Mestrelab Research

Gears MANIQ 1.1

USER MANUAL

Document Number

P/N 226 R3

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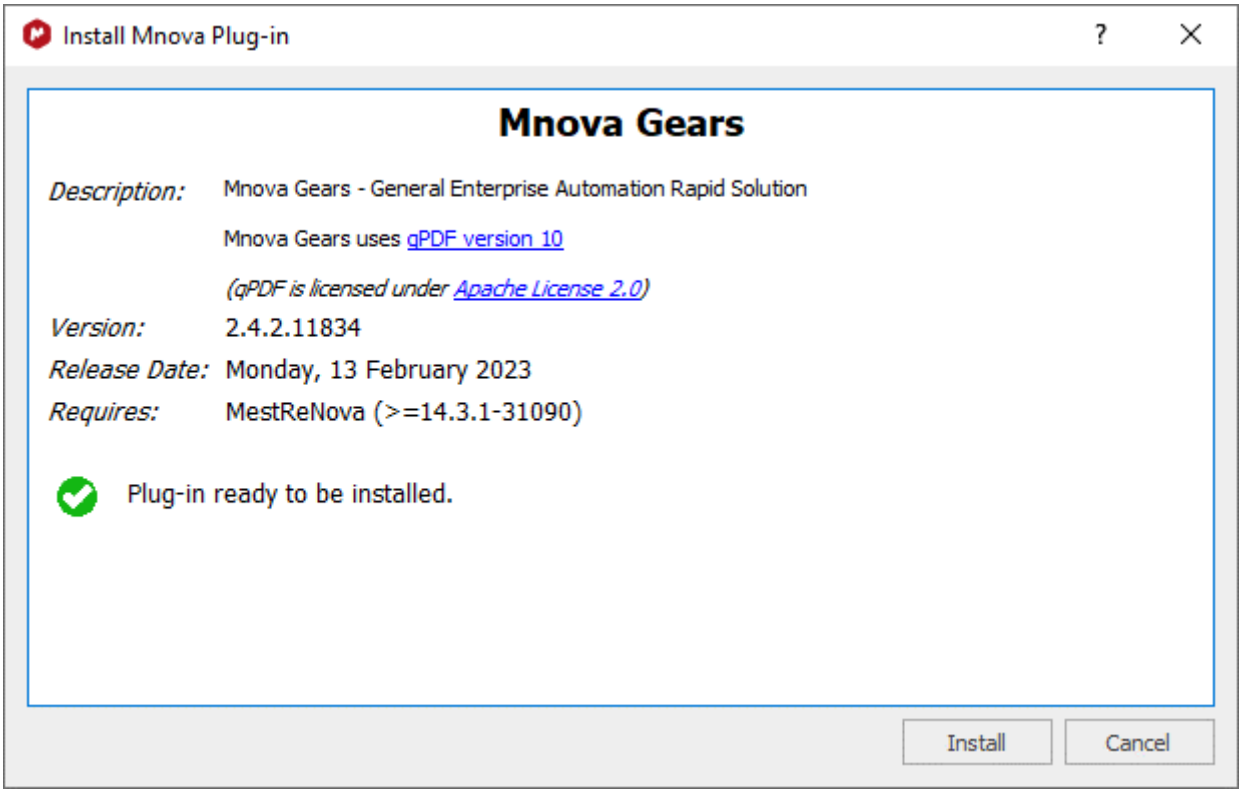
1. Installation

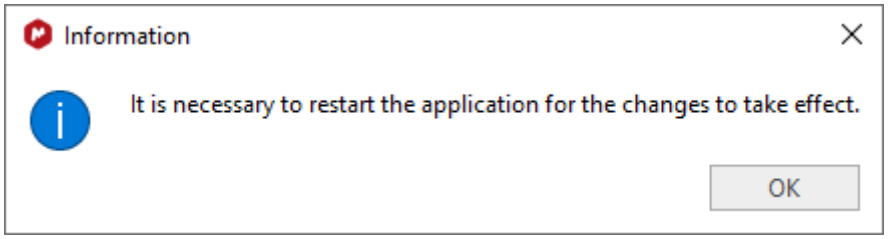
First, three different products need to be installed to run the MANIQ plugin for Mnova, and in the following order:

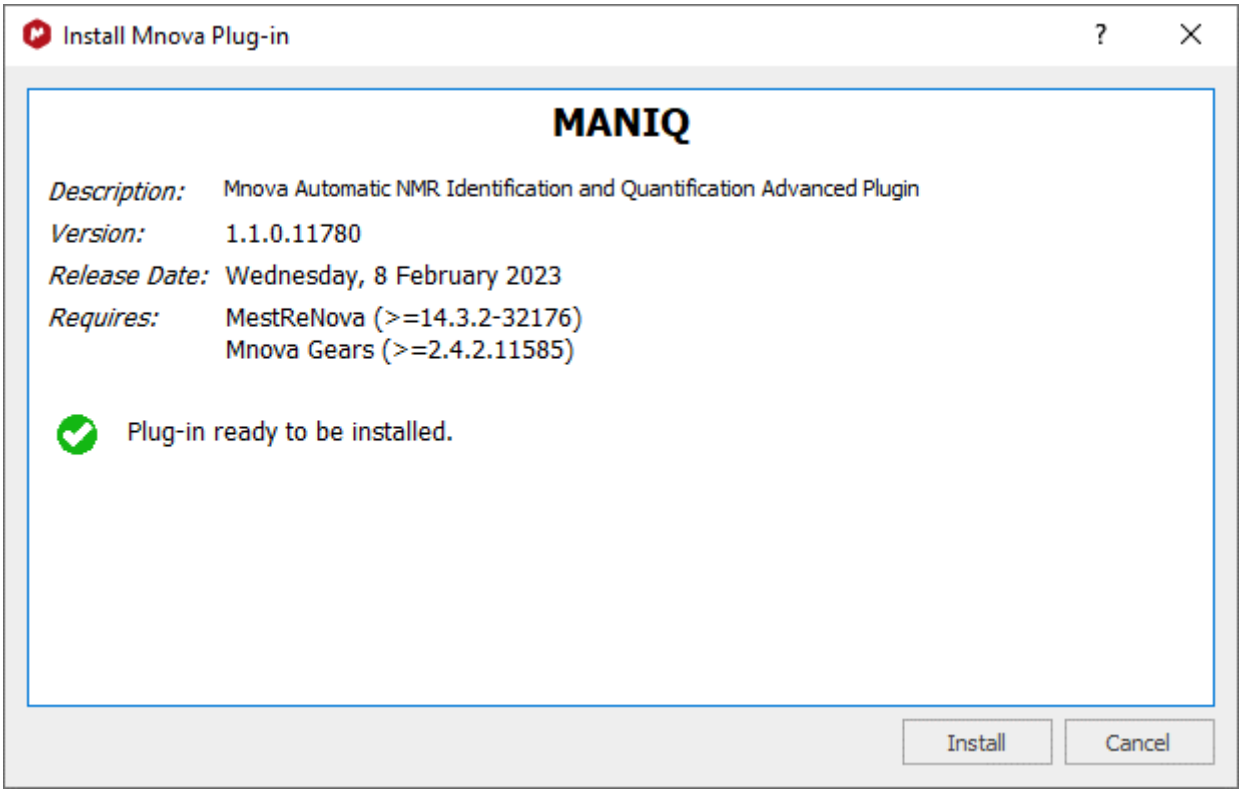
- **Mnova** (minimum version: 14.3.2.32716)
- **Mgears** (minimum version: 2.4.2.11585)
- **MANIQ** (minimum version: 1.1.0.11780)

Nombre	Fecha de modificación	Tipo	Tamaño
MestReNova-14.3.2-32299_x64.msi	15/02/2023 12:03	Paquete de Windows Installer	468.996 KB
Mnova-Gears-2.4.2.11834.zip	15/02/2023 12:02	Carpeta comprimida (en zip)	11.384 KB
Mnova-MANIQ-1.1.0.11780.zip	15/02/2023 12:02	Carpeta comprimida (en zip)	355 KB

While Mnova has a regular installer, Mnova Gears and MANIQ need to be installed via the Mnova interface. To this end, you can just drag and drop the installers into the Mnova interface (please note that MANIQ must be installed *after* Mnova Gears).



