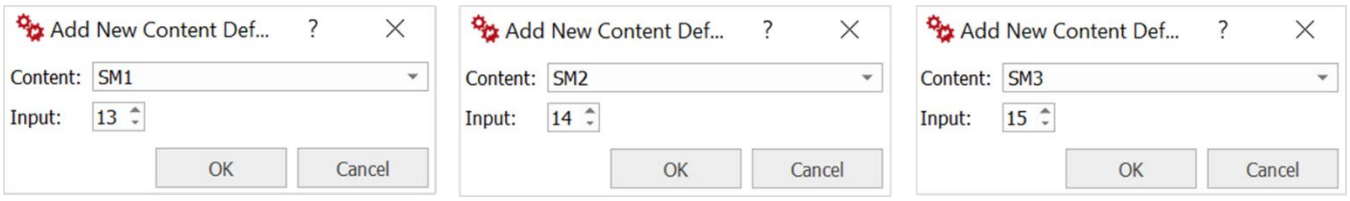
1- Add Input columns:

Click on **Add a content** to enter new content and select each desired column. For this example, we are going to add three contents for each input group, “Final Product” and “Starting Material”.

Final Product Rows: **FP1**, **FP2**, and **FP3.**



Starting Material Rows: **SM1**, **SM2**, and **SM3.**

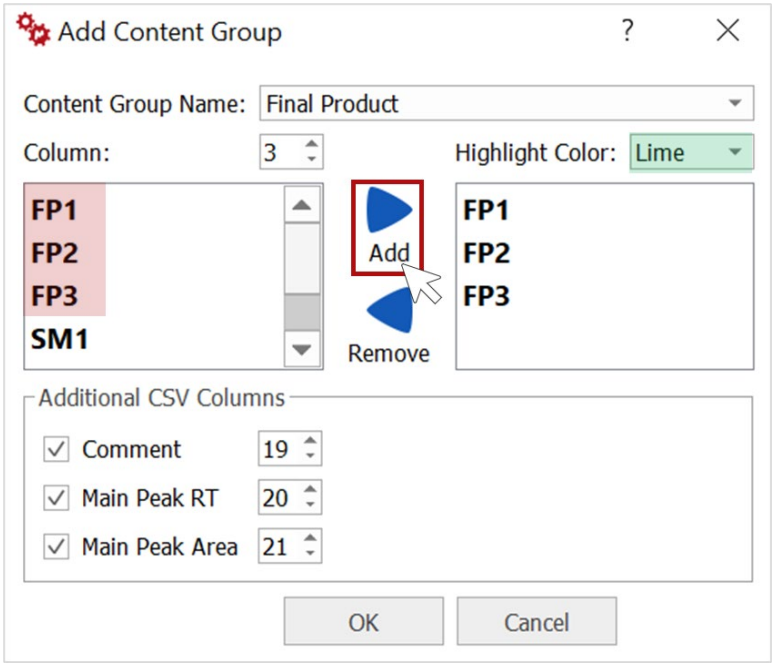


Note: *Remember that each row of the table matches a column in the .csv file.*

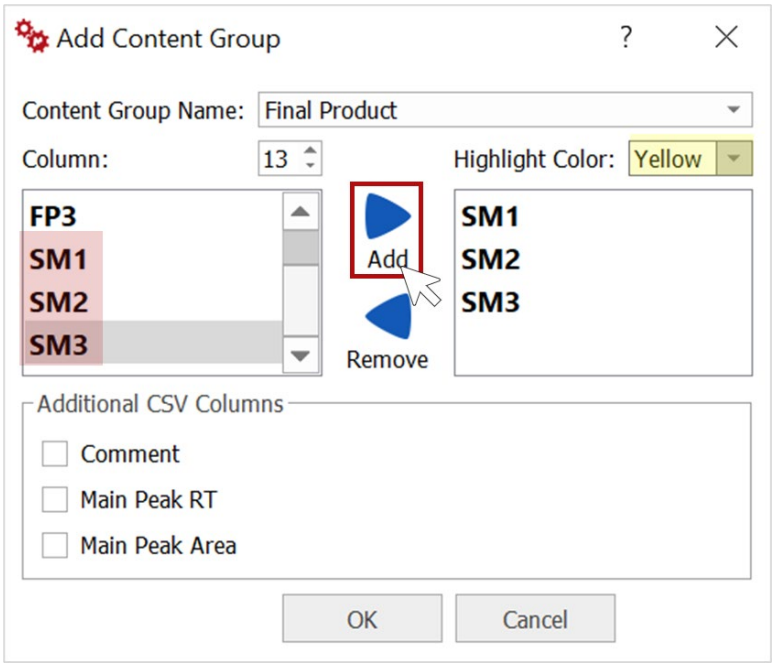
2- Create an Output group for each content group:

Click on **Add a group of contents** to create an Output Content Group.

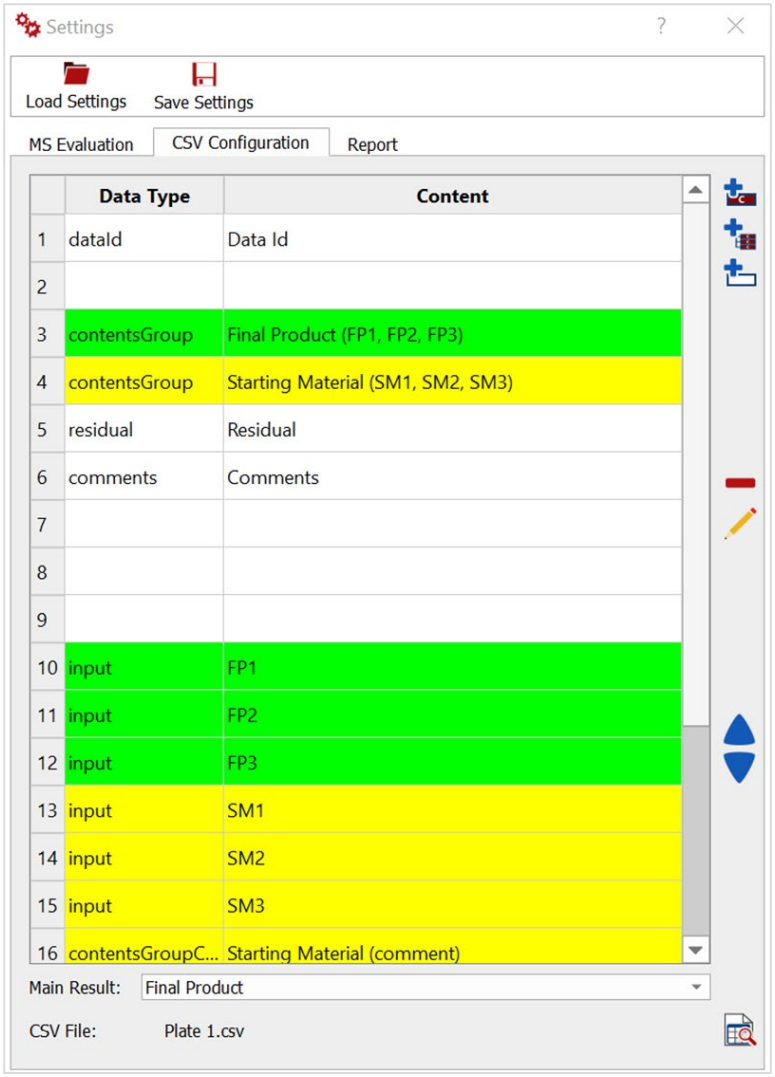
In this case, we are going to create two Content Groups. The first group is configured as per the following figure for **Final Product** contents.



