

MAQ-S USER GUIDE v2.1

Manual for use of the MAQ software

IFU510
v170791

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Chapter 1 Installation

MAQ-S can be downloaded from the Multiplicom website at [www.agilent.com/en/products/next-generation-sequencing/amplicon-target-amplification-\(multiplicom\)/maq-s](http://www.agilent.com/en/products/next-generation-sequencing/amplicon-target-amplification-(multiplicom)/maq-s). The application works right away after decompression, so you do not need to install anything. This should exclude any permission issues of the operating system, regarding the installation of software on your computer. MAQ-S works anywhere, so you can put the MAQ-S folder anywhere you like.

The MAQ-S folder also contains a subfolder named DemoFiles in which some sample (.fsa) files can be found together with their assay description file (.enc). Using these files, you can trial the program fully.

MAQ-S is a cross-platform application and thereby also available for Linux users.

Chapter 2 MAQ-S: An overview

2.1. Background

MAQ-S was developed to simplify the analysis of a new method called Multiplex Amplicon Quantification (MAQ).

MAQ is a multiplex PCR (mPCR)-based method for the detection and analysis of copy number variations (CNVs) in a genomic region or gene of interest. This method consists of the simultaneous PCR amplification of several fluorescently labeled target and control sequences. The comparison of normalized intensities between the test individual and reference individuals results in a dosage quotient indicating the copy number of the target amplicon.

The PCR primers of the MAQ assays are designed by proprietary software that allows the design of mPCR primer sets with a very high multiplexing degree (> 40). In this way, several genomic regions, genes or even exons of interest can be simultaneously analyzed. MAQ is highly comparable to Multiplex Ligation-dependent Probe Amplification (MLPA), with the important difference that the experimental setup consists of a single mPCR reaction followed by fluorescent fragment analysis, resulting in a considerable reduction of labour and time.

Note:

MAQ-S also supports the analysis of experimental data obtained with Multiplex Ligation-dependent Probe Amplification (MLPA).

2.2. The software

The application compares normalized peak areas between a test individual and reference individuals resulting in a dosage quotient indicating the copy number of the target amplicon. Data analysis is straightforward and starts with importing raw data files generated by the fragment analysis (.fsa files) together with the assay description file. Next, dosage quotients are automatically calculated and visualized. The interface itself is kept very simple and is thereby very user-friendly. Chromatogram data as well as dosage quotients can be graphically represented and exported.

The MAQ-S main window (Fig. 1a) serves as a platform to all its other features and consists of three parts:

- Buttons to open the other interesting windows (see bold text)
 - Import:** selection of the folder containing the raw data files and the assay description file
 - Save:** to save a project
 - ListBox:** selection of reads you want to view or set as reference
 - DosPlot:** graphs containing the calculated dosage quotients
 - Export:** exporting of your data/graphs
 - Settings:** mostly changing visual appearance of MAQ-S
 - '?' or Help:** enables/disables help through the use of text balloons (a text balloon will appear if you hold the mouse pointer over an object)
- One or more graphs showing the raw chromatogram data
- The 'comment bar' showing comments/help/notes from the application itself

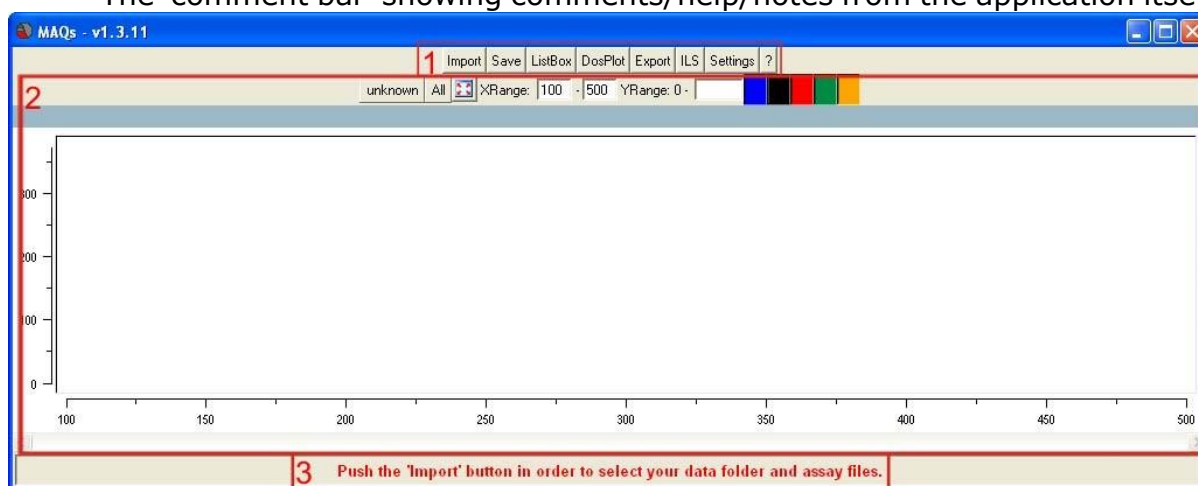


Fig. 1a: The different parts found in MAQ-S main window

Note:

MAQ-S holds a guide that will try to help you by giving hints of what you should do next. This guide is on by default, but it can be switched off in Settings.

Chapter 3 Starting a new project

To have a look at the dosage quotients, you will only need to do two things (if your settings are correct):

- Select the data folder.
- Select the correct assay description file.

!

If your settings are not optimal, MAQ-S will not be able to identify the correct control reads automatically. In this case, you will need to set these manually by selecting them and pushing the 'SetRef' button found in the ListBox.

Chapter 4 Import

4.1. Selecting the data folder and the assay description file

The import window (Fig. 4a) mainly serves as a platform to select the folder containing your raw data files and the assay description files. It also holds the key to start the demo version and the first step(s) for acquiring a license. Here we will focus on the importing of your data.

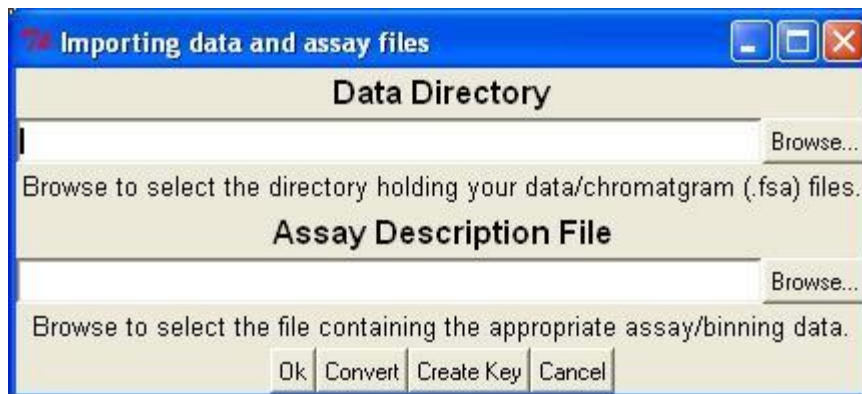


Fig. 4a: Importing data and assay files

The upper part of the window expects you to select the directory containing your raw data (.fsa files). MAQ-S will, by default, select all the reads found in this directory. You can deselect files in the MAQ-S application afterwards, but it's better to divide the different projects over different folders before you start.

In the middle part you select the appropriate assay description file (.enc file). This file holds the information of every amplicon used in the assay (e.g. color, length, type). Assay description files are created by us and come alongside the MAQ kit or can be made on request (for MLPA).

The lower part contains a number of buttons, of which only the "Ok" button is important (for now). The "Create Key" button is for licensing purposes and

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starting the demo version. The "Convert" button is for creating the assay files but only works if you have a valid account.

Although the assay files are created solely by us, the user is still able to change bin position and/or width.

4.2. Import/export projects

Opening previously saved projects can be performed by:

- Using the "Import" button, after which you browse the directory in which your project file(s) can be found.
- Double-clicking the project file (.mqs).

Saving a project is performed by clicking the "Save" button. A project file holds information about:

- The location of the data files.
- The location of the assay description file.
- The currently activated reads AND assay.
- The currently set reference reads.

!

At the moment, only the locations of data/assay files are kept. So, a project will only work if you do NOT move the assay and data files.

4.3. ABI import issues

Depending on the software you are using, the analysis of the internal lane standard is done automatically. This analysis results in a correlation between retention time and base length.

A correct representation of your chromatogram relies on this correlation. Unfortunately, the ABI software sometimes fails to give a correct correlation, usually with overloaded samples. This will result in chromatograms that are shifted.

In that case, you can let MAQ-S do the analysis:

- by clicking the "AnaILS" (Analyze Internal Lane Standard) button in the ListBox.
- by default, with the option "always overrule standard" in Settings (which is disabled by default).

It can also happen that one or more sizes of the internal lane standard are not found, resulting in one or more ranges with uncertain base lengths.

