Rotor-Gene Q Manual

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Rotor-Gene Q Manual

CREATE THE TEMPLATE

Before starting the run, create a template. To perform further analysis of results with the Genvinset® Report Viewer software, it is important to correctly create the template before the run.

The steps to create the template are the following:

1. Click on *File>New*. On the *New Run* window select the option *Empty Run*, which allows to create a new template from scratch. Then click on *New*.

2. Next, select the rotor and check the *Locking ring attached* box. Click on *Next*.

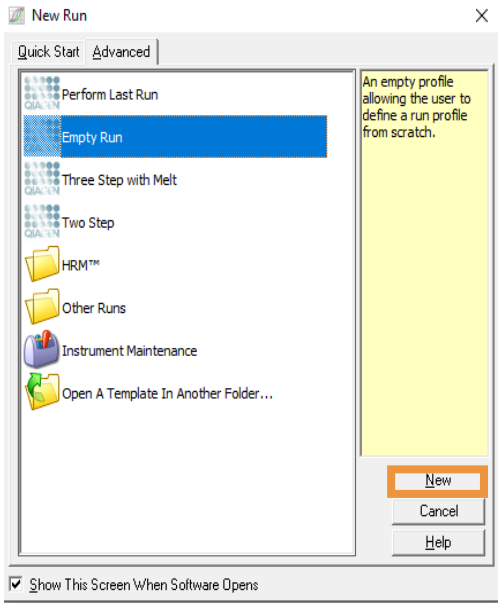

***Figure 1**: Open a template*

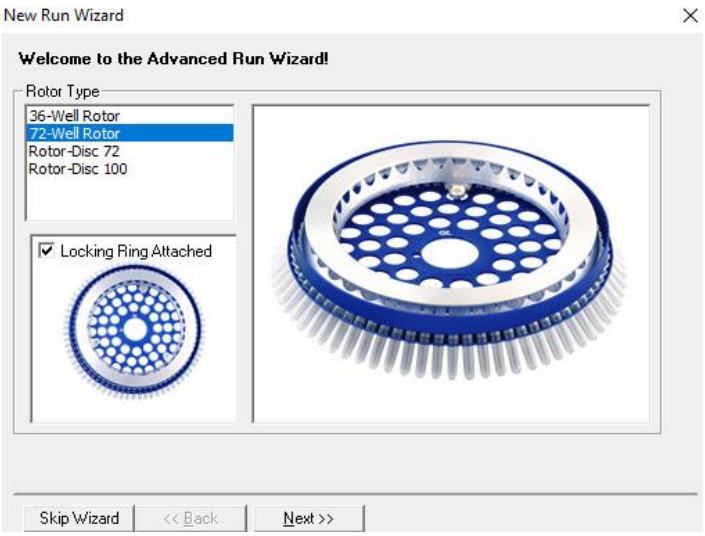

***Figure 2**: Type of rotor*

3. Enter the Operator and Reaction Volume information in the *New Run Wizard* window.

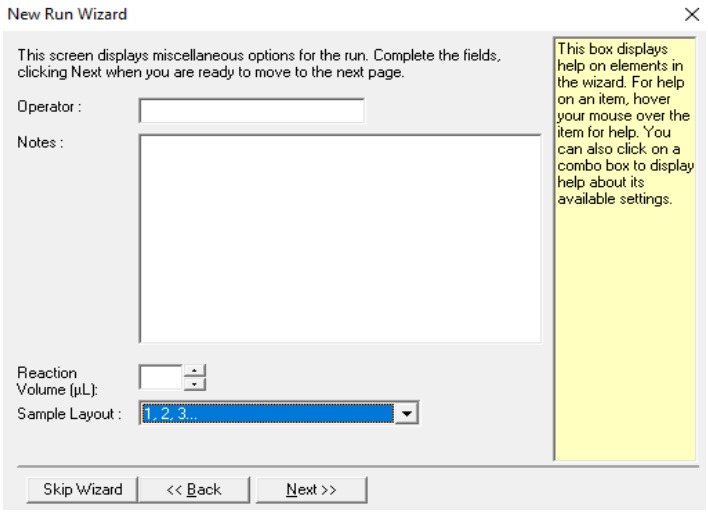

***Figure 3**: New Run Wizard*

4. In the *Channel Setup* option within the *Run New Wizard* window, click on the *Create New* button, as shown in Figure 4. In the *Edit Channel* window, set up the specific channels, naming the genes and setting the Source and Detector as specified in Figure 5 and the table below. Set *Gain* at 5.