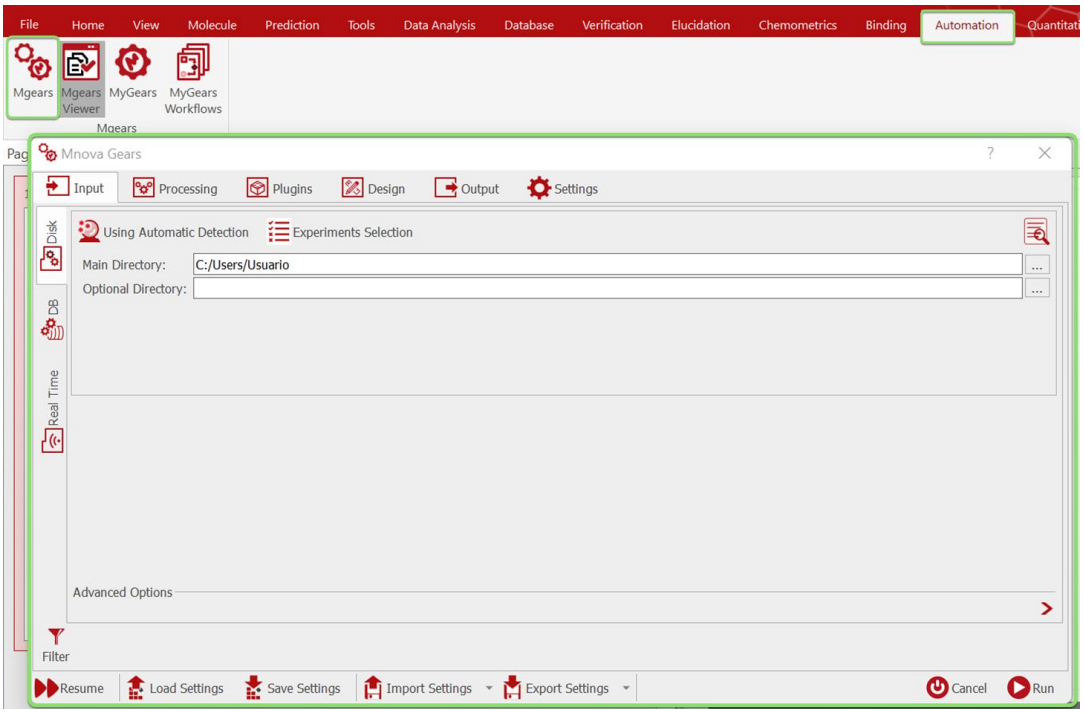

Information
It is necessary to restart the application for the changes to take effect.
OK


Install Mnova Plug-in
MANIQ
Description: Mnova Automatic NMR Identification and Quantification Advanced Plugin
Version: 1.1.0.11780
Release Date: Wednesday, 8 February 2023
Requires: MestReNova (>=14.3.2-32176)
Mnova Gears (>=2.4.2.11585)
Plug-in ready to be installed.
Install
Cancel

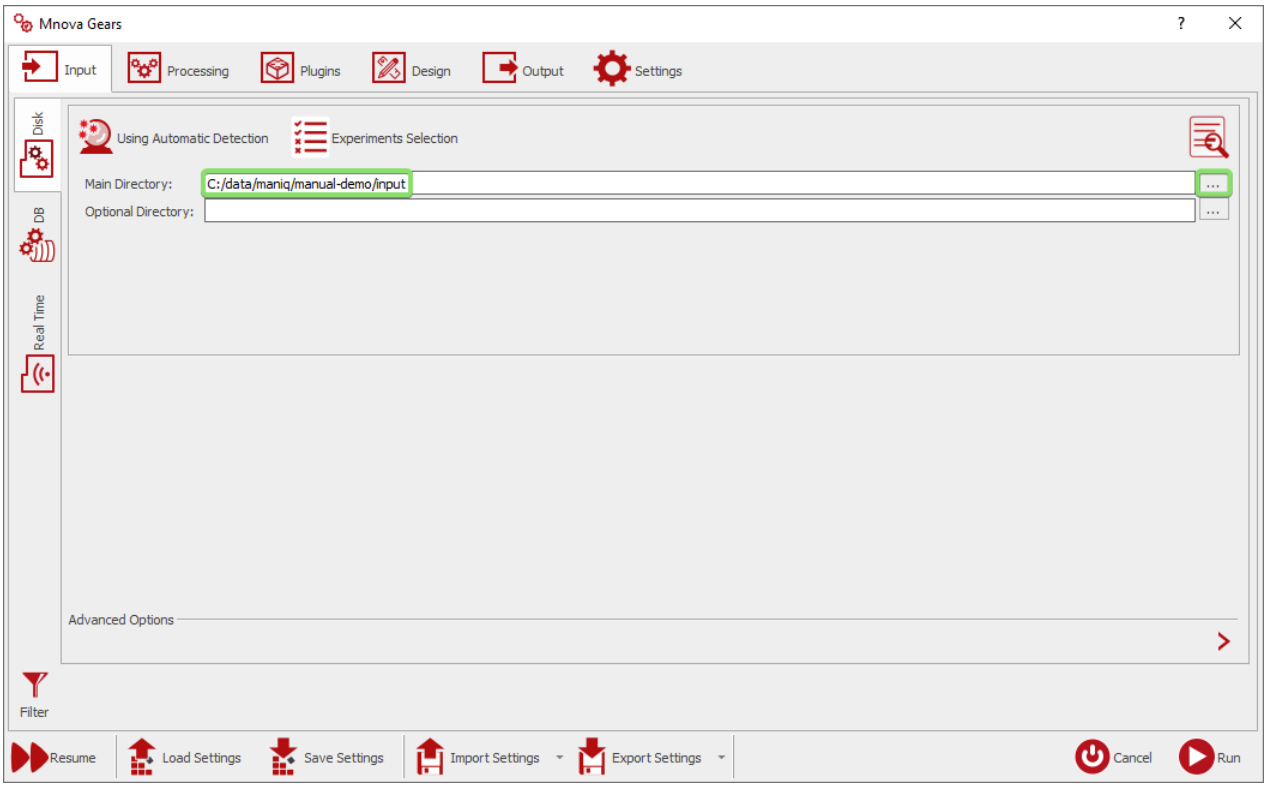
2. Configuration

2.1.Configuring Mgears

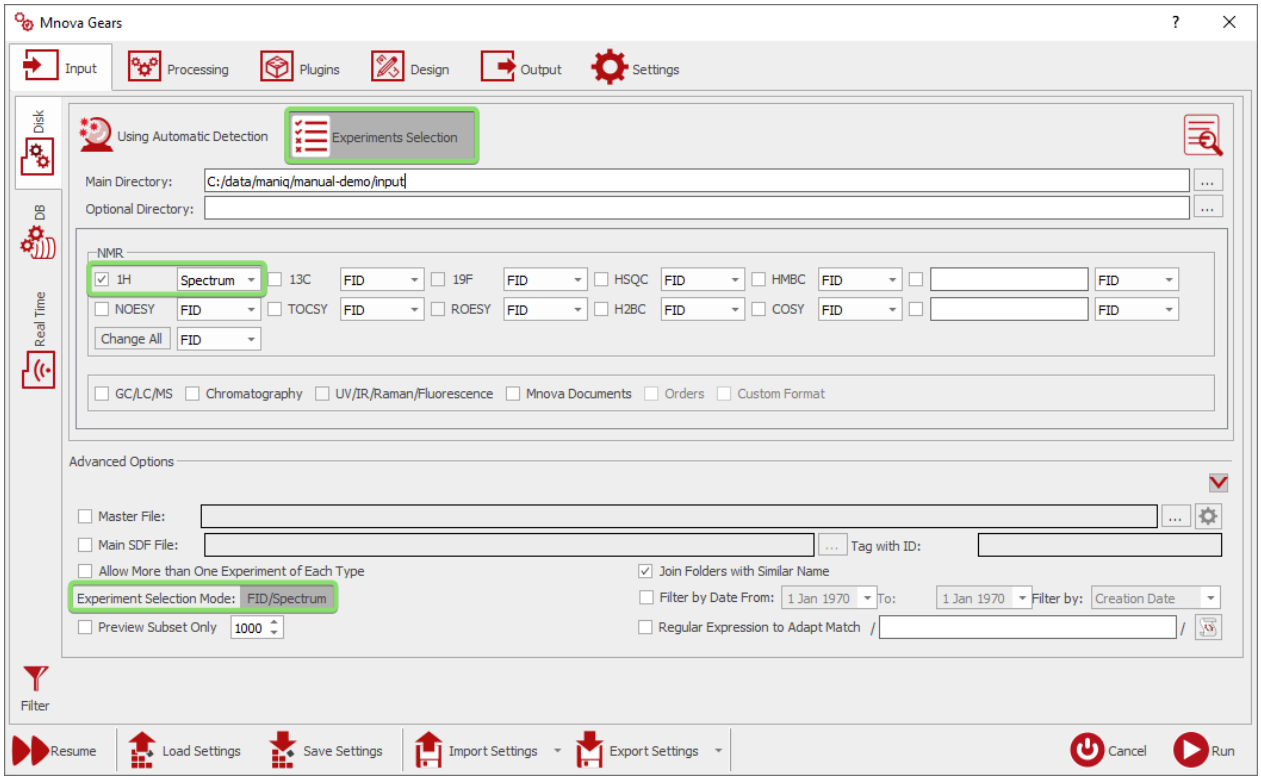
Before running the MANIQ plugin, Mgears must be appropriately configured. First, open **Mgears** from the **Automation** menu in Mnova.



