7500 Real-Time PCR System Manual

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AMPLIFICATION CURVES ANALYSIS

CREATE THE TEMPLATE

Open the software and select *New Experiment*. To perform further analysis of results with the Genvinset® Report Viewer software, it is important to correctly create the template before the run.

1. Set up the experiment properties and in *Experiment type,* select *Quantitation - Standard Curve*.

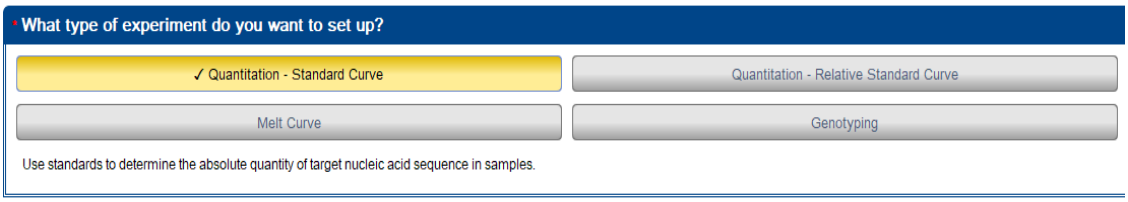

***Figure 1**: Quantification analysis*

2. Go to *Setup* > *Plate Setup*,
    a) In the *Define Targets and Samples* tab, add new targets, name the genes and select the reporter as specified in Figure 2. For multi-reaction kits, add the target genes of all reactions.
    b) In *the Assign targets and Samples* tab, select none as passive reference.
3. In the *Setup* > *Run Method* tab set the amplification program.
4. To save the setup of the experiment to use it on further occasions, click ok *File* > *Save As Template*.

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 2**: Exact gene spelling*

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 2).

SET UP THE EXPERIMENT FROM DESKTOP SOFTWARE

1. Start the software and create a new experiment from template. Select the template previously created.
2. In the *Plate Setup* tab add samples in the *Define Targets and Samples* tab. Click on *Assign Targets and Samples* to set up the plate.
3. Select the wells and tick the box of the corresponding targets and samples.

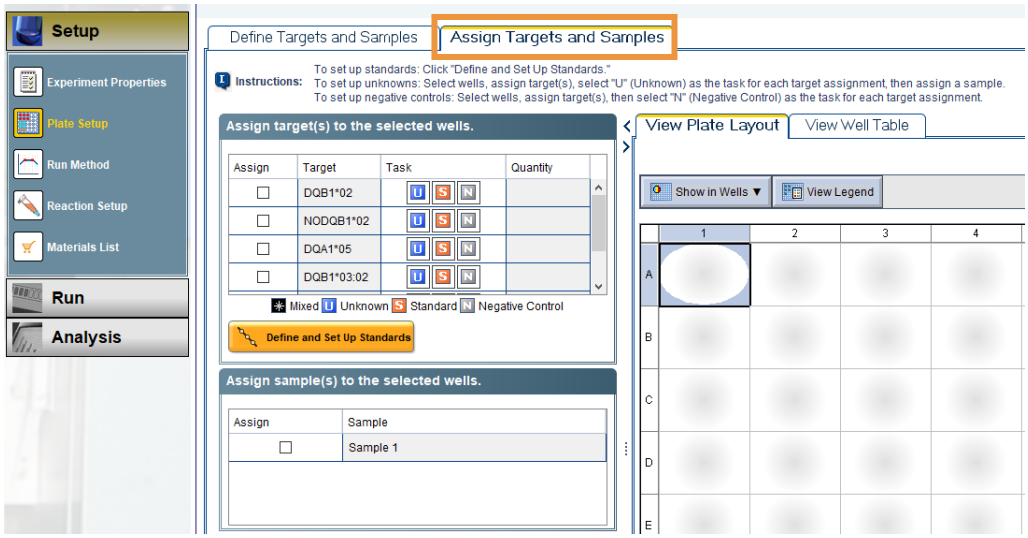

***Figure 3**: Plate set up*

4. Go to the *Run* tab and click on *Start Run*.

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.