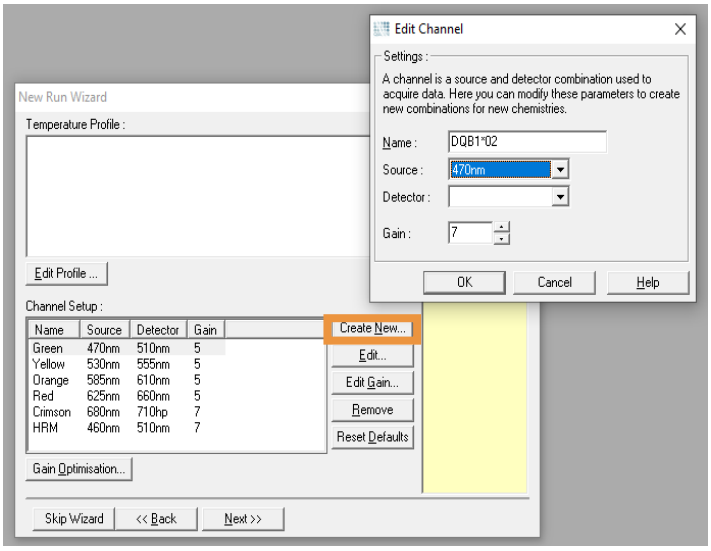

***Figure 4**: Channels setup in the Celiac and Celiac Plus kit*

Kit	Exact spelling		Kit	Exact spelling	
	HEX/VIC channel	FAM channel		HEX/VIC channel	FAM channel
HLA B27	B-GLOBIN	B*27	HFE C282Y	C282Y_wt	C282Y_mut
HLA B5701	B-GLOBIN	B*57:01	Factor II	FII_wt	FII_mut
HLA A29	B-GLOBIN	A*29	Factor V	FV_wt	FV_mut
HLA Narcolepsy	B-GLOBIN	DQB1*06:02	MTHFR C677T	MTHFR_wt	MTHFR_mut
HLA BEHÇET	B-GLOBIN	B*51/52	MTHFR A1298C	A1298C_wt	A1298C_mut
HLA CELIAC (PM1)	NODQB1*02	DQB1*02	PAI-1 4G/5G	PAI_wt	PAI_mut
HLA CELIAC (PM2)	B-GLOBIN	DQA1*05	Lactose Intolerance (C13910T)	C13910T_wt	C13910T_mut
HLA CELIAC (PM3)	B-GLOBIN	DQB1*03:02	Lactose Intolerance (G22018A)	G22018A_wt	G22018A_mut
HLA CELIAC (PM4)	B-GLOBIN	DQA1*03	HLA Diabetes Mellitus (PM1)	B-GLOBIN	DRB1*03
HLA C06	B-ACTIN	C*06	HLA Diabetes Mellitus (PM2)	B-GLOBIN	DQB1*02:01
HFE H63D	H63D_wt	H63D_mut	HLA Diabetes Mellitus (PM3)	B-GLOBIN	DRB1*04
HFE S65C	S65C_wt	S65C_mut	HLA Diabetes Mellitus (PM4)	B-GLOBIN	DQB1*03:02
deltaF508	deltaF508_wt	deltaF508_mut			

***Figure 5**: Exact gene spelling*

	FAM	HEX
Source	470 nm	530 nm
Detector	510 nm	555 nm

IMPORTANT: To perform further analysis with the Genvinset® Report Viewer software, genes must be named exactly as it is specified in the software manual (Figure 5).

5. Edit the run profile. Click on *Edit Profile*. Select *Cycling (default)*, as shown in Figure 6. Next, set up the amplification program.