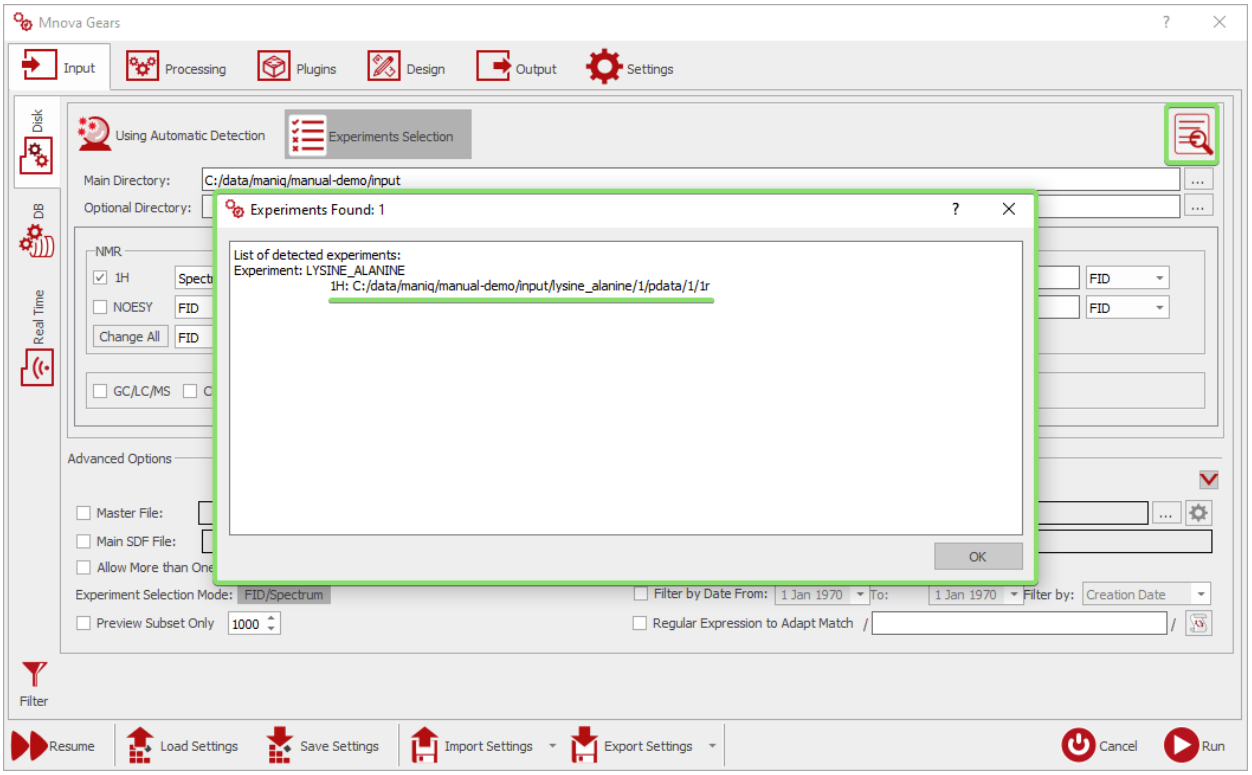
Then, proceed to set a **Main Directory** in the **Input** tab.



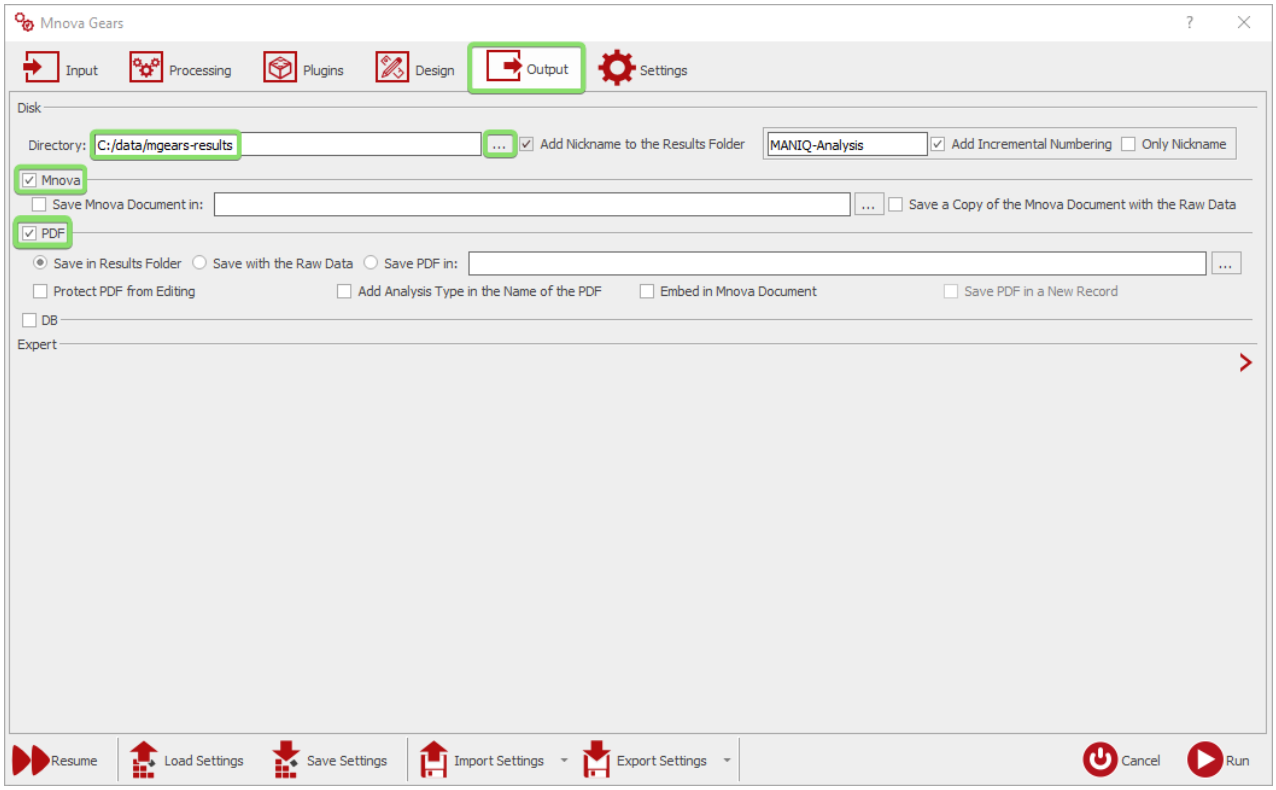
The **Main Directory** folder should contain files with the NMR spectra of the samples you want to analyze. Additionally, you can add different filters to select between the **FID** and the **Spectrum** files (it is strongly recommended that you choose the latter for the calculation of absolute quantifications).



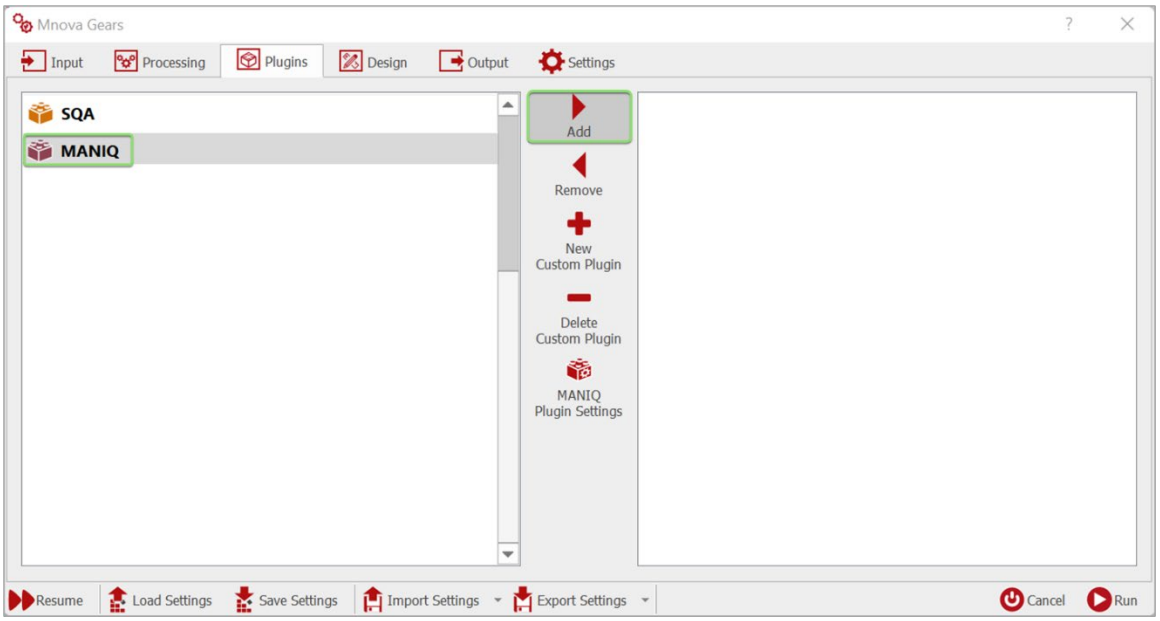
If you want to check that the documents you want to analyze are being correctly detected by Mgears from the **Main Directory** folder, you can use the **Automatic Detection** tool.



In the **Output** tab, it is necessary to set up the output folder in which the results will be created. It is also possible to configure whether you want Mnova and/or PDF files with the analyzed samples to be copied into the results folder, but this is not immediately necessary as the MANIQ analysis results will also be output in another file.