To show the amplification curves, click on *Analysis* > *Amplification Plot*. The data collected during the cycling stage of PCR amplification will be displayed.

PLOT CONFIGURATION

The following settings may help in the results visualization and interpretation:

- Plot settings
  - *Plot Type*: ΔRn vs. Cycle
  - *Graph Type*: Linear
  - *Plot colour*: *Target*

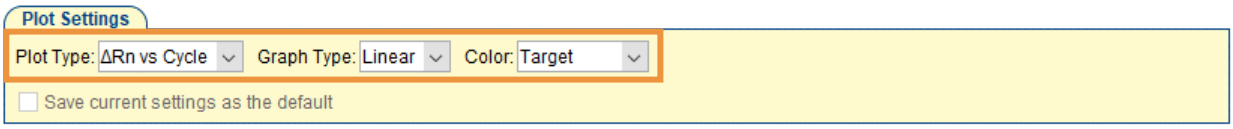

***Figure 4**: Plot Settings*

- Plot Properties: Within the *Amplification Plot* window, click on the icon shown in Figure 5 to edit *Plot Properties*.

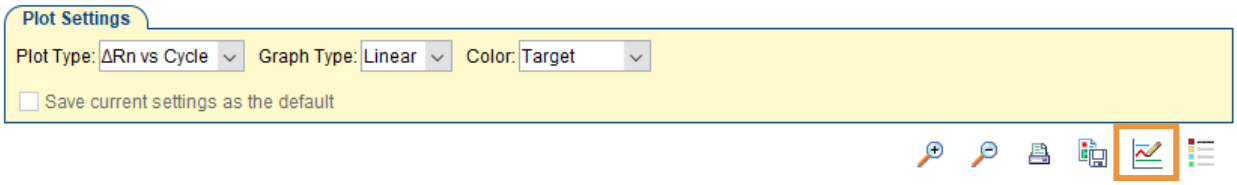

***Figure 5**: Plot Properties settings*

- Options: In the lower side of the *Amplification Plot* window, the following parameters can be set:
  - *Target*: this option allows to select which target is displayed in the graph. All targets can be displayed at once or separately.
  - *Threshold*: uncheck all the boxes, as shown in Figure 6.
  - *Show*: check only the threshold box, as shown in Figure 6.

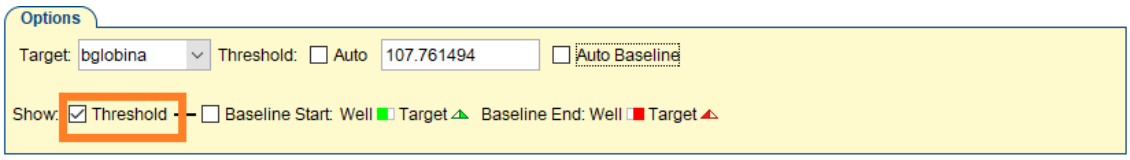

***Figure 6**: Options menu*

SET THE THRESHOLD

For each target gene, follow the next steps:

1. Click on *Analysis Settings* (in the top-right corner).
2. Deselect *C_T Settings to Use: Default Settings*.
3. Deselect *Automatic Threshold*.

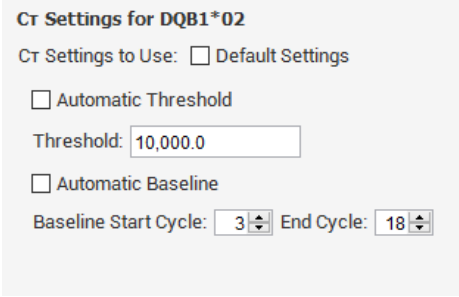

***Figure 7**: C_T settings*

4. Deselect *Automatic Baseline*. Set 3 as the Baseline Start Cycle and 18 as the Baseline End Cycle.
5. Click *Apply*.
6. Click on Re*analyse* to reanalyse to experiment with the new settings.

Once the threshold line is on display, it can be manually moved up and down to find the desired position.

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.