Example:

Suppose size 200 is missing in the GS500LIZ standard, which usually contains sizes "35 50 75 100 139 150 160 200 300 340 350 400 450 490 500." => All sizes between 160 and 300 are uncertain and are covered by a pink (by default) rectangle.

!

It's not recommended to let MAQ-S do the analysis of the internal lane standard by default. It can take a lot of time, and in most cases it's not necessary.

4.4. Unknown internal lane standard

In case the ABI software does NOT analyze the internal lane standard, MAQ-S will do this automatically for you. If, however, the internal lane standard used is unknown to MAQ-S, it will ask you to enter the sizes of every peak within this standard (Fig. 4b).



Fig. 4b: Picture of the window for adding new internal lane standards.

Fig. 4b:

- Displays the size standard that is unknown to MAQ-S.
- You can use another known internal lane standard as template, so you do not have to fill in all sizes yourself (click to select one).
- All sizes found within this internal lane standard:
The lengths associated with this new internal lane standard are saved, so you only have to do this once for every new internal lane standard.

!

Entering wrong sizes will give bad results, as amplicons will have incorrect lengths!

Chapter 5 ListBox

The ListBox window serves as an overview of your experiment as well as a platform to select and deselect reads and references.

5.1. De-Selecting reads and references

5.1.1. In-Activation of reads

MAQ-S frequently uses the term 'active read'. Active reads are, generally speaking, the reads of interest. Only those will be shown or can be browsed in the graphs. The first time you start with an assay, all reads will be active by default. Inactivation is performed by clicking on the checkboxes (Fig. 5a, rectangle 2) or the "Inactivate" or "Activate" buttons (Fig. 5a, rectangle 1). Both of these buttons will set the selected reads as inactive or active. Selecting reads can be performed using the SHIFT (subsequent selection) or CONTROL (individual selection) keys. The button "ActSel" (Active to Select) will select the reads that are currently active, which is very handy when you want to alter the current set of active reads without first selecting the ones that already are.

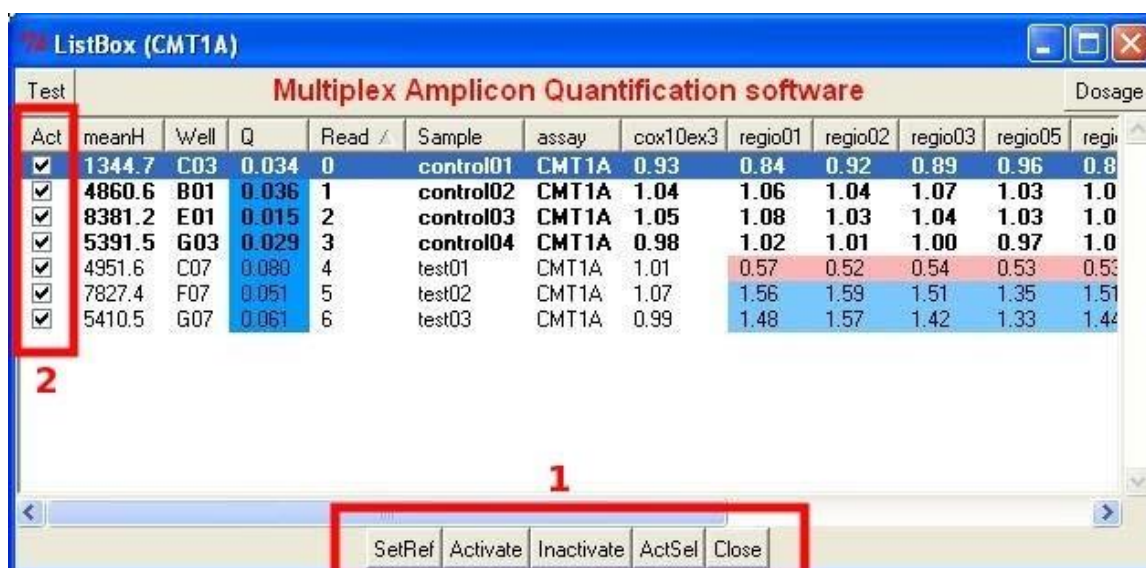


Fig. 5a: De-selecting reads and controls

Important:

All graphs are linked with one another. If you change the read in one window, the others will follow. This linking can be forced by clicking with the right mouse button on the "Read" button in the ListBox.