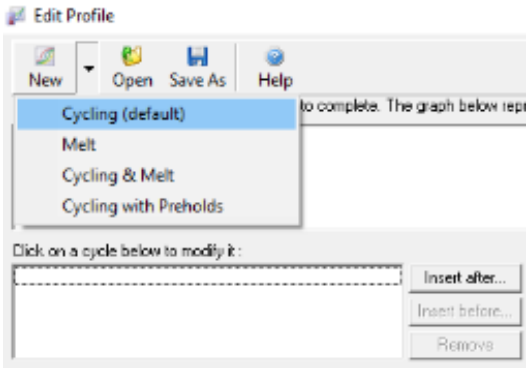

***Figure 6**: Edit Profile*

Select *Cycling*, select the last step and click on *Not Acquiring*, as shown in Figure 7. Then, in the pop-up window that appears, set the acquiring channels accordingly to the kit being used, as shown in Figure 8.

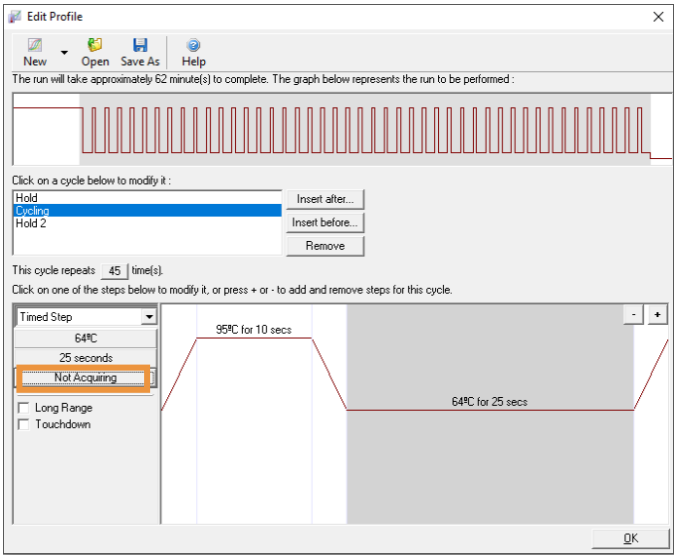

***Figure 7**: Channel Acquisition*

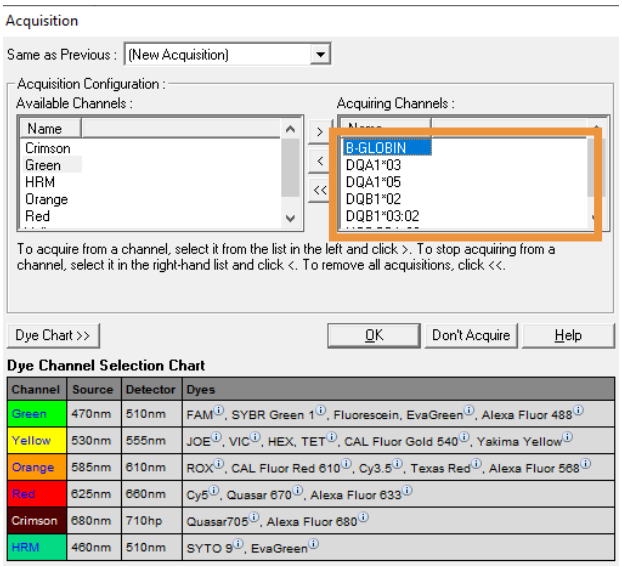

***Figure 8**: Acquiring Channels*

6. Next, click on *OK*, *Next* and *Save Template.*

SET UP THE EXPERIMENT

Start the software and click on *New*. Then, click on *Open a Template In Another Folder*, as shown in Figure 9.

The New Run Wizard window appears. Click *Next,* checking that all the parameters are correctly set, and finally click on *Start Run*. Save the experiment and another *New Run Wizard* window appears to define the setup of the samples.