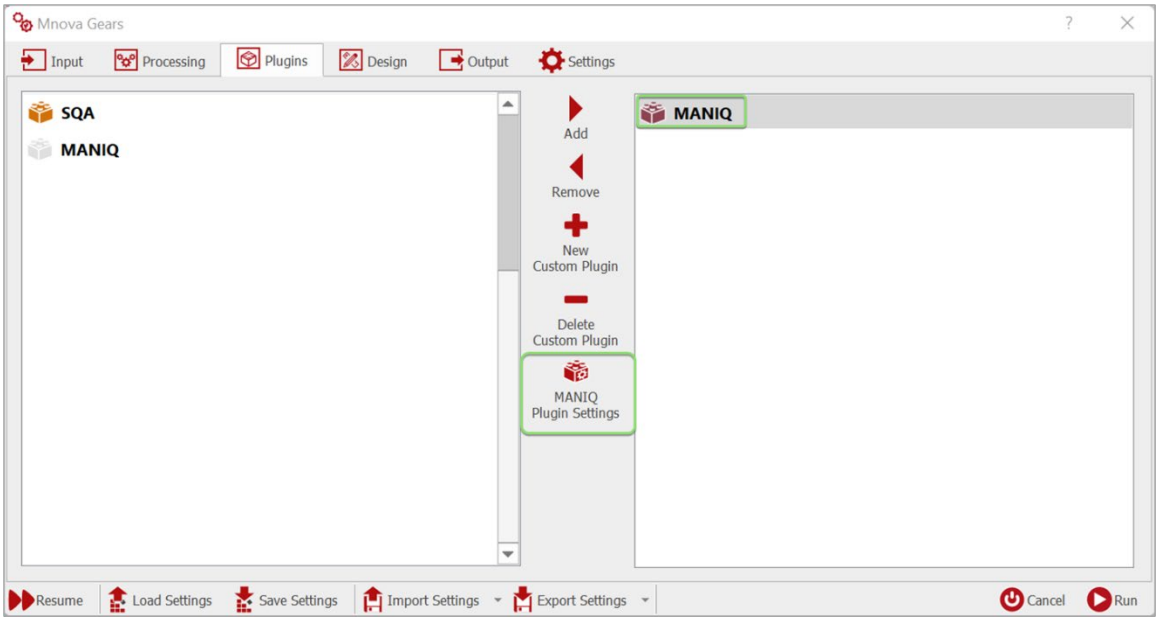
Finally, you must add the MANIQ plugin to the set of plugins that will be run with Mgears. For this, go to the **Plugins** tab, find and click on the corresponding MANIQ brick in the left-hand side list, and click on **Add**. Following that, the MANIQ brick should appear on the right-hand side, which is the list of plugins that will be used in the Mgears analysis.



For more details on the Mnova Gears general configuration, please refer to the Mnova Gears manual.

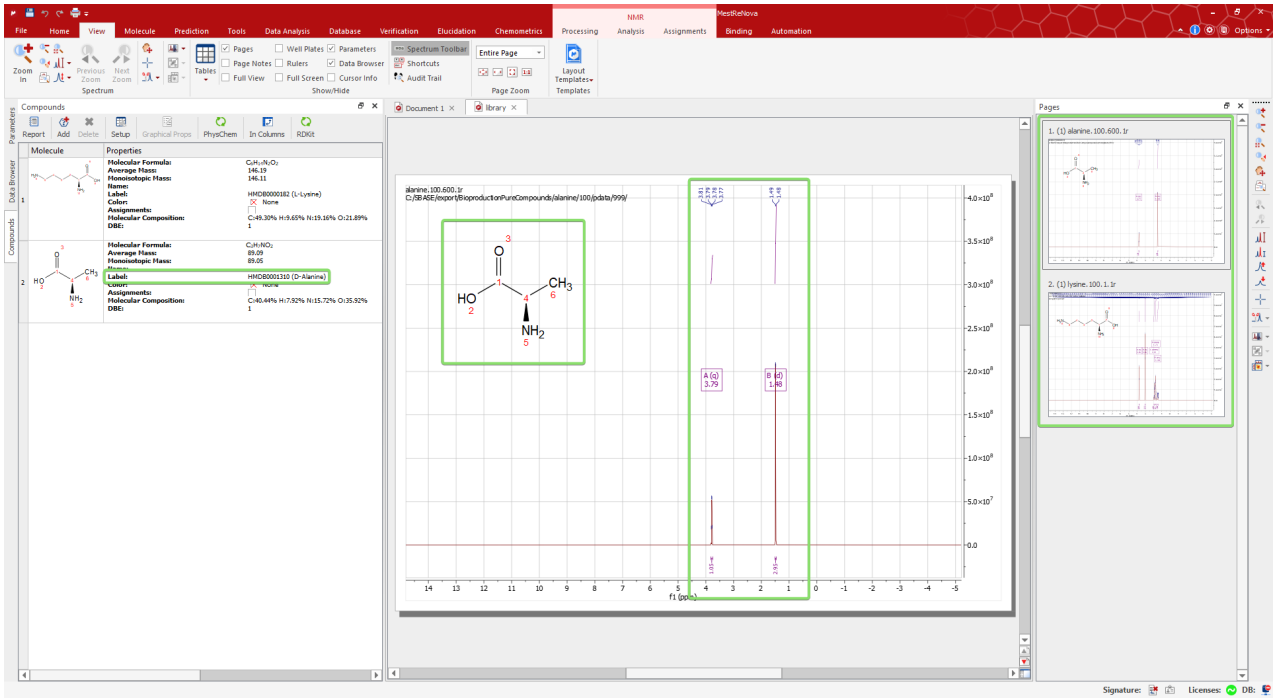
2.2.Configuring MANIQ

You can now proceed to set up the MANIQ plugin. In the **Plugins** tab, click on **MANIQ** and then click on **MANIQ Plugin Settings** in the middle column. This will open the **MANIQ Settings** dialog.

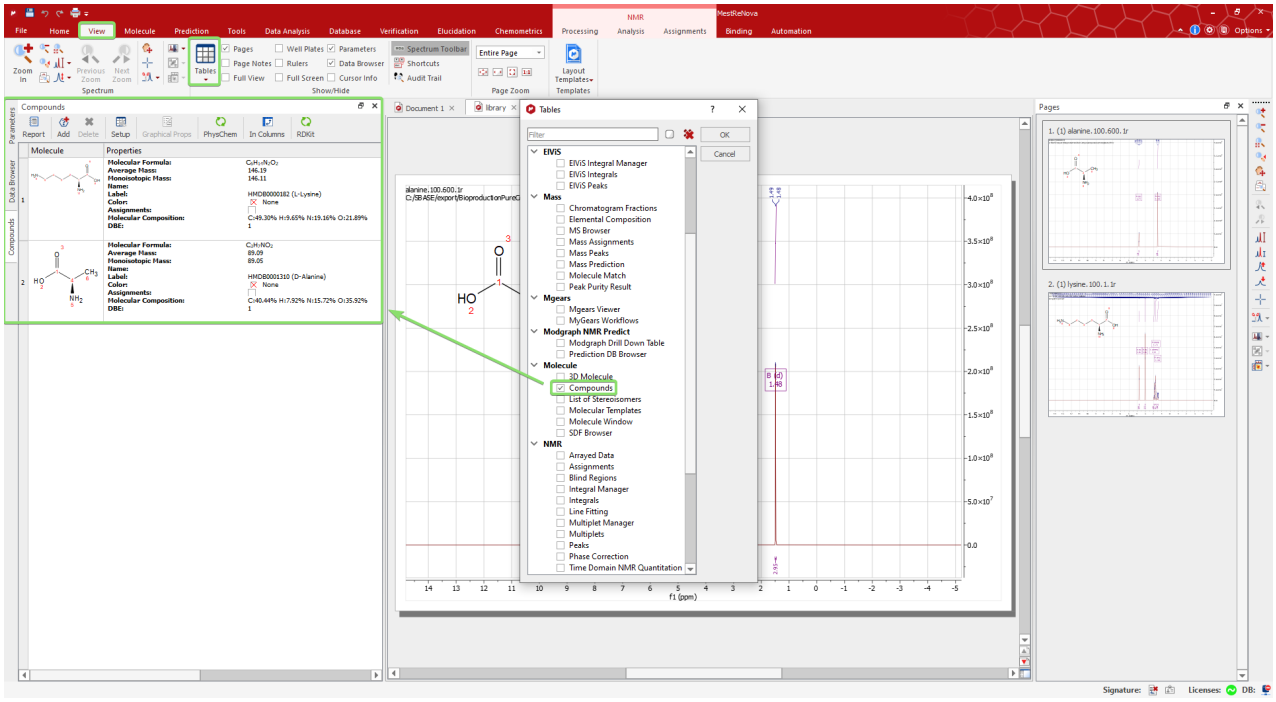


2.2.1. The Compounds Library tab

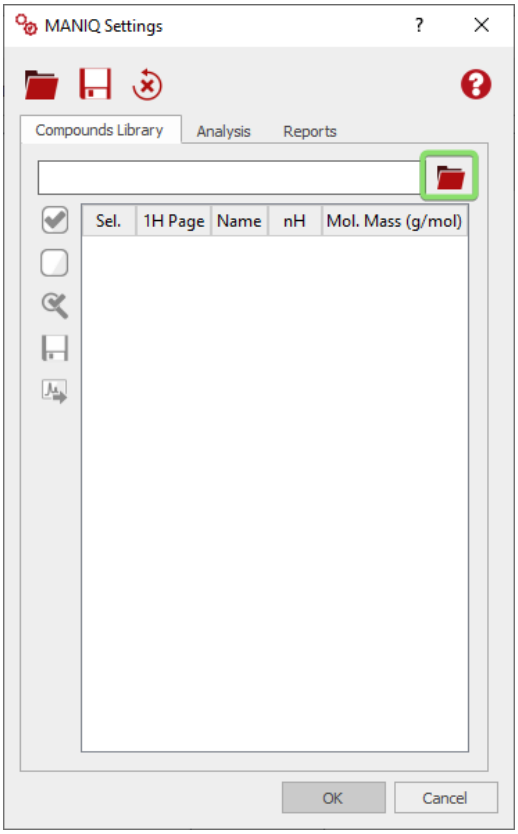
The most important part of the MANIQ plugin configuration is the **Compounds Library**. This library consists of an Mnova document with the NMR spectra of the reference compounds that the user wants to include in the identification and quantification analysis. The structure of this document must be configured as follows: on each page of the document, there should be an NMR spectrum of a compound along with its corresponding molecular structure. An example of a compound library document is as follows:



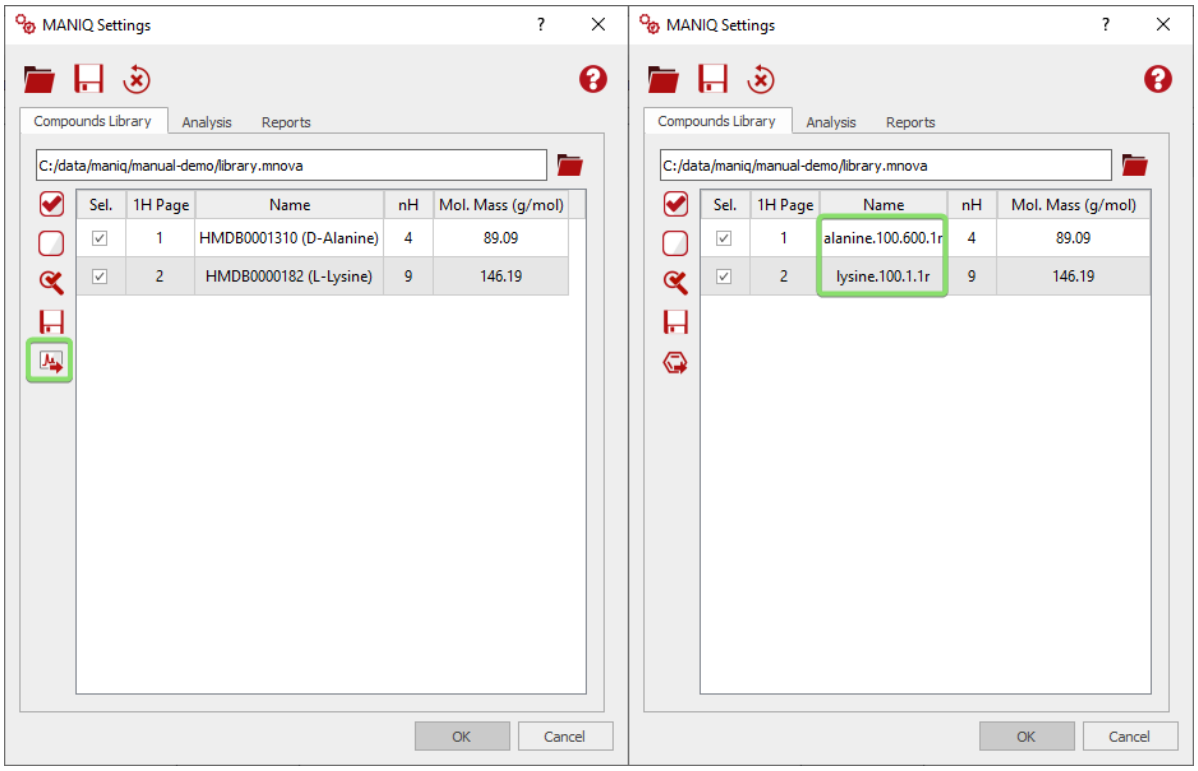
It is also important that each molecular structure is correctly labeled, as the name assigned to each compound is the label given to the corresponding structure (to view this information, you can open the **Compounds table**: **View → Tables → Molecule** and then check **Compounds** and click **OK**; you can then review the **Label** field for each compound).



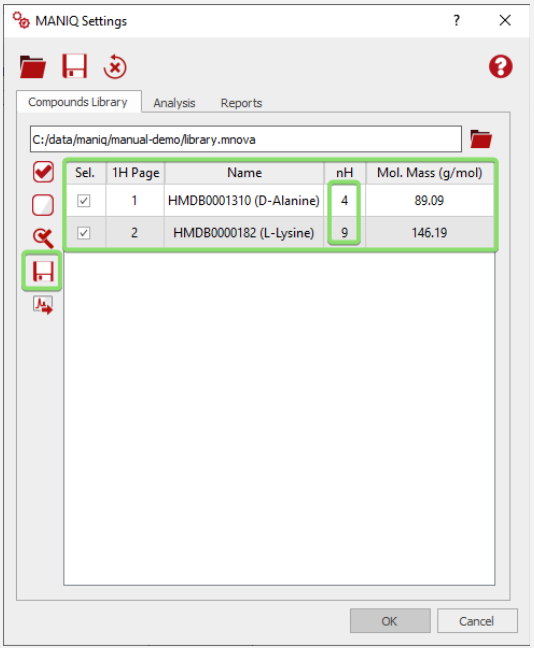
When the compounds library is ready, select it from the **MANIQ Settings** dialog. A **table** will appear with the information for each compound taken from the document.



With regard to the default compound names, you can switch between the molecule names and the spectra titles by clicking on the following button. The compound names can also be edited manually.

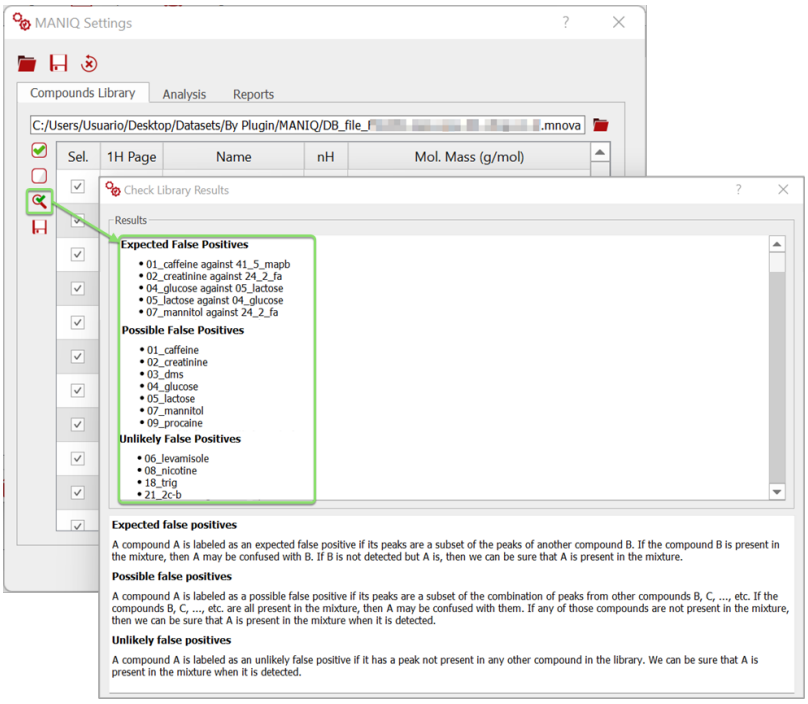


Important. When opening a library for the first time, Mnova will show the number of observable protons (nH) for each molecule, as calculated according to a set of rules. However, it is advised that the user reviews these values the first time the library is loaded as they are critical to a correct quantification. If required, the nH values (but also the Molecular mass and Name fields information) can be manually edited by clicking on each field and typing the new values. After editing, the user must click on the **Save** button.



Note that it is possible to use anything from the entire compound library to a particular selection of compounds for the analyses. The user can easily check/uncheck the compound rows to include/exclude them from the analysis, respectively.

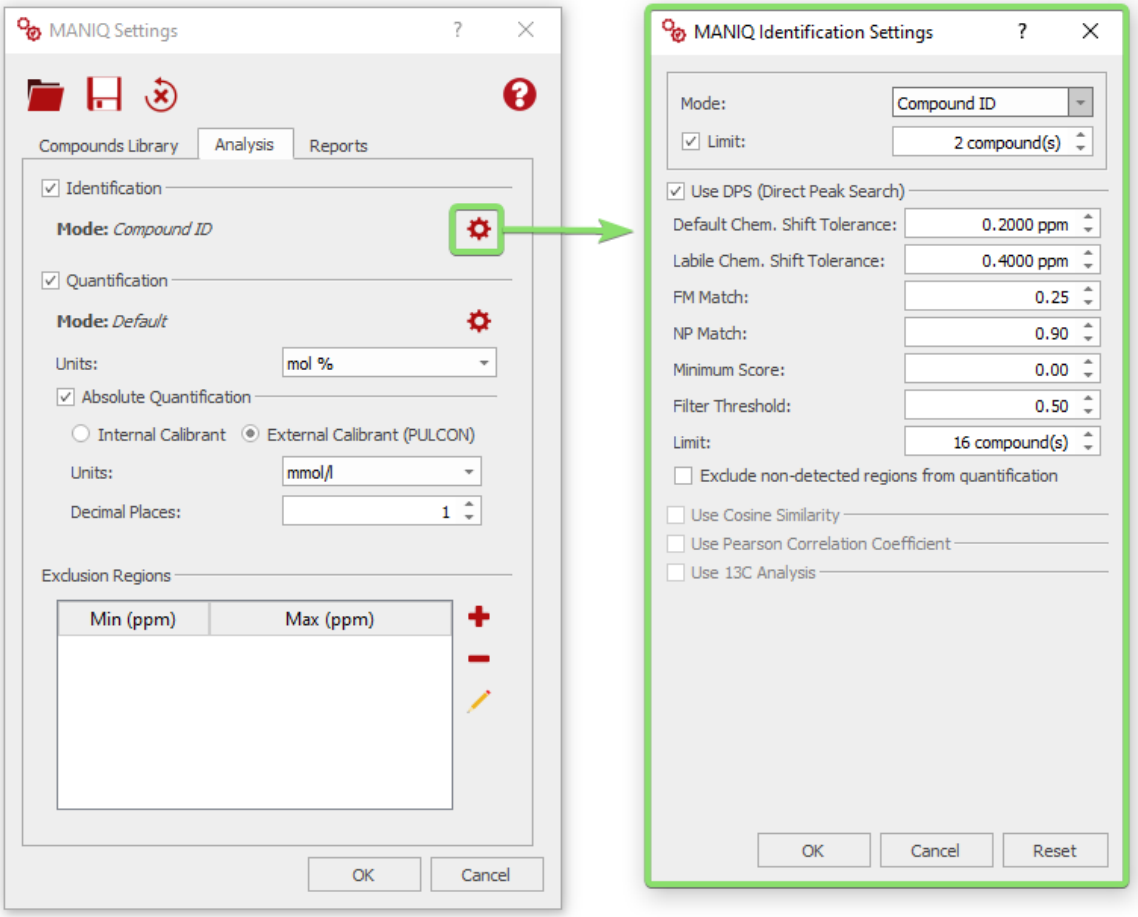
It is also possible to run an analysis of the reference library to detect compounds that can potentially be falsely identified as components of certain samples by the Direct Peak Search algorithm (further information about this algorithm can be found in the *Analysis tab* section). The analysis will generate a list with **Expected False Positives**, **Possible False Positives**, and **Unlikely False Positives**, as shown in the image below.