5.1.2. Setting the reference reads

The reference reads are extremely important, because they will determine whether you'll have a deletion, duplication or nothing at all. These references are automatically set after you load an experiment. This is done based upon a certain pattern in the file name. This pattern can be set or changed in Settings. If MAQ-S can't find any matching reference individuals, you can still set those manually by first selecting them, followed by pushing the "SetRef" button (Fig. 5a, rectangle 1). After the reference reads are known, dosage quotients can and will be calculated.

Notes:

- You should always use more than one reference.
- A good reference should always have at least one copy of every amplicon. Otherwise, you cannot have any clue about the copy number, because the dosage quotient can't be calculated.
- The reference reads set are listed in bold.

5.2. The data

The ListBox (Fig. 5b) holds useful information about the reads:

- **meanH** : average height of all the amplicons
- **standard** : ILS used by MAQ-S to analyze the raw data
- **ILSA**: tells you if the ILS was known to MAQ-Sv or unknown and selected by the user
- **Q**: a quality value of the control amplicons within the read, after setting the reference samples
- **sample** : name of the sample, extracted from the name of the raw data file name
- **assay** : assay used at the moment to analyze the data
- **DQ** : dosage quotients
- **file**: raw data file used to load into the program

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ListBox (CMT1A)

Multiplex Amplicon Quantification software

Test	meanH	Well	Q	Read	Sample	assay	cox10ex3	regio01	regio02	regio03	regio05	regio06
✓	1344.7	003	0.034	0	control01	CMT1A	0.93	0.84	0.92	0.89	0.96	0.84
✓	4860.6	001	0.036	1	control02	CMT1A	1.04	1.06	1.04	1.07	1.03	1.03
✓	8381.2	002	0.015	2	control03	CMT1A	1.05	1.08	1.03	1.04	1.03	1.03
✓	5391.5	003	0.029	3	control04	CMT1A	0.98	1.02	1.01	1.00	0.97	1.03
✓	4951.6	001	0.080	4	test01	CMT1A	1.01	0.57	0.52	0.54	0.53	0.53
✓	7827.4	002	0.051	5	test02	CMT1A	1.07	1.56	1.59	1.51	1.35	1.51
✓	5410.5	003	0.061	6	test03	CMT1A	0.99	1.48	1.57	1.42	1.33	1.44

1 2 3

SetRef Activate Inactivate ActSel Close

Fig. 5b: Browsing the data

5.2.1. MeanH

MeanH holds the average height of every amplicon within the read. Reads with low average heights (less than 500) tend to have varying ratios and are thereby instable, so beware if you want to assess these results.

5.2.2. Q

Q stands for quality and is actually the stability of the control amplicons within the read. It's the mean of the standard deviation of the control amplicons. This means, **the lower the better**. The better the quality, the higher chance the target amplicons will give a good impression of the dosage.

5.2.3. DQ

The dosage quotients are the CNV for each amplicon. To ease the assessment, these DQs are colored. Colors and their respective ranges can be changed and verified in Settings.

5.3. There is more...

Figure 5c:

- Through the use of this drop-down button, you can select the amplicons for which you want to see the results.
- Sorting can be performed by clicking on the column header.
- This drop-down button provides the possibility of switching among height, area and dosage quotient values.

ListBox (CMT1A)

Test **1** Multiplex Amplicon Quantification software **3** Dosage

Act	meanH	Well	Q	Read	Sample	assay	cox10ex3	regio01	regio02	regio03	regio05	regio06
<input checked="" type="checkbox"/>	1344.7	C03	0.034	0	control01	CMT1A	0.93	0.84	0.92	0.89	0.96	0.84
<input checked="" type="checkbox"/>	4860.6	B01	0.036	1	control02	CMT1A	1.04	1.06	1.04	1.07	1.03	1.03
<input checked="" type="checkbox"/>	8381.2	E01	0.015	2	control03	CMT1A	1.05	1.08	1.03	1.04	1.03	1.03
<input checked="" type="checkbox"/>	5391.5	G03	0.029	3	control04	CMT1A	0.98	1.02	1.01	1.00	0.97	1.03
<input checked="" type="checkbox"/>	4951.6	C07	0.080	4	test01	CMT1A	1.01	0.57	0.52	0.54	0.53	0.53
<input checked="" type="checkbox"/>	7827.4	F07	0.051	5	test02	CMT1A	1.07	1.56	1.59	1.51	1.35	1.51
<input checked="" type="checkbox"/>	5410.5	G07	0.061	6	test03	CMT1A	0.99	1.48	1.57	1.42	1.33	1.44

SetRef Activate Inactivate ActSel Close

Fig. 5c: Changing content

Chapter 6 The chromatogram graph

6.1. Content

This region of the main window (Fig. 6a) holds and shows the:

- Raw chromatogram data (dark blue line)
- Label of the read (test01)
- Bins of the target amplicons (yellow area)
- Bins of the control amplicons (grey area)
- Areas (light blue) used for calculating the dosage quotient
- Area (pink) with fixed height for getting a quick and general idea about the intensity (without having to look at the Y-axis)
- Reference chromatogram (grey line), which can be put on every read after the references are set. This chromatogram represents the mean of all references currently set and can be shifted or hidden in Settings.