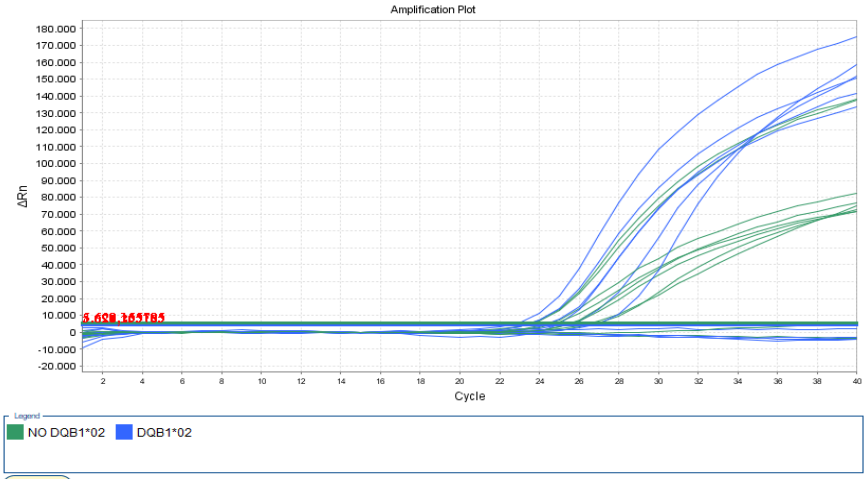

***Figure 8**: Amplification plot*

EXPORT THE FILE

The process to obtain a file to export to the Genvinset® Report Viewer software is the following: *File* > *Export*

An Export Data window will appear. Make sure that the *Results* box is selected by default. If not, check it. Name the file and chose an export file location. Finally, select the (*.xls) type of file and click on *Start Export*.

> **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.

ALLELIC DISCRIMINATION (SCATTER PLOT)

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

CREATE THE TEMPLATE

The steps to design the experiment are the following:

1. Open the software and follow the next route: *File* > *New Experiment*
2. In the *Setup* > *Experiment Properties* tab, select the *Genotyping* option, as shown in Figure 9.

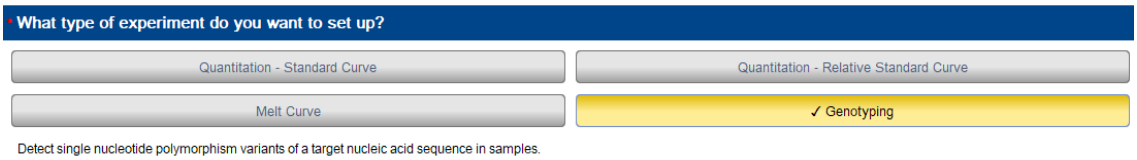

Figure 9: *Genotyping analysis*

3. In the *Setup* > *Plate Setup* tab, edit the SNP Assay introducing the name of the experiment, the name of each allele to be detected and the fluorophores assigned, as shown in Figure 10. The mutated allele will be detected in the FAM channel, whereas the wild type allele will be detected in the HEX/VIC channel.

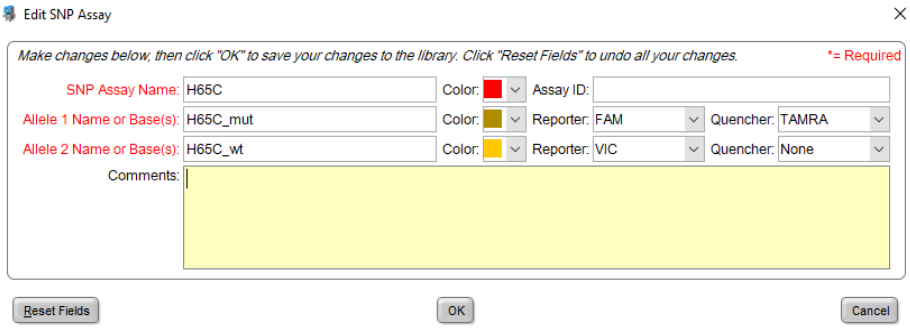

Figure 10*: Allele setup*

4. In the *Setup* > *Run Method* tab set the amplification program.
5. Last, click ok *File* > *Save As Template*.

SET UP THE EXPERIMENT FROM DESKTOP SOFTWARE

1. Start the software and create a new experiment from template. Select the template previously created.
2. In the *Plate Setup* tab, add the samples to be analysed.