The same process can be repeated with **Starting Material** contents, selecting a different **Column**, **Highlight Color**, and **Comment** column.



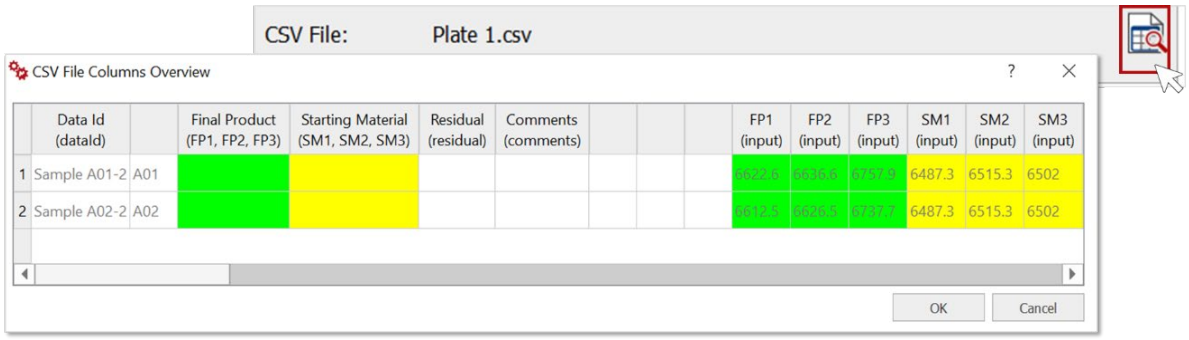
Added groups will be displayed in the main table



After creating the table, we need to ensure that the rows from the table match the columns of our .csv file.

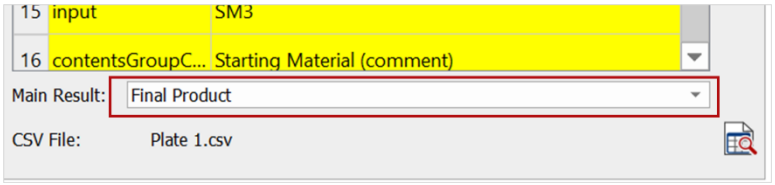
3- CSV inspection:

Click on the **Magnifying Glass button** to open the **CSV File Columns Overview** and inspect.



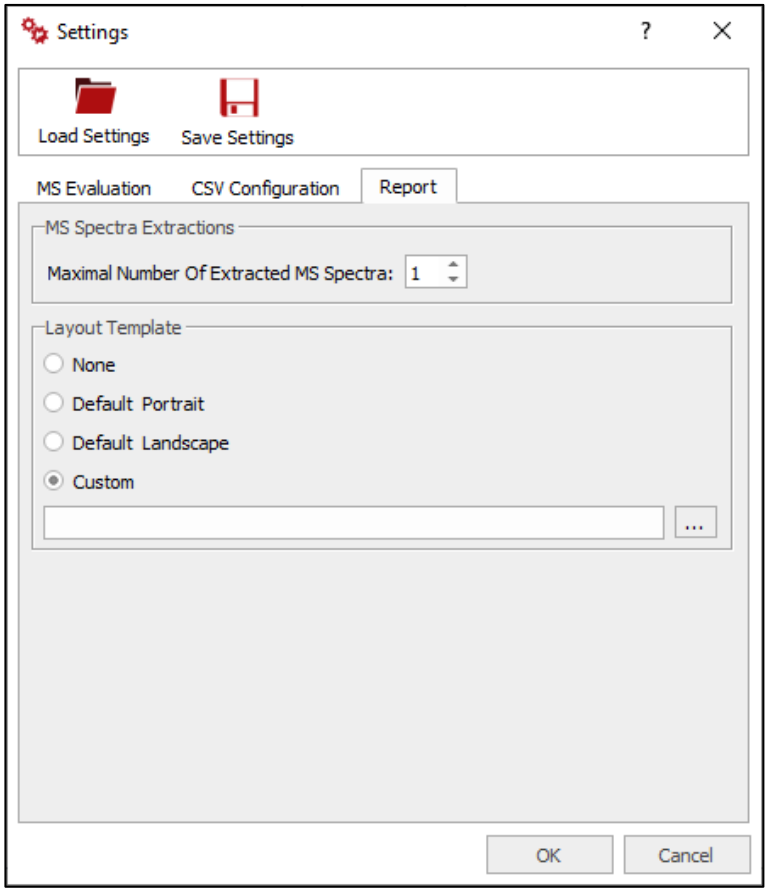
Select **Main Result**:

Open the Main Result menu, choose Final Product and click OK or continue with QC Profiling configuration.



3.2.4 Report

Define the number of **MS Spectra Extractions** and Layout **Template** to apply in the output Mnova Files.



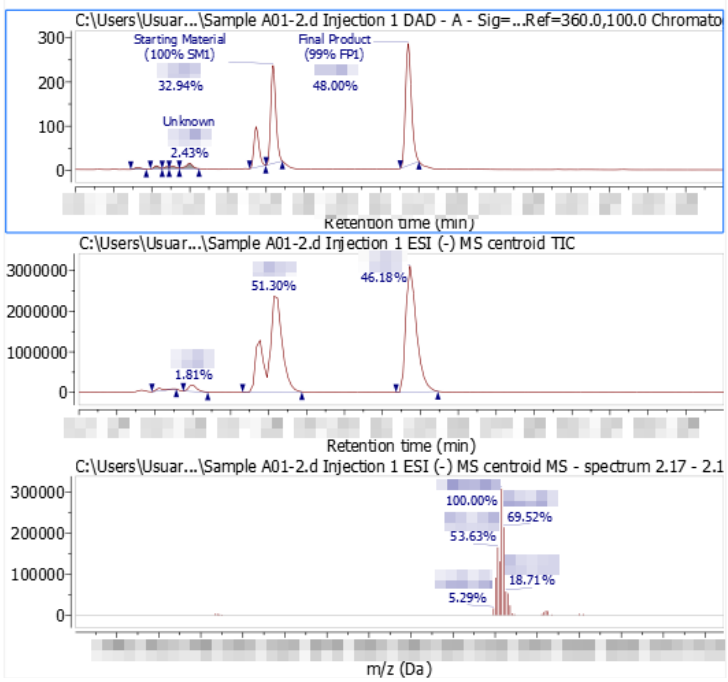
MS Spectra Extractions

- **Maximal Number Of Extracted MS Spectra:** Define the number of MS Spectra for the report.