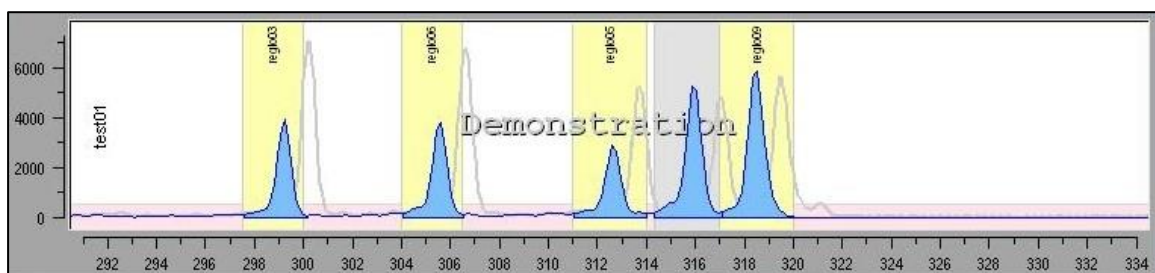


Fig. 6a: Peak areas of the test individual in blue, and the average reference peaks in grey

Note:

- Many properties (e.g. color and font) of the objects shown in the chromatogram can be altered using Settings.
- Ranges that are not fully covered by the internal lane standard are shown in the same color as the 'low height marker.'

6.2. Changing bin width or position

If you are unhappy about the position or the width of a certain bin (because the peaks are somewhat shifted, or the background is high), you can very easily move or resize the bin. To enter the editing state, just double click on the bin you want to change. By default, MAQ-S will zoom into the region so you can move with more precision (this can be turned off in Settings). To move the bin, click and drag with the left mouse button. To resize the bin, click and drag with the right mouse button. To leave the editing state, just double click. By default, MAQ-S will zoom out again.

After the bins are in place, you can:

- re-analyze your data by re-selecting the assay and/or
- export the assay for future use.

6.3. Other functions

In addition to just looking at the default range, you can also (Fig. 6b):

- Select another assay (if the assay description file contains more than one).
- Quickly zoom into a certain region (default width can be adjusted in Settings).
- Change grid layout (number of rows and/or columns).
- Change the X (base length) and Y (peak intensities) axis.
- View the intensities of other dyes.

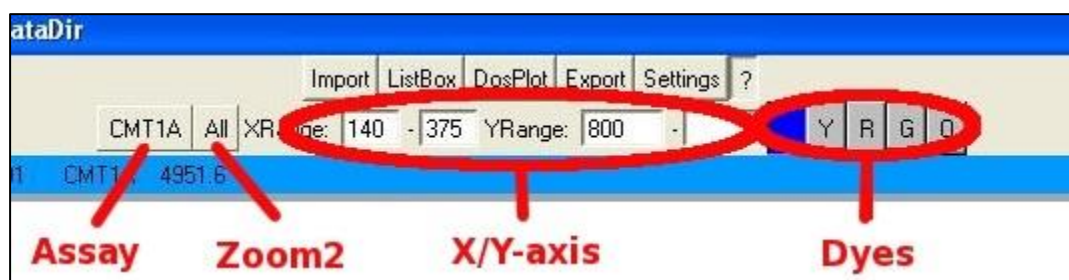


Fig. 6b : The different chromatogram functions

Note:

- All graphs are linked with one another. If you change the read in one window, the others will follow. This linking can be forced by clicking with the right mouse button on the chromatogram graph.
- You can use bin editing (double clicking) to quickly zoom into a region around the bin. To zoom out, just double click outside the bin.

Chapter 7 DosPlot

The DosPlot is a graph showing one or more dosage quotients (only the activated reads are shown).