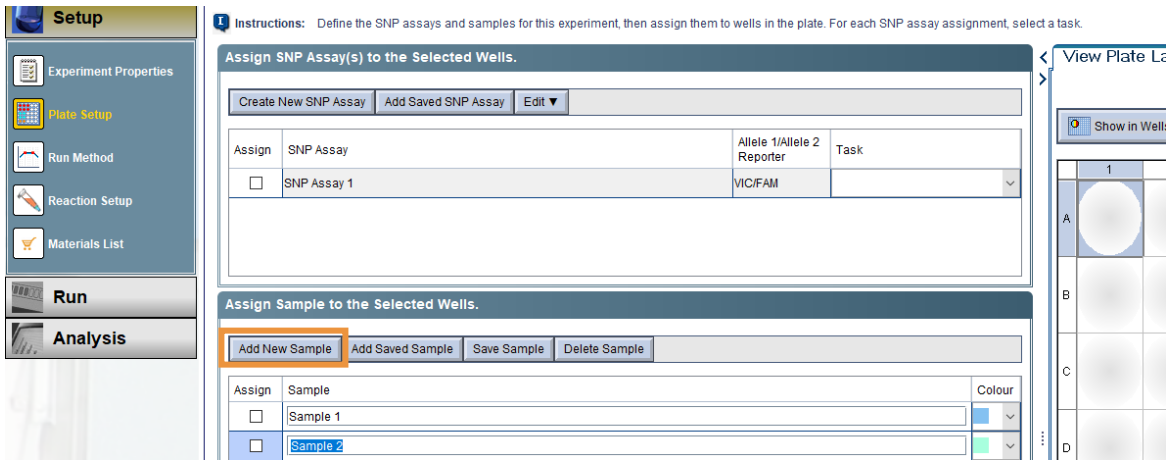

Figure 11*: Plate set up in genotyping analysis*

3. Select the wells and tick the box of the corresponding SNP assays and samples.
4. Go to the *Run* tab and click on *Start Run*.

RESULTS ANALYSIS

Once the run is finished, visualize the amplification curves in linear scale to check the correct shape of the amplification curves, as stated in the homologous section in *Amplification curves analysis*.

SCATTER PLOT

The Allelic Discrimination Plot is displayed in *Experiment Menu* > *Analysis* for the selected SNP assay. Once you click anywhere on the graph, the data points in the plot change to the call colours. Use *Plot type:* Cartesian.

To perform manual calls, click-drag to select the samples in the plot and select the allele call from the *Apply Call* drop-down list.

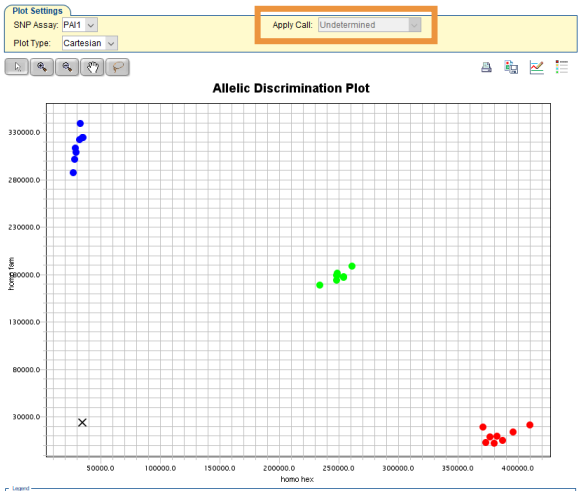

***Figure 12**: Allelic discrimination plot*

EXPORT THE FILE

On the top-right toolbar, click *File* > *Export*

An Export Data window will appear. Make sure that the *Results* box is selected by default. If not, check it. Name the file and chose an export file location. Finally, click on *Start Export*.