2.2.2. The Analysis tab

The **Analysis** tab is divided into three different sections: **Identification**, **Quantification**, and **Exclusion Regions**.

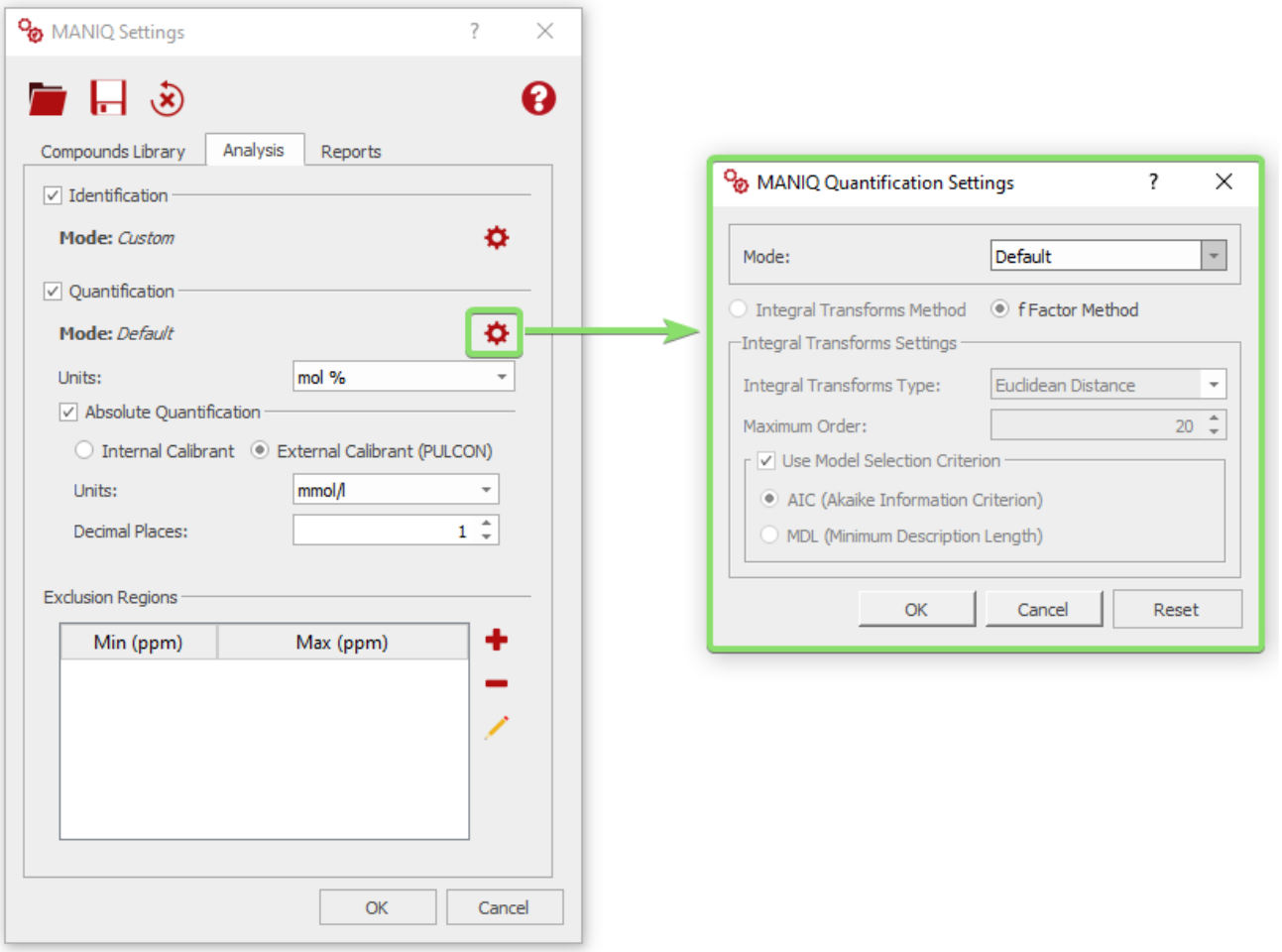
In the **Identification** section, there are two preconfigured modes: **Compound ID** and **Spectrum ID**. Compound ID is for mixtures of samples in which the compound signals may be overlapped with those of other compounds. Spectrum ID, by contrast, is for samples in which only one compound is present. Although it is advised that one of these two settings be used in the general case, an additional mode called **Custom** enables the user to select which method(s) they want to use.



To date, there are four different identification methods, which can be used separately or jointly:

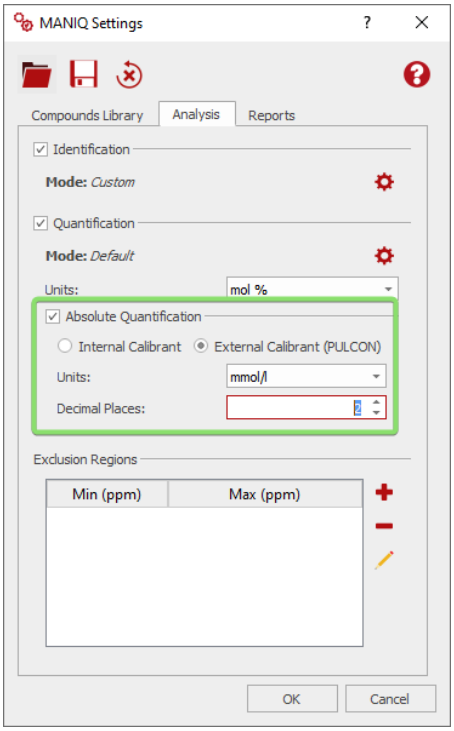
- **DPS (Direct Peak Search)**. This method matches all the peaks for each compound with the corresponding peaks in the sample spectrum. If one or more peaks cannot be matched, then it discards the compound. This is the recommended method for use with mixture samples.
- **Cosine Similarity**. This method uses cosine similarity to compare the compounds' spectra with the sample spectrum, otherwise discarding compounds according to a threshold set by the user. Additionally, the user can enable Cross Correlation or Align Multiplet Regions Separately to improve the results in the presence of shift changes.
- **Pearson Correlation Coefficient**. This method uses the Pearson correlation coefficient to compare the compounds' spectra with the sample spectrum, discarding compounds according to a threshold set by the user.
- **13C Analysis***. This option uses Mnova Verify to compare the 13C spectra associated with the compounds with the 13C spectrum of the sample (if any). It enables an additional column in the compounds' library table. If no 13C spectrum is found for a compound, it will use the 13C prediction from the corresponding molecule instead. *(*Using this method requires valid licenses for Mnova NMR Predict and Mnova Verify plugins.)*

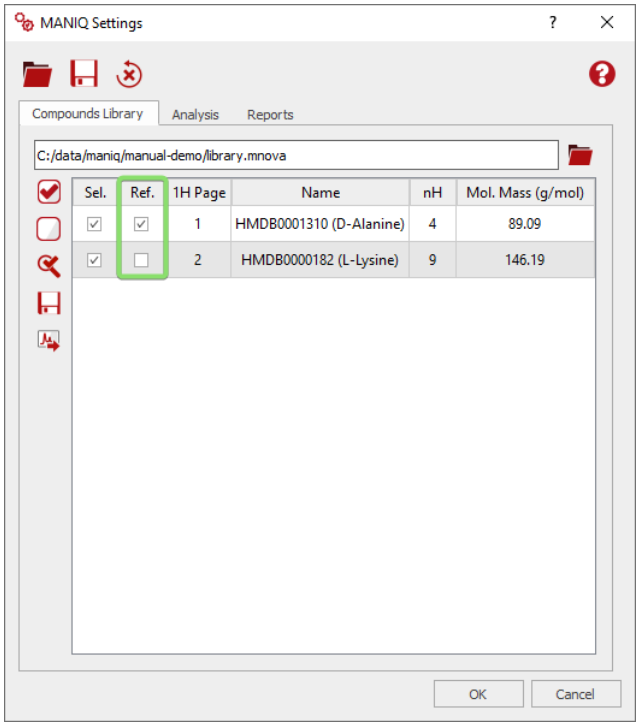
In the **Quantification** settings, there are two modes: **Default** and **Custom**. The first works in most situations, and uses the **f Factor Method**, an analysis algorithm internally developed by Mestrelab. The second allows the user to select which method they want to use, and in the case of the **Integral Transforms Method**, there are different settings to choose from. The relative concentrations reported in the results can be shown in g % or mol %.