7.1. What's on the DosPlot

Figure 7a:

- Holds the reads [label](#)
- Shows how many (if any) [controls are set](#) at the moment
- One data value (its intensity resembles the [mean height](#); the more dark the better)
- The type of amplicon:
 - gray: good, stable control amplicon
 - striped gray: bad, unstable control amplicon
 - white: target amplicon (no dosage change)
 - colored: target amplicon (dosage change)
- General information of the current read (Q, label, assay, meanH)
- One data value and its standard deviation error flag

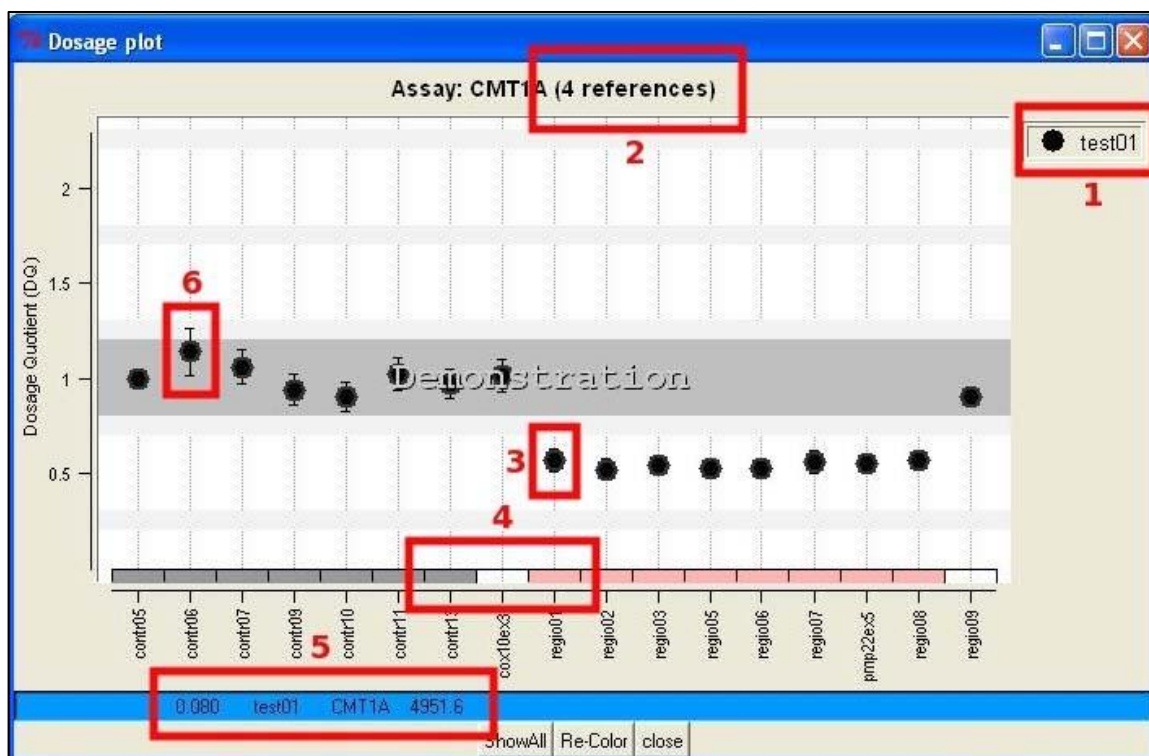


Fig. 7a : The DosPlot

The "ShowAll" button will put all currently active reads on the graph. By default, the intensity (grey scale) of the data points will reflect the mean height of the amplicons within the read. If "ShowAll" is pressed, the data points belonging to a certain read will have the same color. This coloring is done at random. If you are unhappy with this choice of colors, you can change those again (and again) by pressing the "Re-Color" button.

Note:

- Left clicking a data point will show/hide the data point's value.
- Right clicking will force the other windows to focus on the same read.

Chapter 8 Export

You can select one of following types:

- current assay (txt): exports all numerical data to a tab-delimited text file that can be opened in any spreadsheet
- current assay (html): exports the dosage quotients together with all the chromatogram and dosage plots
- current chromatogram: only exports the current chromatogram (the way it looks at that moment)

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- current DosPlot: only exports the current DosPlot (the way it looks at that moment)
- assay description file: exports the current (changed) assay to a file

Note:

- It's imperative that you know that if you export a graph, it will export it the way it looks at THAT moment (e.g. colors and size). Altering the look is done by changing the Settings.
- Only the first chromatogram graph will be exported, even if you have more than one chromatogram graph in the main window.

Chapter 9 Settings

The Settings window is the interface for altering many settings. Most settings change the look of the application, which makes for nice pictures if you want to use those in a presentation or document.