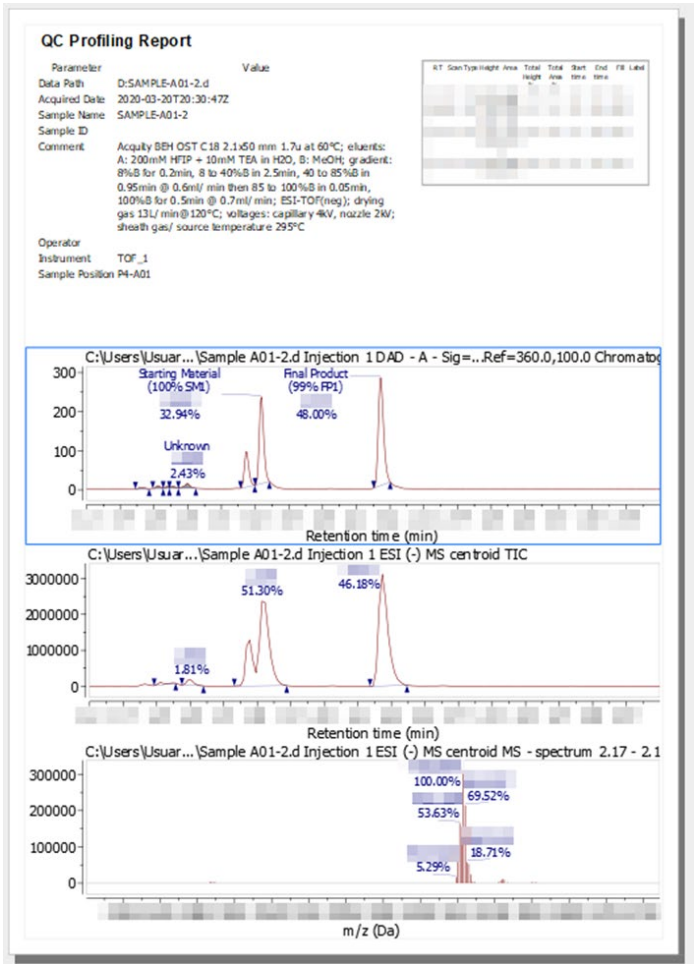
Layout Template

No template and two default templates are available in the plugin; a custom mode is also available to configure the report according to user preferences.