For multi-reaction kits, samples for each reaction must be selected. For example, if 3 samples were to be analysed, (Sample 1, 2 and 3), the setup should have to be made in the following manner:

- Create a page for each reaction, naming them accordingly (in figure 10: Rx1, Rx2).
- In each one of the pages, determine the position of the samples according to the well they have been loaded in and choose *Yes* on the *Selected* column. In the rest of the positions, choose *No*.

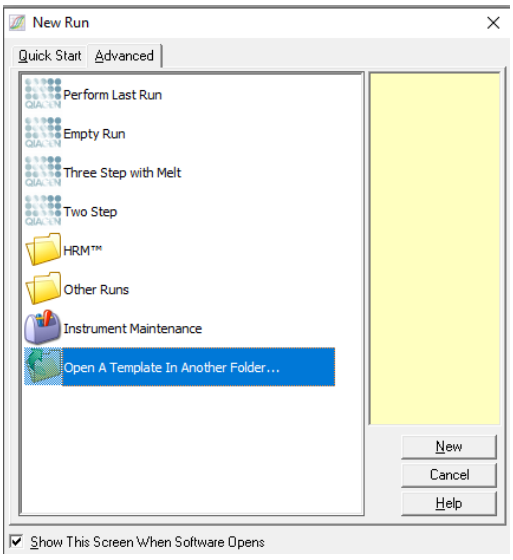

***Figure 9:** Open A Template In Another folder*

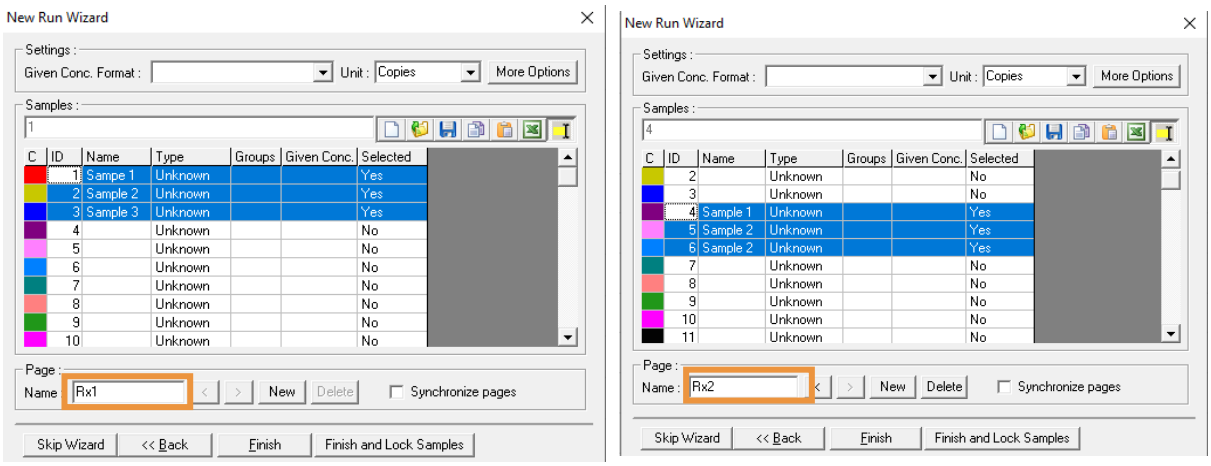

Figure 10: *Samples setup*

Click on *Finish* and the amplification starts.

RESULTS ANALYSIS

Once the run is finished, visualize the amplification curves in linear scale to check the correct shape of the amplification curves:

- An amplification curve is considered positive if a quick and regular (exponential) increase of fluorescence values is observed (sigmoidal amplification) with Ct<35.
- A weak fluorescent or background lineal or exponential signal with Ct>35 should not be considered a positive amplification.

The Ct value is the cycle number at the point where the amplification curve crosses a threshold of detection. By setting a threshold line and calculating the intersection with each of the curves, the Ct value for each sample is established.

When setting a threshold manually, it should be set in the exponential phase of the run, **significantly above the background level** to avoid noise and below the onset of the plateau phase in later cycles.