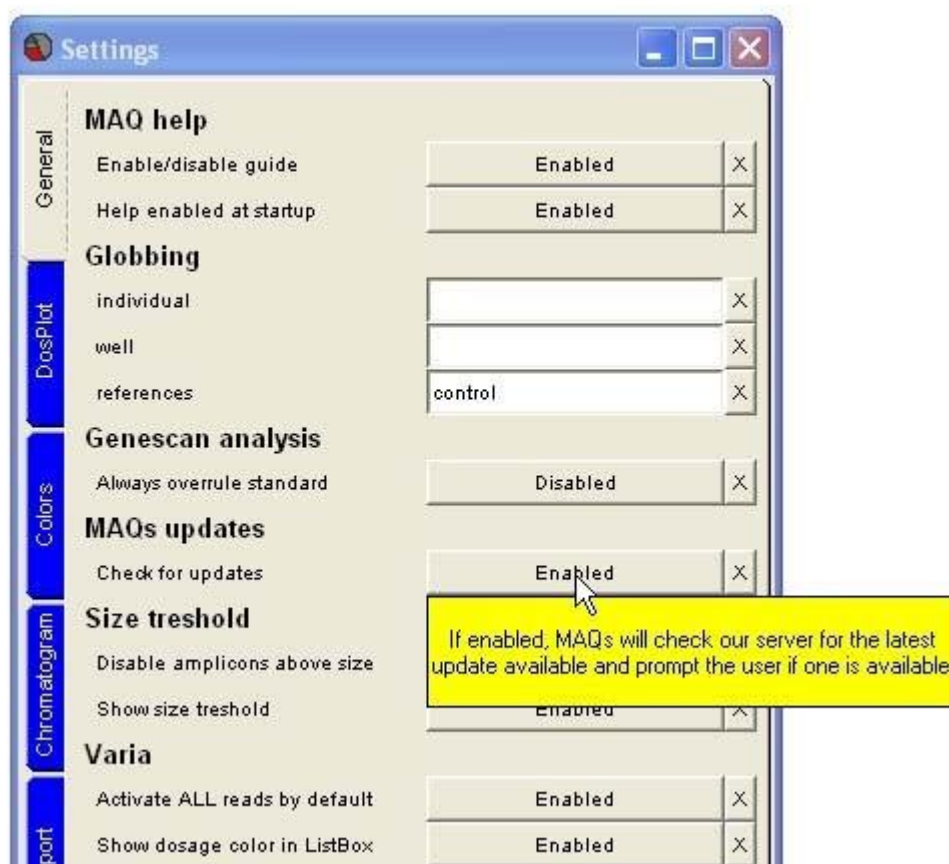


Fig. 9a : Part of the Settings window

Important:

If you have questions about a certain setting, or you are not sure what it does, turn on the help feature and move your mouse over the setting. Most likely, it will give a brief description.

Chapter 10 License

MAQ-S is license-free for Multiplicom customers.

Note:

- The License File is created based upon the Key.bin file and thereby only valid for the computer the Key.bin file was created on.
- MAQ assay description files will be handed over together with the kits.
- MLPA assay description files can be obtained after contacting us.

Chapter 11 Demo

You can also trial the application in its full functionality using a limited set of data files. This limited set of data files are files that are delivered with the package and can be found in the DemoFiles folder. Before you can start the MAQ-S demo, you first must create a 'Key.bin' file by pushing the "Create Key" button found in the Import window. After this, the application will ask you for a License key, which you probably will not have. You can ignore this comment if you want to analyze the demo files. Without a valid License File, however, no other data files besides these demo files can be analyzed.

Note:

- While using the Demo, all graphs will display the Demonstration tag. This tag will be removed after a valid license has been obtained.

Chapter 12 More help

12.1. MAQ-S Help features

MAQ-S holds 2 types of help: the guide and help balloons.

- The guide will try to determine what you should do next, and it will notify you through flashing objects and comments in the Comment Bar.
- The help balloons will show more information (if available) about the object currently under the mouse pointer.

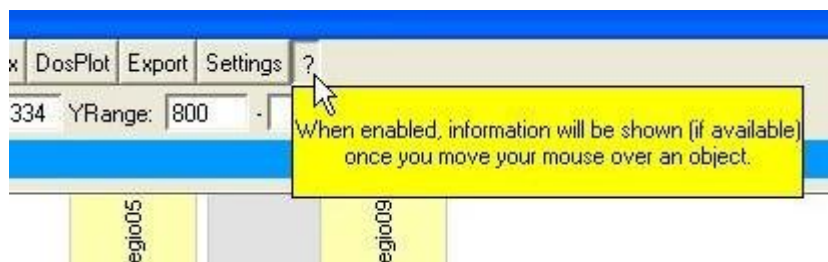


Fig. 12a: Help through the use of text balloons

Note: Both types of help can be disabled in Settings.