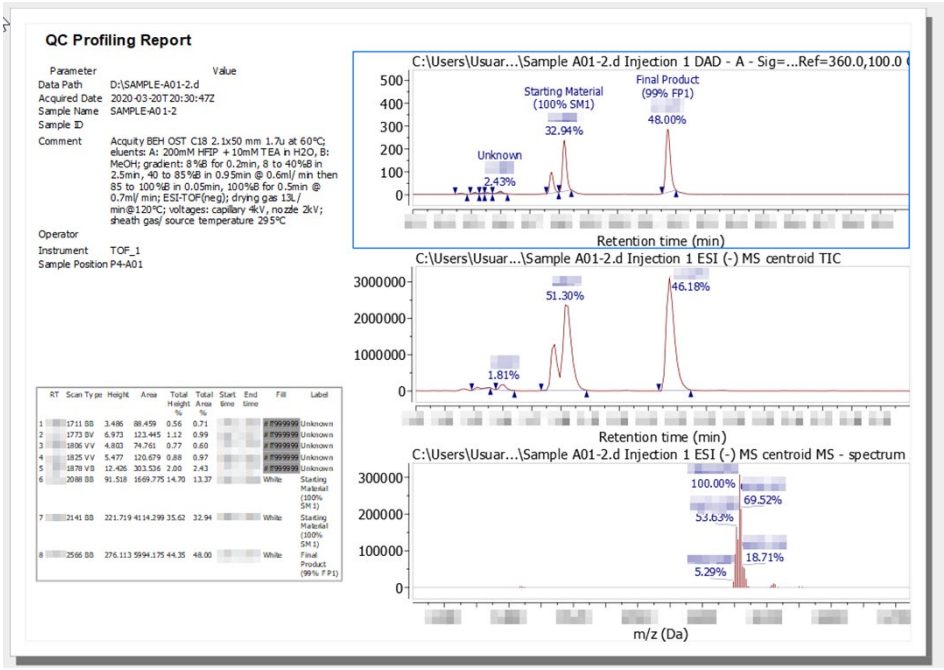
- **None**



- **Default Portrait**



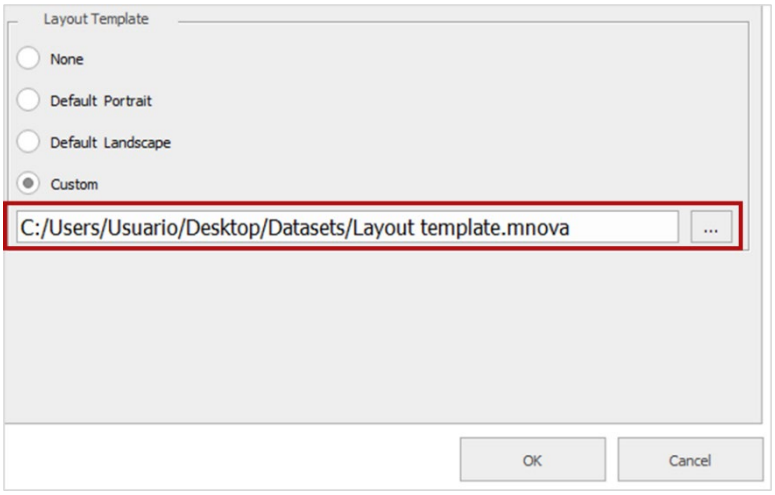
- **Default Landscape**



-

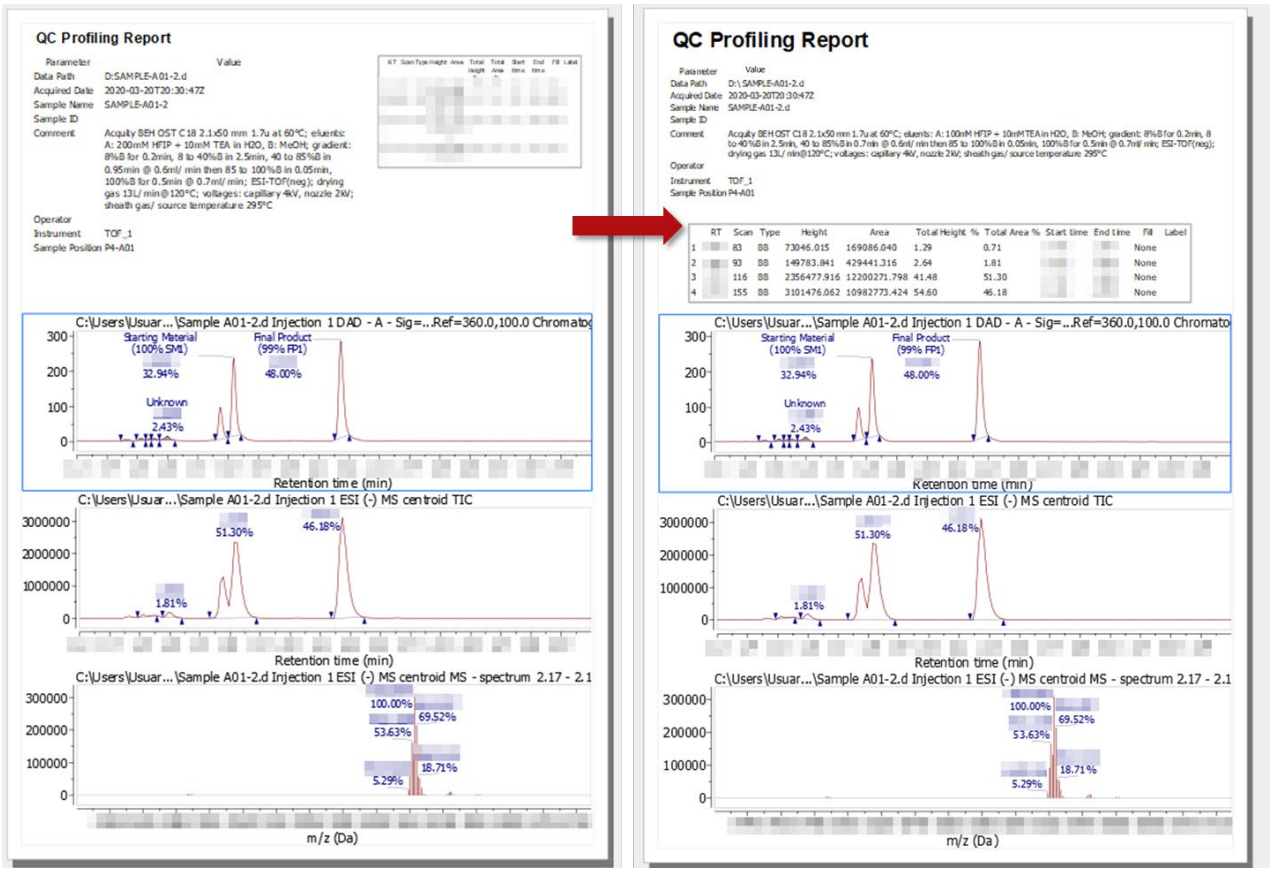
- **Custom**

The user can load their own Layout Template, which can be created in Mnova. Find the directory with a saved custom layout template file and load it into the plugin.

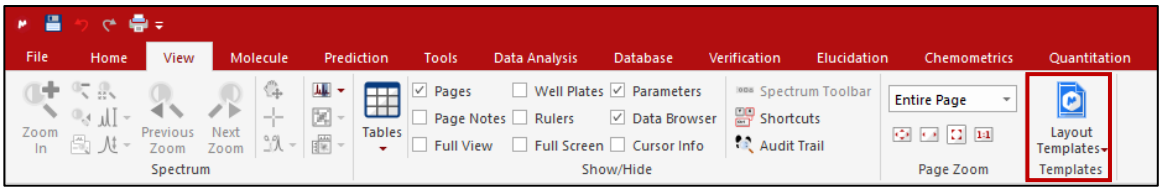


How to create a Layout Template report and save it:

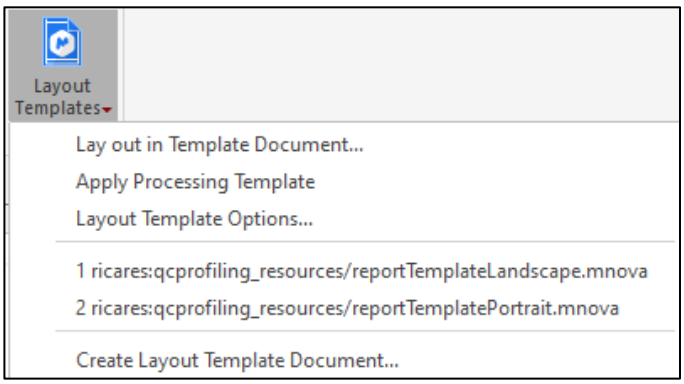
- Open a QC Profiling report, for example with the “Default Portrait” template, and place the objects on the page as desired.



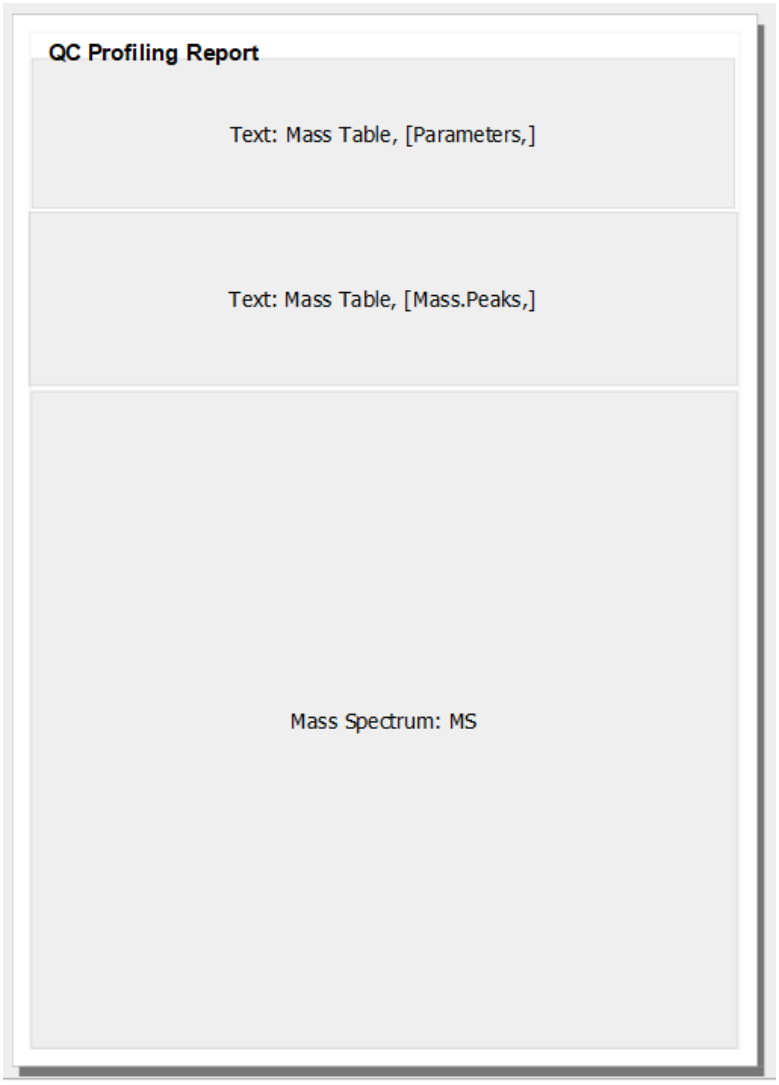
- Go to the **View** tab.