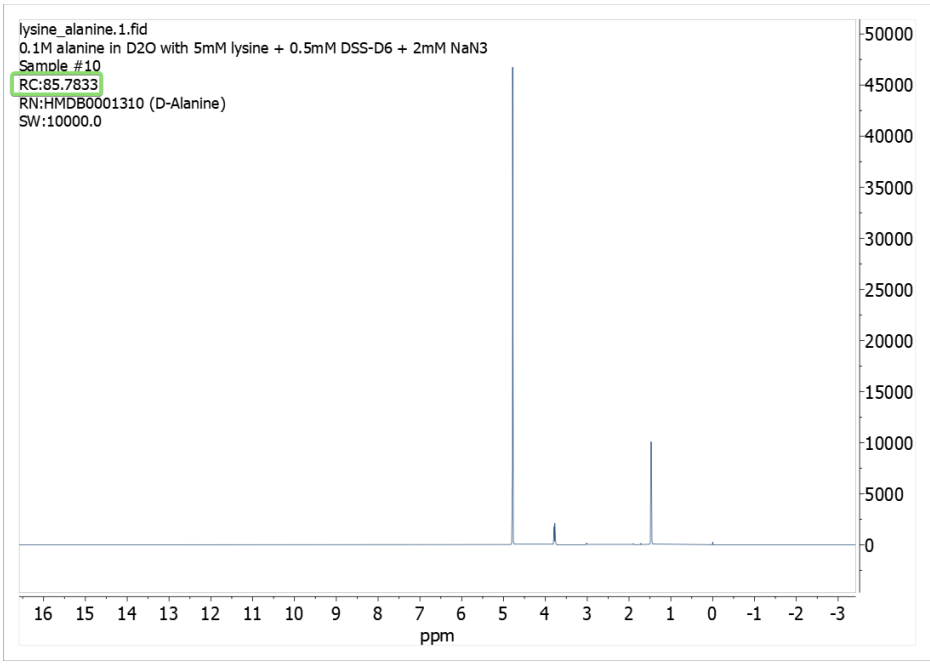


You can enable the **Absolute Quantification** —the units and the decimal places for the returned values can be configured— but some information needs to be provided for each analysis mode:

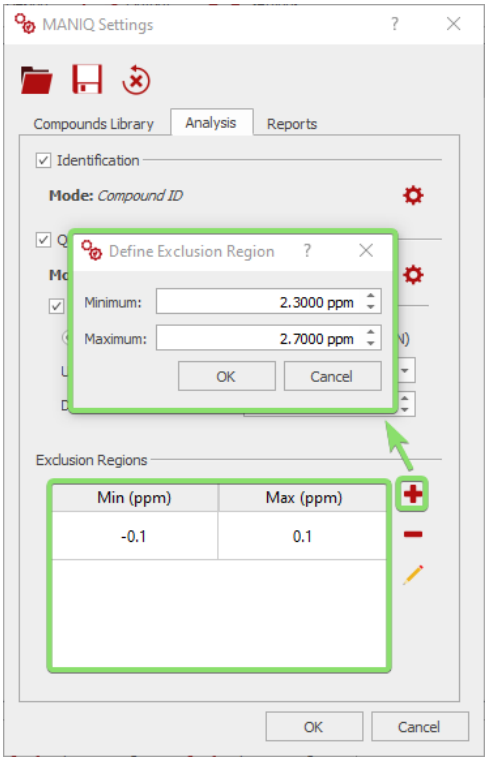
- In the **External Calibrant** mode, the "eretic" file provided by TopSpin must be available inside the sample spectrum directory.
- In the **Internal Calibrant** mode, a reference compound must be selected in the **Compounds Library** table (the **Reference** column is enabled once this mode is selected). Its actual concentration must be specified in the comment field of the sample spectrum, as shown below (this value must be in mmol/l).





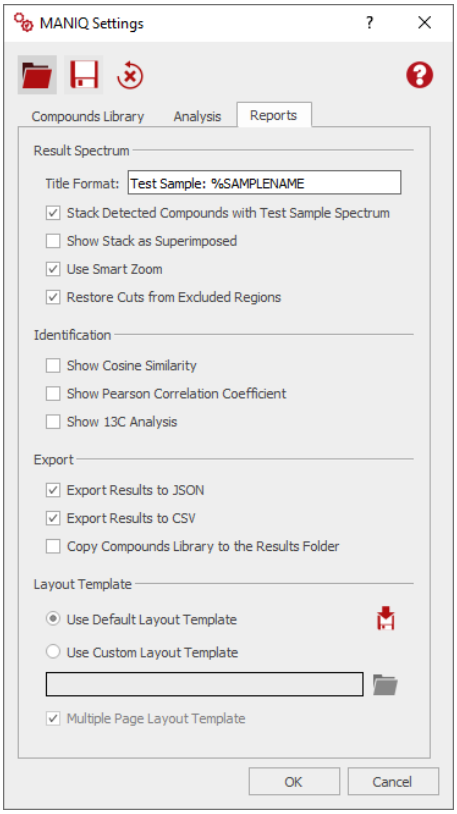


On the lower part of the **Analysis** tab, there is a section where **Exclusion Regions** can be added to the MANIQ analysis. As their name suggests, those regions will be completely excluded from the identification and quantification analyses.



2.2.3. The Reports tab

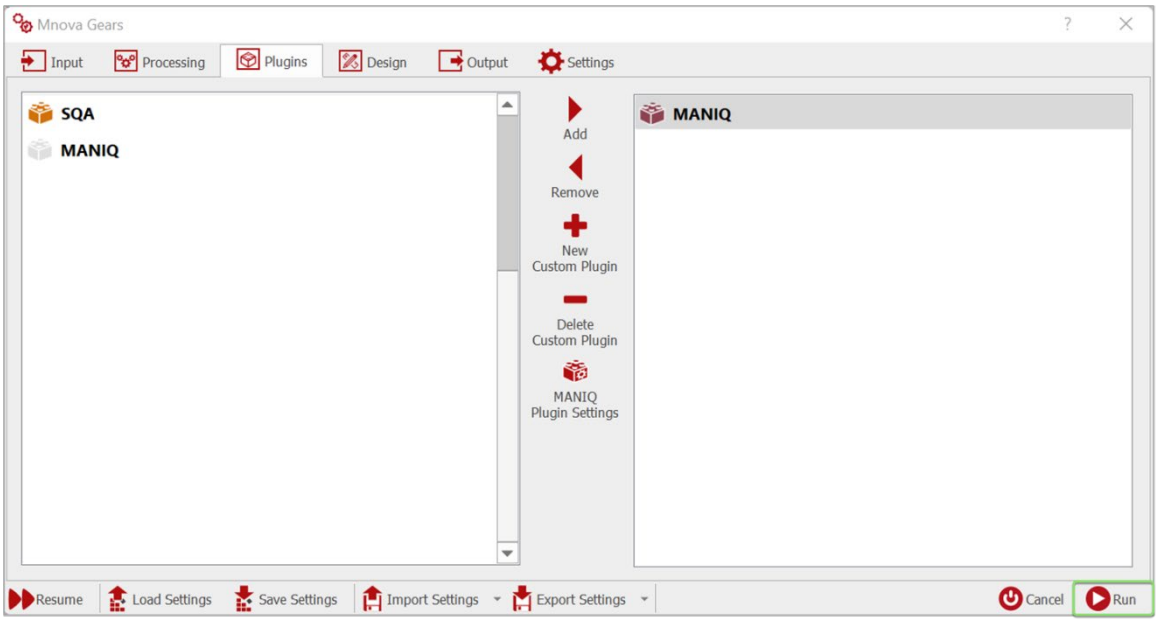
In the **Reports** tab you can find additional settings, all of which are self-explanatory. You can also export the default layout template file for using it as basis for your own one. More information about these settings is available in the corresponding tooltips.



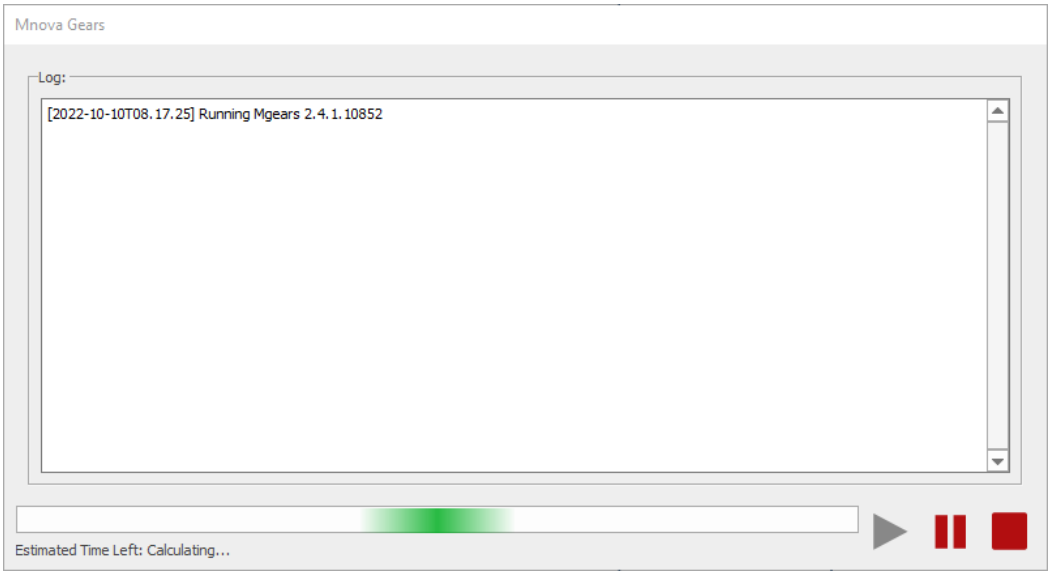
3. Running the MANIQ analysis

3.1. Running Mgears

After Mgears and MANIQ have both been set up correctly, you are now in position to run the analysis. To do this, just click on the **Run** button in the main Mgears dialog.