AMPLIFICATION CURVES ANALYSIS

To show the amplification curves, follow the next steps:

1. On the main upper-left toolbar, click the *Analysis* button.
2. Select the *Quantitation* tab.
3. Select the channel to be analysed.
4. Click *Show*. The *Quantitation Analysis* window appears.

Channels that have already been analyzed have a green checkmark next to them. This means that threshold and normalization settings have been saved for this analysis.

Note that for multi-reaction kits, the channels should be selected accordingly to the kit being used, as shown in Figure 12.

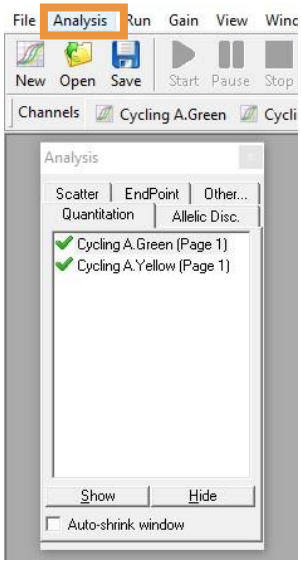

Figure 11*: Analysis window*

***Figure 12**: Channels involved in each reaction*

PLOT CONFIGURATION

In the Quantitation Analysis window of each channel, follow the guidelines below to configure the amplification plot.

- On the bottom toolbar, deselect *Log. Scale* to visualize the amplification curves in linear scale.

- On the upper toolbar, several settings may help in the results visualization and interpretation. These settings should be validated for each assay/reaction:

  Dynamic tube. Leave this option selected. It determines the average background of each sample before amplification commences. Note that for some data sets, background fluorescence is not consistent during the cycles before amplification begins, and in these cases, it may be necessary to deselect dynamic tube normalization.

  ***Slope Correct**:* Select this option when the background fluorescence shows a gradual increase or decrease over several cycles. Noise slope correction improves the data when raw data backgrounds are observed to slope upward or downward before the take-off point (Ct).

  ***NTC Threshold**:* Select this option by clicking on the *Outlier Removal* button. Setting the NTC threshold to 5-15% is recommended to correct or exclude non-specific signals This option is suggested for most applications although it should be validated.

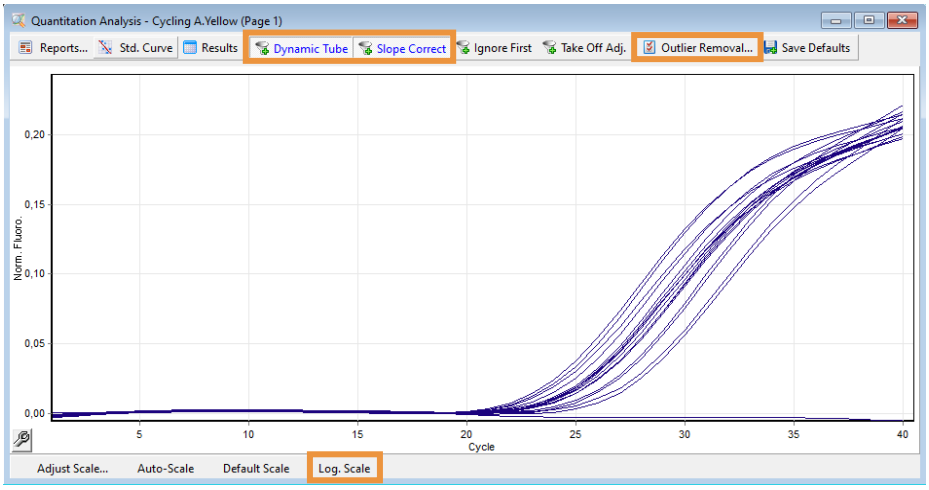

***Figure 13**: Results analysis options.*

SET THE THRESHOLD

Adjust the threshold line above the background signal, so that it crosses close to the inflexion point of the amplification curves. The threshold line should **slightly exceed the value of the highest fluorescence obtained with negative samples** for the allele detected in this channel.