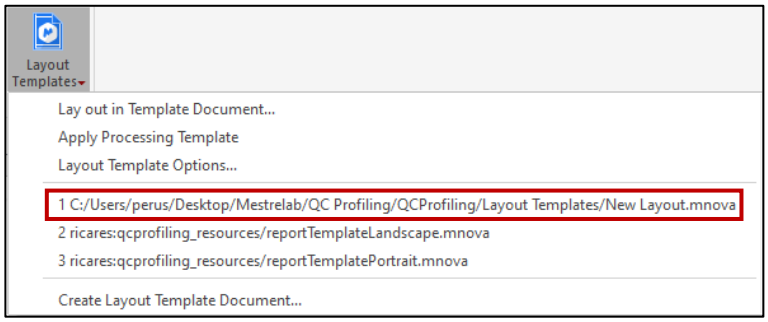
- Click on **Layout Templates**.



- Select **Create Layout Template Document** and save the template in your preferred directory.
- The content of all page items is removed to leave a pure template with the user's desired placeholders.



- A new Layout Template is created and made available in the template list, and can be loaded as a **Custom Layout Template** in the QC Profiling report settings.



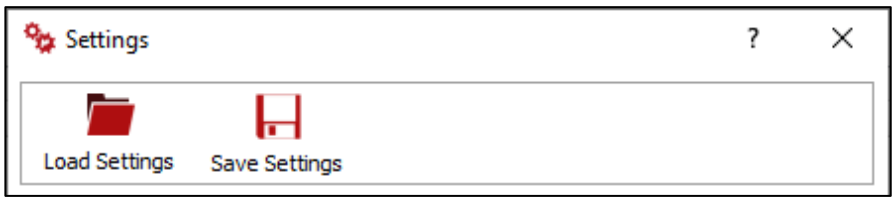
3.3 Save and Load Settings

Saving your settings in files can save a lot of time the next time the automation is executed. These files can also be used to exchange settings with other users within your organization or with our Mnova Support Team. Settings can be saved and loaded using two different functions, each saving a different form of settings file:

- **".mgrs":** contains Mnova Gears and QC Profiling settings. This can be saved or loaded from the Mgears window.

Note: *Each evaluation generates an .mgrs file in the output directory (3.4.1 Output Folder and Files).*

- **".data":** contains QC Profiling settings. This can be saved or loaded from the QC Profiling Settings window.



Load saved settings files, ".mgrs" or ".data", to simplify the plugin configuration and modify them as required to save time.

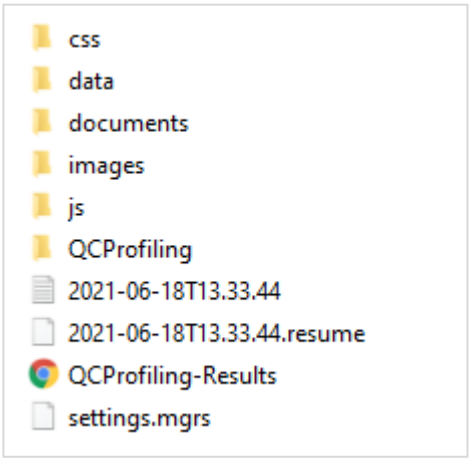
3.4 Results

During the evaluation, a new directory is created in the output directory declared in Mgears. This directory carries a time stamp for the start of the evaluation. All the results generated during the evaluation are created there. Among these results, the most important are illustrated in the following point.

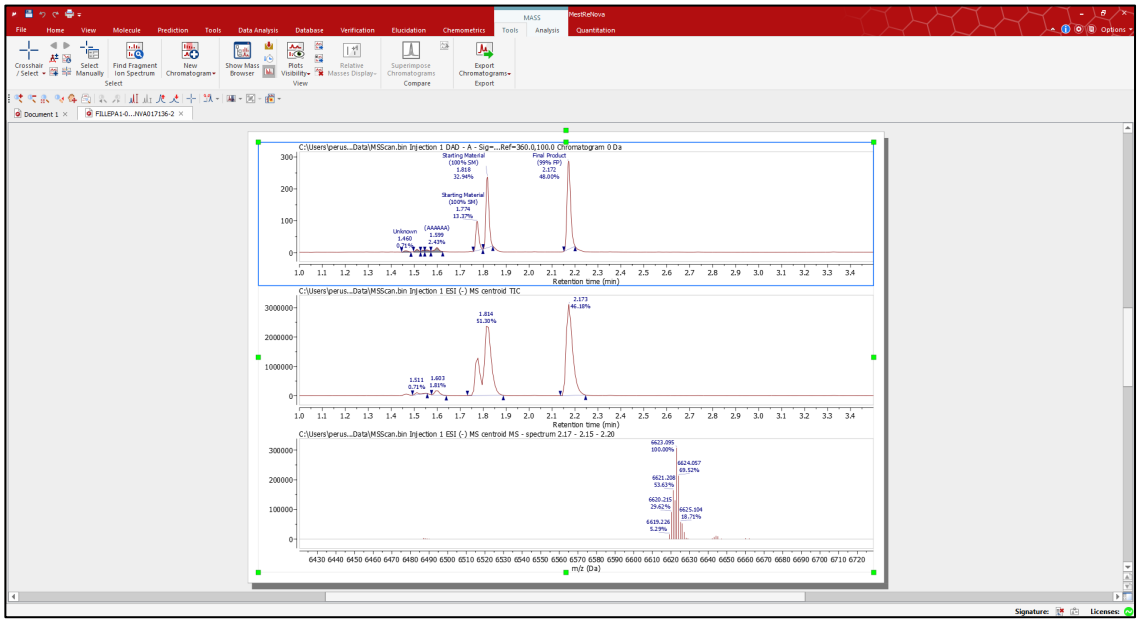
3.4.1 Output Folder and Files

Each evaluation generates a folder as per the figure, named according to its own time stamp.