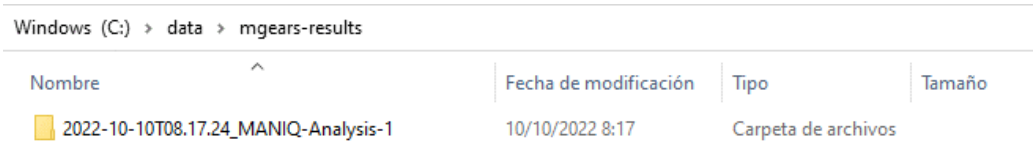


A new dialog will then be shown in which the user can watch the progress of the analysis file by file.

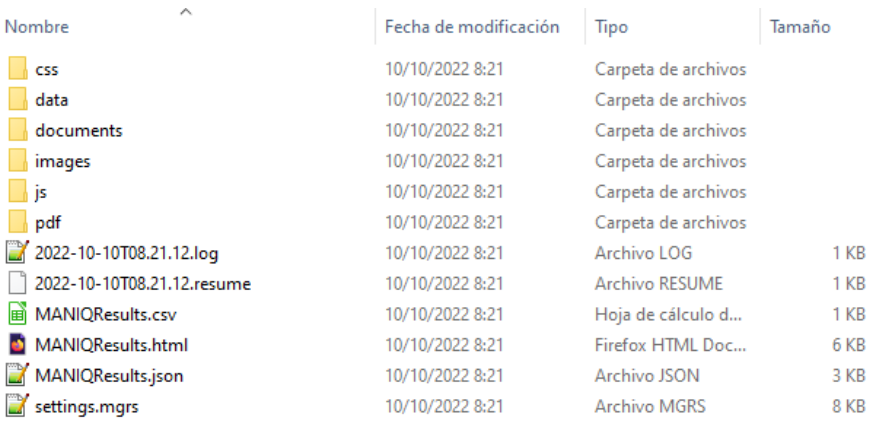


3.2. Checking the results

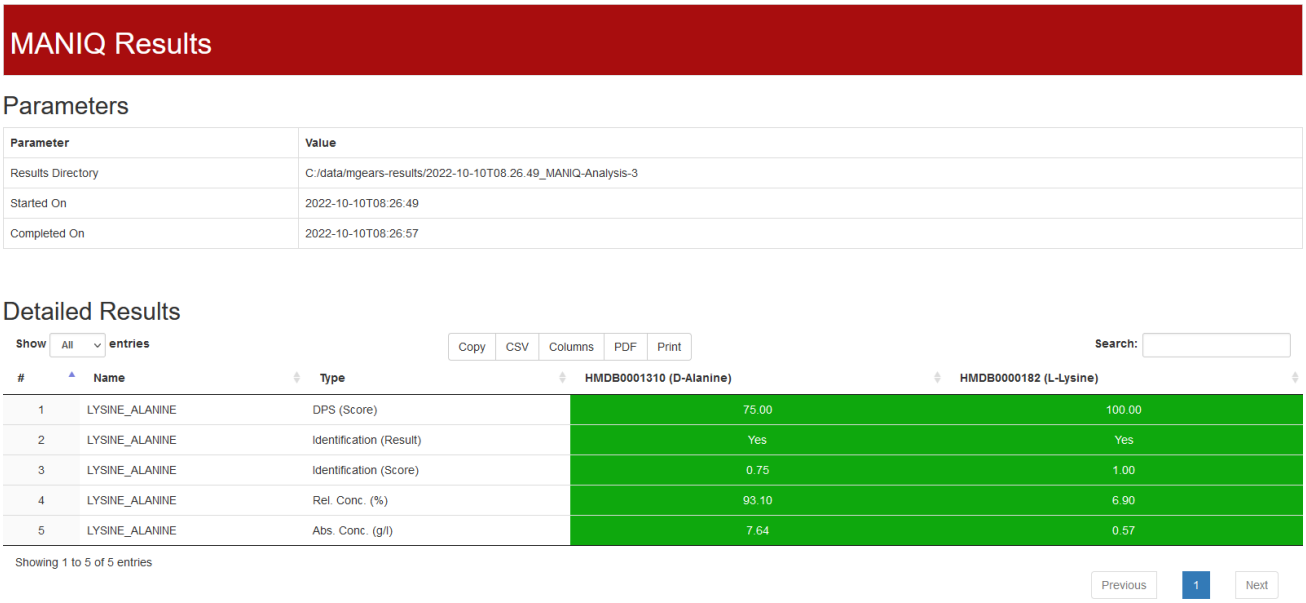
After Mgears has finished the analysis, a new folder will appear in the **Output** directory configured in the Mgears settings, named after the date and time of the analysis.



Inside this folder, there are Mnova documents and PDF files containing the analyzed mixture spectra (assuming the user checked the appropriate options in the **Output** tab). The actual results of the MANIQ analysis can be found in the MANIQResults.html file.

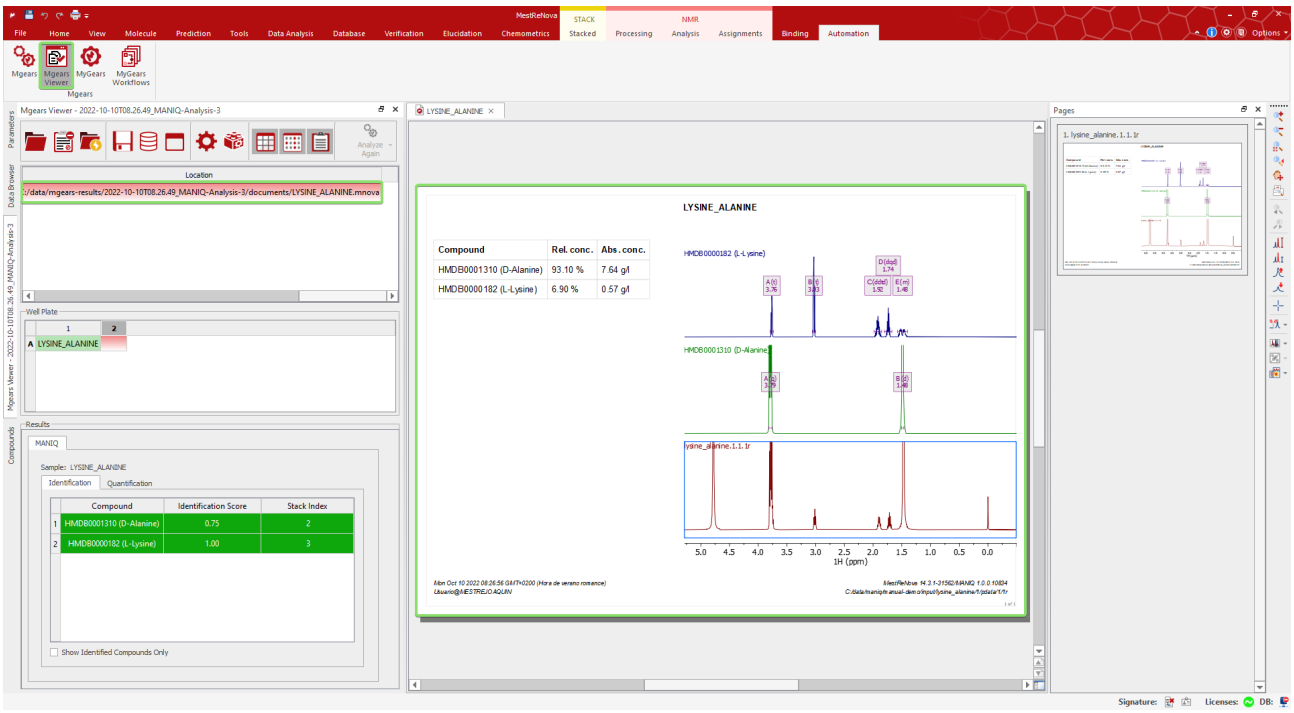


This HTML file includes a table. The rows represent the mixture files, and the columns represent the reference compounds in the **Compounds Library**. The results of the MANIQ analysis are shown within the table, where green indicates that the compound was detected in the mixture, and white otherwise. The values and results for each identification method used are shown, along with the relative quantification results. If you enable the **Absolute Quantification**, the absolute concentrations will also be shown here.



Alternatively, you can view the results from the **Mgears Viewer** tool that you can launch from the **Automation** tab in the Mnova ribbon. To add the results to the viewer, you must press the **Load Data** button, and choose the output folder.

The results will be loaded and can be reviewed on a per sample basis. The **Well Plate** will show each sample in green if at least one compound was identified, and in red otherwise. In the **Results** section, several tabs will show the different partial results for each sample: Identification, Quantification, Cosine Similarity, etc.



For more details on the Mnova Gears options, please refer to the Mnova Gears manual.