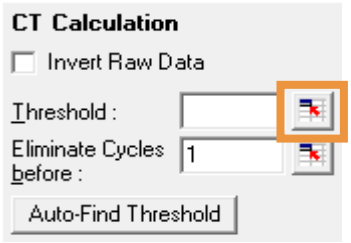

***Figure 14**: How to display the threshold.*

To show the threshold line, follow the next steps:

1. Click on the graph of one channel.
2. In the bottom right of the main window, the "*CT Calculation*" options are displayed.
3. Click on the icon that appears beside the *Threshold* option (Figure 14).
4. Click anywhere on the respective channel graph, and the threshold line appears. It can be moved up or down until finding the right position for each experiment.
5. Repeat the process for the other channel.

The Ct values based on the adjusted threshold appear in the *Quant. Results* window for each sample. This window is opened by default, but if it has been closed it can be reopened by clicking *Results* on the upper toolbar of the channel graph.

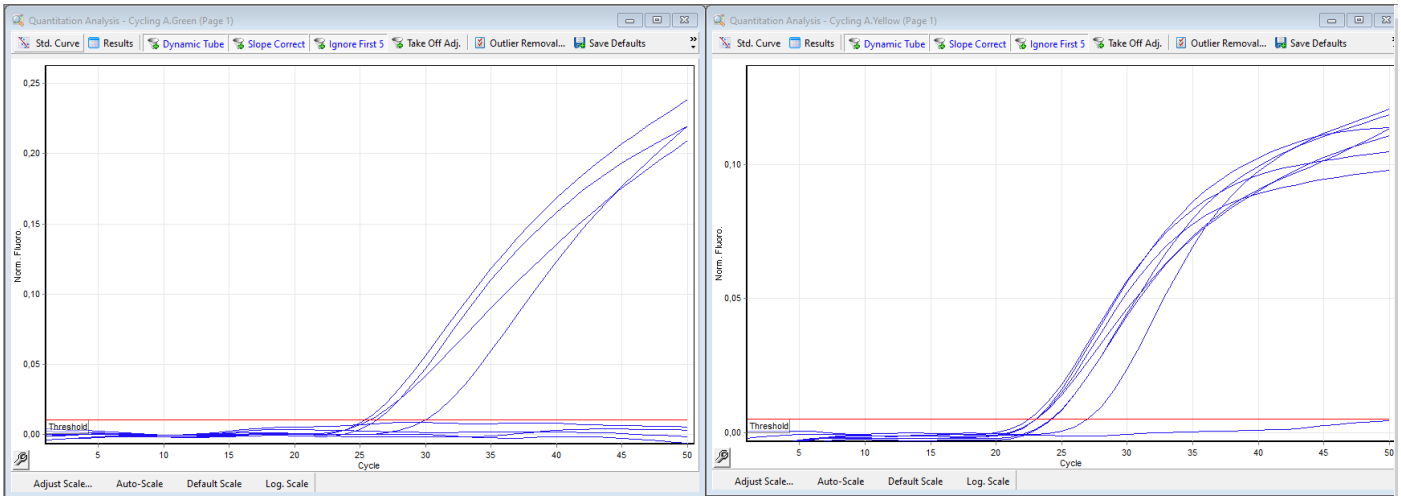

***Figure 15**: Amplification plots showing the threshold line in channels FAM and HEX.*

ALLELIC DISCRIMINATION (SCATTER PLOT)

IMPORTANT: Genvinset® Report Viewer software is only able to analyse experiments interpreted by amplification curves in genotyping experiments.

SCATTER PLOT

To show the Scatter Analysis plot, click on the *Analysis* button and select *Other* > *Scatter Analysis*. To perform this analysis, either hold down CTRL and click to highlight each channel you wish to analyze or drag the mouse pointer over these channels. Once the desired channels have been highlighted, click *Show*.

Mind the settings indicated in the *Plot Configuration* section to adjust the results visualization and interpretation.

To perform genotyping, define regions by clicking and dragging a selection on the graph. The selection can then be labelled based on the genotypes configured in the *Genotypes* window. Wild-type samples are detected in the HEX channel ("*name*"_wt / Cycling A. Yellow) and mutant samples in the FAM channel ("*name*"_mut / Cycling A. Green).