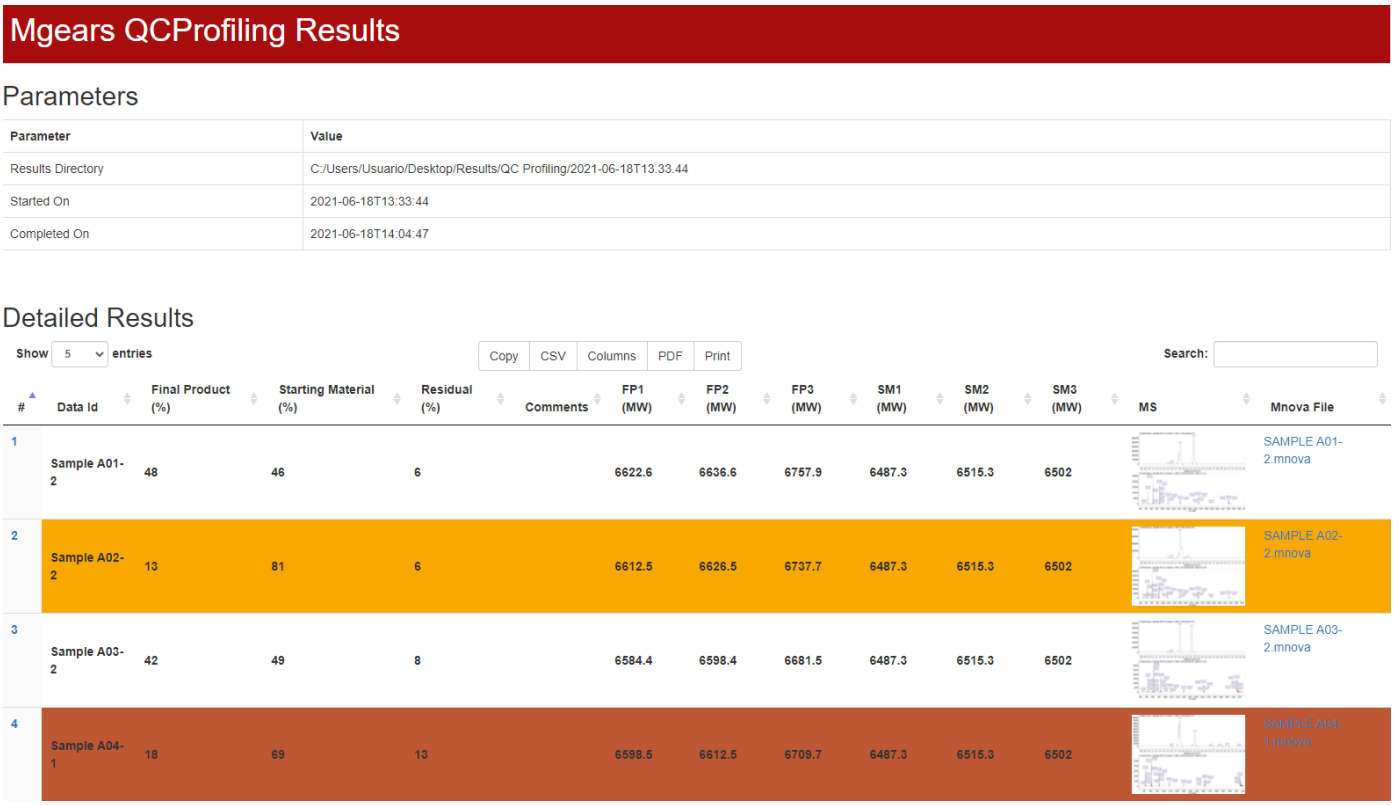
Within this folder, the user can find the evaluation results, such as different types of files and some important files types:



- **Mnova Files:** open the "**documents**" folder and click on any file. A window similar to that in the figure below will open and the user can use Mnova tools to analyze the report document.



- **HTML File:** click on **QCProfiling-Results.html** and a website will open in an internet browser window.



Mgears QCProfiling Results

Parameters

Parameter	Value
Results Directory	C:/Users/Usuario/Desktop/Results/QC Profiling/2021-06-18T13.33.44
Started On	2021-06-18T13:33:44
Completed On	2021-06-18T14:04:47

Detailed Results

Show 5 entries | Copy | CSV | Columns | PDF | Print | Search:

#	Data Id	Final Product (%)	Starting Material (%)	Residual (%)	Comments	FP1 (MW)	FP2 (MW)	FP3 (MW)	SM1 (MW)	SM2 (MW)	SM3 (MW)	MS	Mnova File
1	Sample A01-2	48	46	6		6622.6	6636.6	6757.9	6487.3	6515.3	6502		SAMPLE A01-2.mnova
2	Sample A02-2	13	81	6		6612.5	6626.5	6737.7	6487.3	6515.3	6502		SAMPLE A02-2.mnova
3	Sample A03-2	42	49	8		6584.4	6598.4	6681.5	6487.3	6515.3	6502		SAMPLE A03-2.mnova
4	Sample A04-1	18	69	13		6598.5	6612.5	6709.7	6487.3	6515.3	6502		SAMPLE A04-1.mnova

Opening this file allows you to:

- Get a brief inspection of the results.
- See small descriptive figures of the results.
- Download Mnova files through links.
- Sort the results by different column names.
- Find any file with the Search tool.
- Copy the website link and share.
- Create simple reports in .csv or .pdf format.
- Print the results.
- Select the columns to show on the website.

This file is available for any device such as PC, smartphone, or tablet that can run an internet browser.