12.2. Globbing

By default, MAQ-S will take the well and label values from within the fsa files. If you are not happy with these data values (for whatever reason), you can use the globbing feature. This feature uses a given pattern and tries to match it on the fsa file names. Thus, by changing the file name and having the correct pattern, you can have any well and label value shown in MAQ-S. The globbing is done using the regular expression syntax of Tcl/Tk.

Examples for file '1234_sample-1_bla.fsa':

```

_([^-]+) => sample
_([^.]+)_ => sample-1
([^_]+) => 1234
_[^_]+_([^.]+) => bla

```

12.3. Dosage Quotient

Dosage quotients are calculated using control amplicons and control reads or individuals.

- The control amplicons are used for calculating the different ratios for a certain target amplicon. In other words, every target amplicon is compared to a set of control amplicons resulting in a set of ratios for that target amplicon.
- To determine a dosage quotient, these sets of ratios are then compared to the ratios found in one or more control reads. This results in a set of dosage quotients for every target amplicon. This set of dosages will give rise to (Fig. 12b) one data value (the mean) and a standard deviation (showing its stability).

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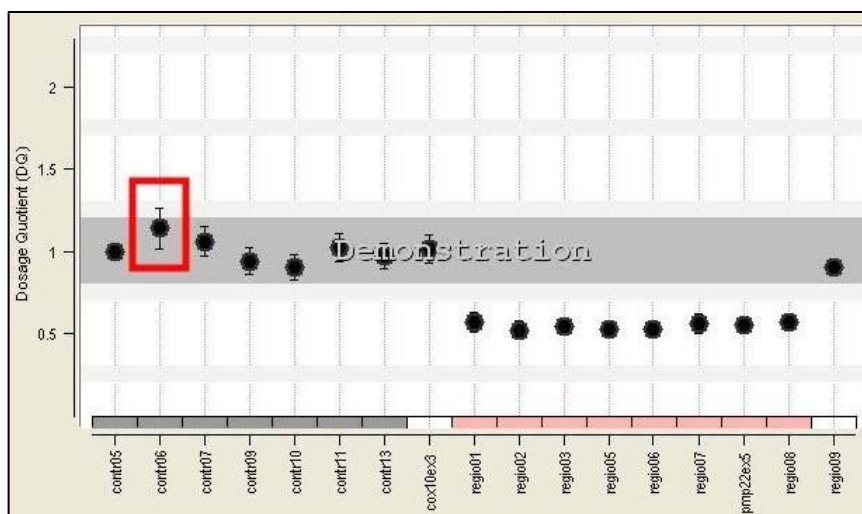


Fig. 12b: The DosPlot showing one data point with its standard deviation

Note:

- Using more than one control amplicon gives more reliable ratios.
- Not every control amplicon is used to calculate the ratios for a particular target amplicon. The control amplicons used are shown in the DosPlot when holding the mouse over the amplicon (Fig. 12c).

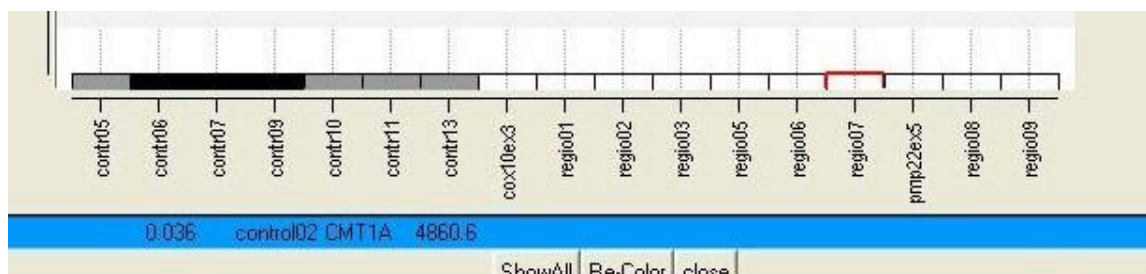


Fig. 12c: Holding the mouse over the regio7 box will highlight the control amplicons (in black) used for calculating the ratios of this target amplicon.

Settings also give you the chance to change the type of data that should be used for calculating dosages. The types you can choose are peak height or peak area (area is selected by default).

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