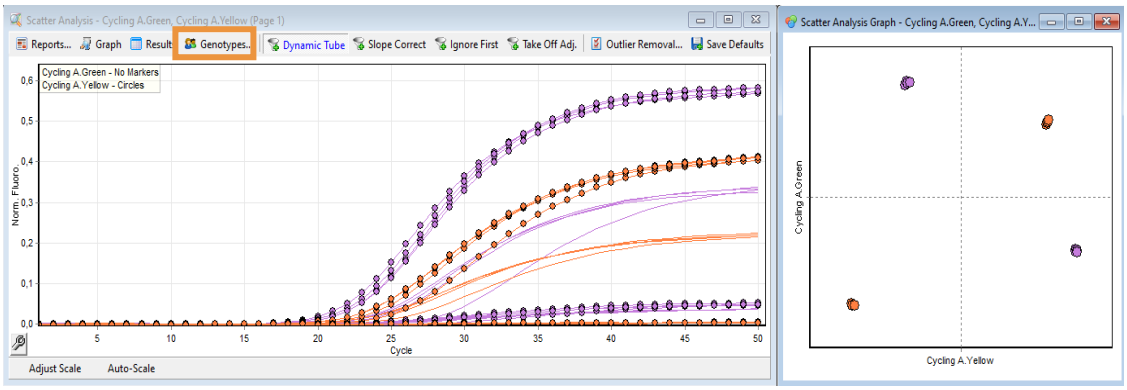

***Figure 16**: Scatter plot analysis.*

ALLELIC DISCRIMINATION PLOT

To show the Allelic discrimination plot, click on the *Analysis* button and select *Other* > *Allelic Discrimination*. To perform this analysis, either hold down CTRL and click to highlight each channel you wish to analyze or drag the mouse pointer over these channels. Once the desired channels have been highlighted, click *Show*.

Mind the settings indicated in the *Plot Configuration* section to adjust the results visualization and interpretation.

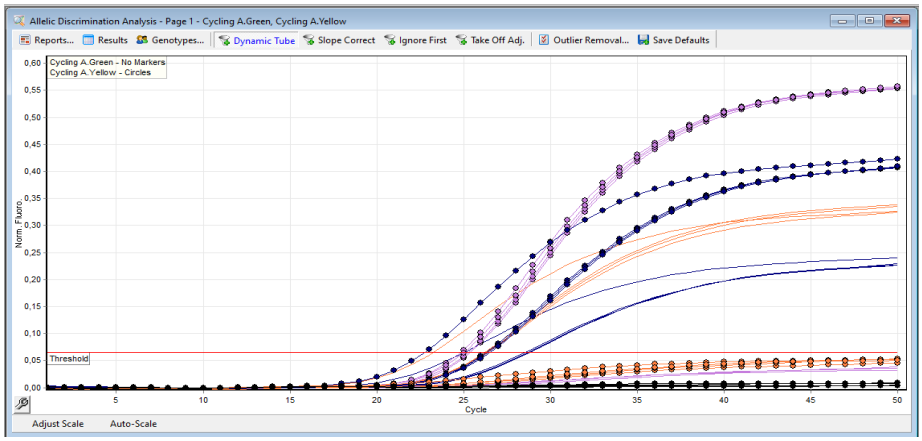

***Figure 17**: Allelic discrimination plot.*

Define which genotype is detected in each channel in the *Genotypes* window:

- Assign the wild-type genotype to the HEX channel ("*name*"_wt / Cycling A. Yellow)
- Assign the mutant genotype to the FAM channel ("*name*"_mut / Cycling A. Green)