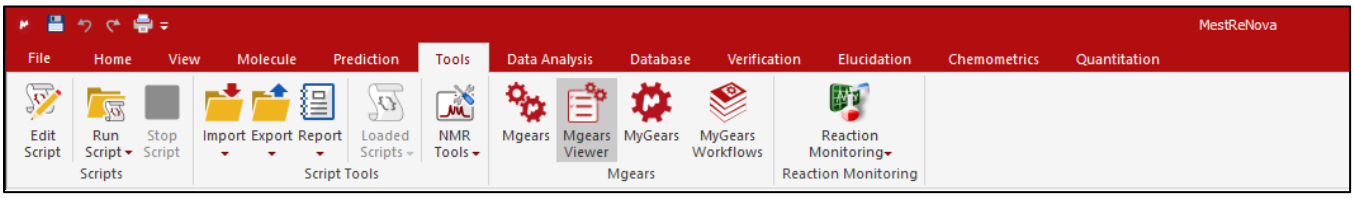
- **CSV File:** open the "**QCProfiling**" folder. A copy of the input .csv file is saved in the QC Profiling directory of the output folder. This file is populated with all the output from the evaluation using the settings defined in CSV Configuration (*3.2.2 CSV Configuration*).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Sample A01-2	A01	48	46	6					6622.6	6636.6	6757.9	6487.3	6515.3	6502	13% SM1	33% SM1	1.8182	4114.3	48% FP1	2.1724	5994.2
2	Sample A02-2	A02	13	81	6					6612.5	6626.5	6737.7	6487.3	6515.3	6502	7% SM1	74% SM1	1.8187	8615.1	13% FP1	1.8629	1543.8
3	Sample A03-2	A03	42	49	8					6584.4	6598.4	6681.5	6487.3	6515.3	6502	49% SM1	1.8136	657.4	42% FP1	2.1236	564.1	
4	Sample A04-1	A04	18	69	13					6598.5	6612.5	6709.7	6487.3	6515.3	6502	34% SM2	34% SM1	1.8162	134.9	18% FP1	2.2079	71.5
5	Sample A05-1	A05	2	90	8					6593.5	6607.5	6699.7	6487.3	6515.3	6502	12% SM1	78% SM1	1.8165	560	2% FP1	2.0715	12.6
6	Sample A06-4	A06	69	22	9					6626.5	6640.5	6765.7	6487.3	6515.3	6502	22% SM1	1.8162	111.4	69% FP1	1.932	346.9	
7	Sample A07-4	A07	91	1	7					6585.4	6599.4	6683.5	6487.3	6515.3	6502	1% SM1	1.7741	13.6	91% FP1	1.8049	863.7	
8	Sample A08-1	A08	74	15	11					6625.6	6639.6	6763.9	6487.3	6515.3	6502	15% SM1	1.8163	121.9	74% FP1	1.9546	587.7	
9	Sample A09-1	A09	45	48	8					6655.6	6669.6	6823.9	6487.3	6515.3	6502	19% SM1	29% SM1	1.8154	231.9	45% FP1	2.0204	361.3
10	Sample A10-1	A10	26	66	8					6636.5	6650.5	6785.7	6487.3	6515.3	6502	52% SM1	14% SM1	1.772	757.2	26% FP1	1.9828	376.1
11	Sample A11-1	A11	19	75	6					6637.5	6651.5	6787.7	6487.3	6515.3	6502	59% SM1	16% SM1	1.7726	4283.5	19% FP1	1.8693	1412.7
12	Sample A12-1	A12	90	0	10					6601.4	6615.4	6715.5	6487.3	6515.3	6502				90% FP1	1.801	2582.1	
13	Sample B01-2	B01	40	54	6					6599.4	6613.4	6711.5	6487.3	6515.3	6502	1% SM3	2% SM1	51% SM1	1.8174	7524.2	40% FP1	1.8691
14	Sample B02-3	B02	13	81	6					6612.5	6626.5	6737.7	6487.3	6515.3	6502	8% SM1	73% SM1	1.8175	9205.4	13% FP1	1.8617	1590.9
15	Sample B03-2	B03	27	68	6					6638.5	6652.5	6789.7	6487.3	6515.3	6502	7% SM1	61% SM1	1.8153	7987.4	27% FP1	1.9562	3469.7

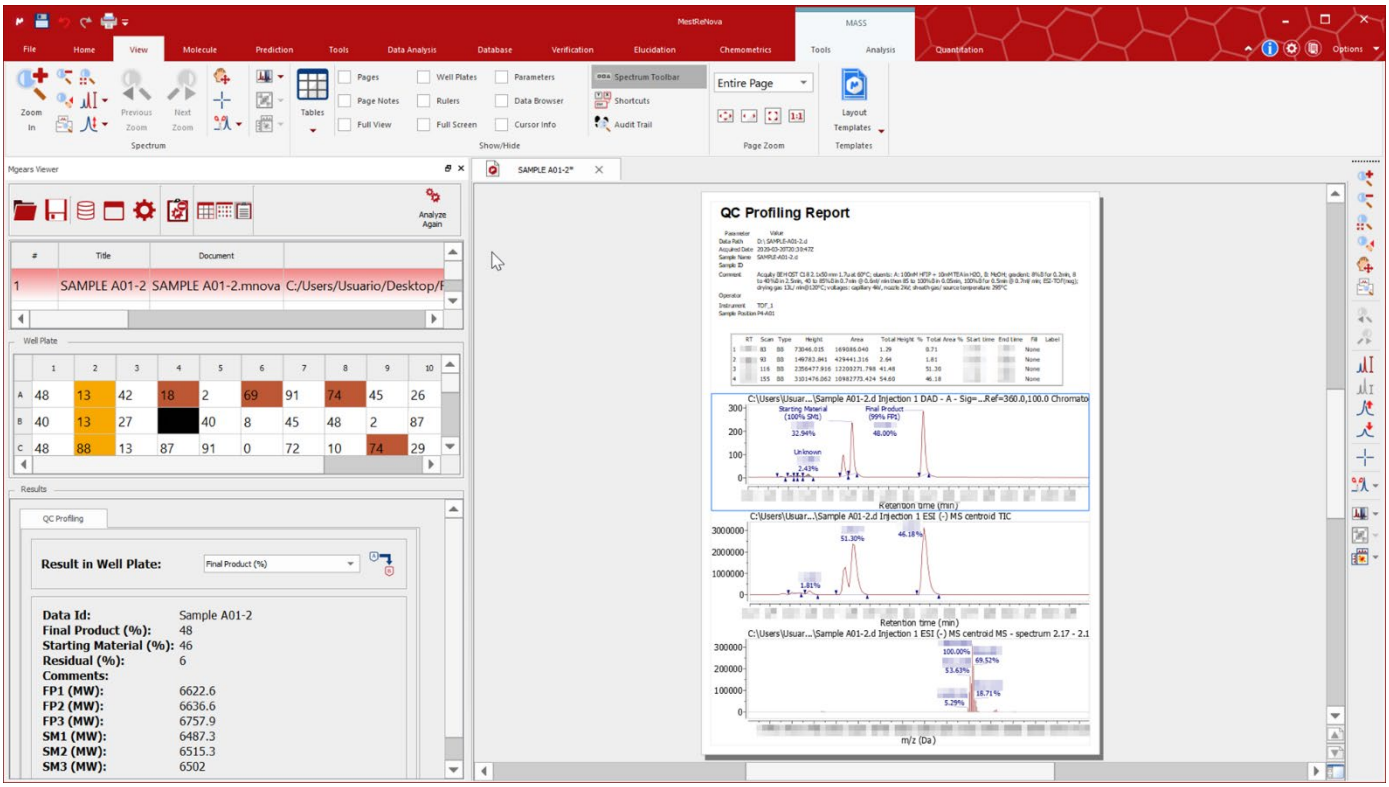
- **.log File:** this file summarizes the entire evaluation. Any error will be reported and consulted on in this document.
- **.mgrs File:** this file contains all the evaluation settings to allow them to be loaded again into QC Profiling Plugin Settings (3.3 Save and Load Settings) for the following evaluations.

3.4.2 Mnova Viewer

The results can be analyzed with the Mnova Viewer, which is a fast and easy way to check the output dataset, make modifications, and analyze again, if needed.

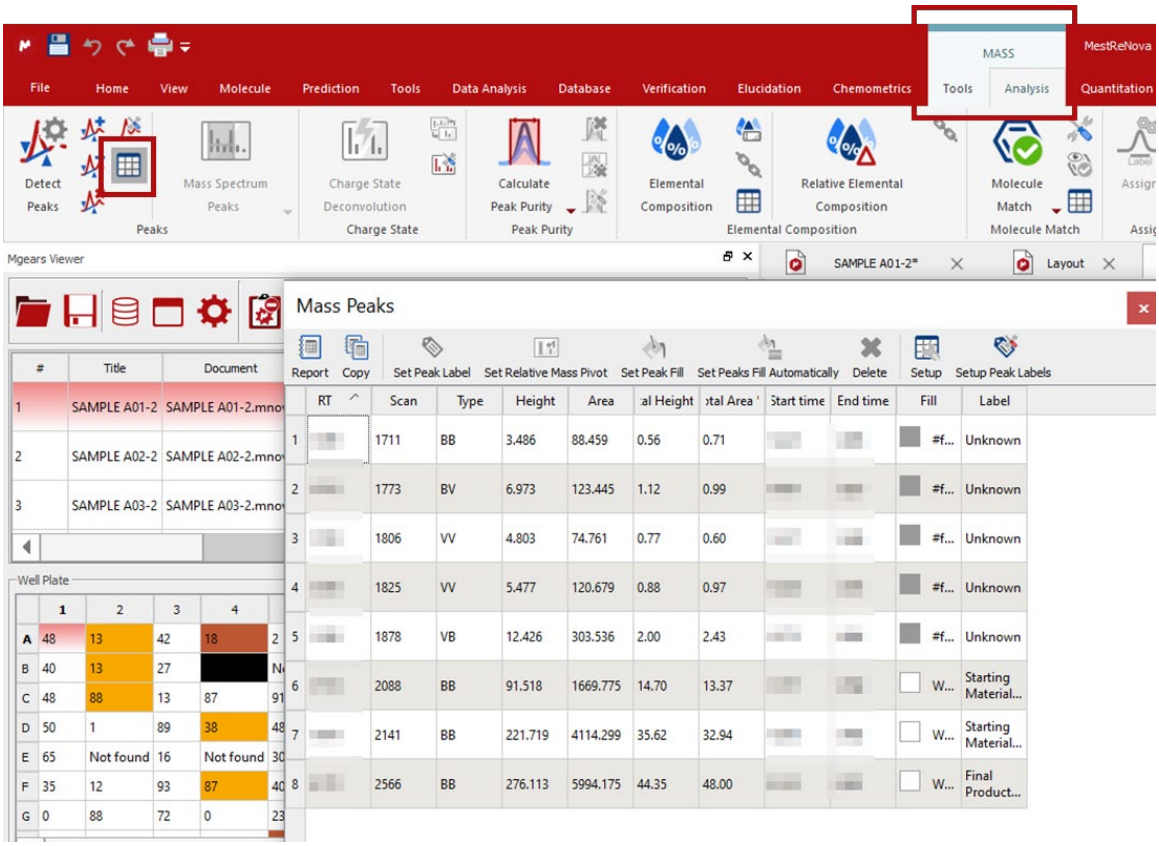


Load the results folder and open in Mgears Viewer.

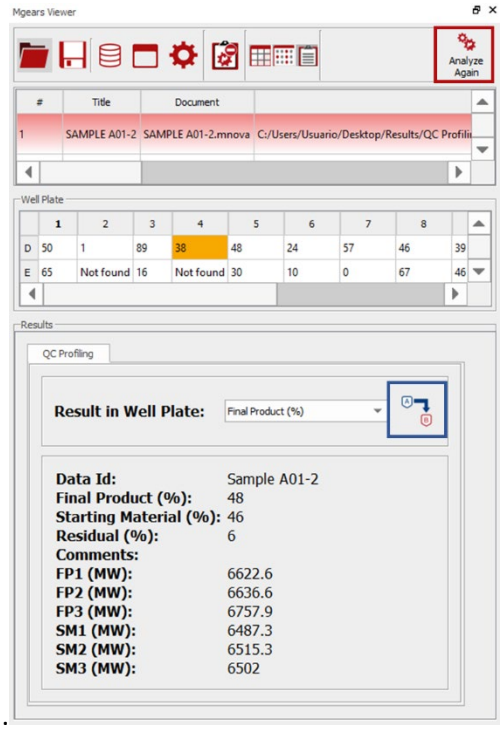


Mnova Viewer Options:

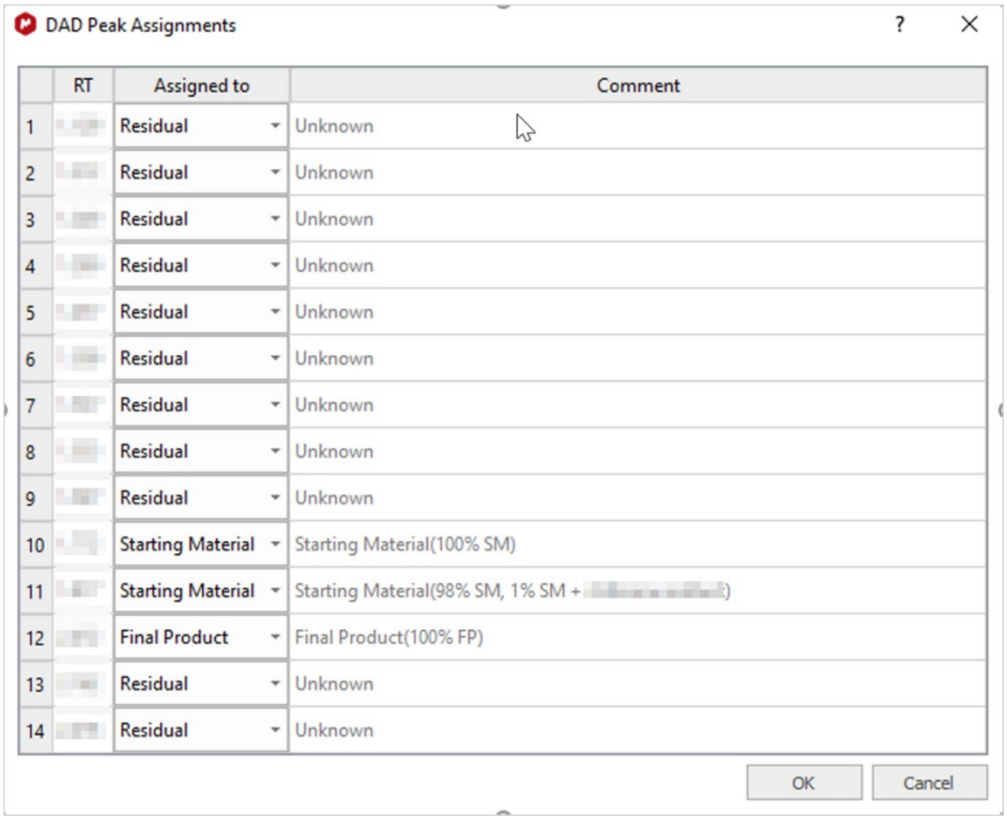
Peak Assignment modifications



After each modification, click on **Analyze Again** to reassign peaks (highlighted red in the figure below).



If you prefer or need to make manual assignments, you can use the **Modify UV peaks assignment manually** option (highlighted in blue in the figure above)



You can save after each modification manually or choose autosave if you prefer to allow a faster results inspection. Click on **Settings** and select **Save Automatically on Clicking Analyze Again** in the **Mgears Viewer Settings** window.