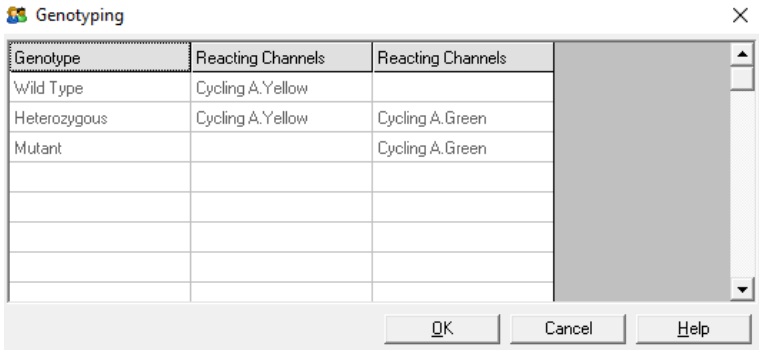

***Figure 18**: Genotyping window.*

To perform genotyping, set the discrimination threshold. All curves passing this threshold are considered to be genotyping samples. The discrimination threshold should be set in the exponential phase of the run, significantly above the background level to avoid noise and below the onset of the plateau phase in later cycles (Figure 17).

To set the threshold, follow these steps:

1. Click on the allelic discrimination plot.
2. In the bottom right of the main window the "*Discrimination threshold*" options are displayed.
3. Click on the icon that appears beside the *Threshold* option (Figure 19).
4. Click anywhere on the graph, and the threshold line appears. It can be moved up or down until finding the right position for each experiment.

***Figure 19**: How to display the threshold.*

Once the threshold has been set, the genotype results appear in the *Allelic Discrimination Results* window.

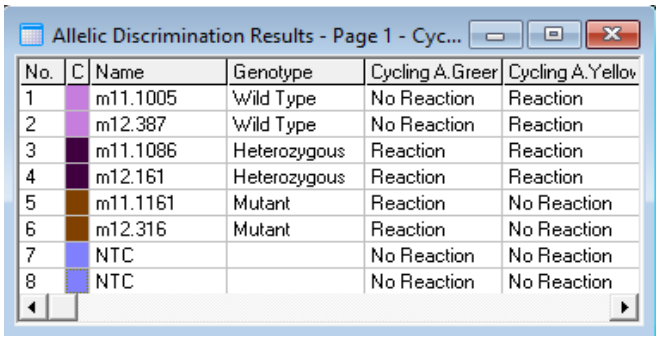

***Figure 20**: Allelic Discrimination Results.*

EXPORT THE FILE

To export the file, follow the next route:

File > Save As… > Excel Analysis Sheet

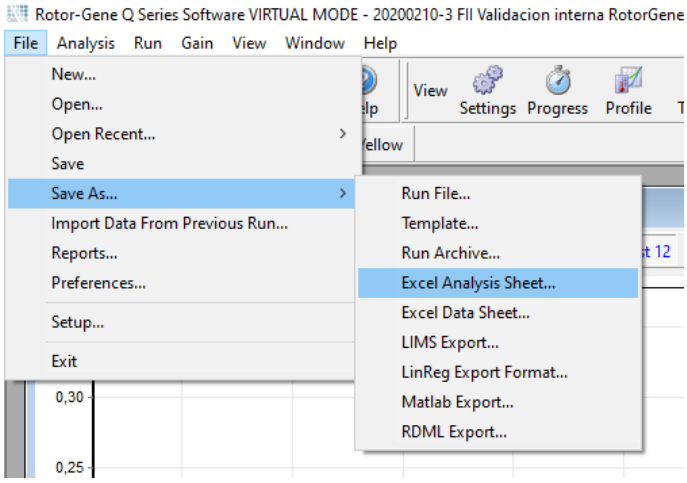

***Figure 20**: Exporting the file.*

Note 1: before exporting the file, beware that genes should be named (exact spelling) as described in the Genvinset® Report Viewer User's Guide. This is important for ensuring the proper working of the Genvinset® Report Viewer software.

For this thermal cycler, it is utterly important to create a template correctly naming the channels **before** the amplification starts. Once the amplification is running, these settings cannot be modified. Therefore, if channels are named wrong, the Genvinset® Report Viewer will not recognise the exported file and will not be able to analyse it.

Note 2: Genvinset® Report Viewer software is only able to analyse experiments interpreted by amplification curves in genotyping experiments.