QuantStudio™ 5 Dx Manual

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Rev. 01 - 2022/06/10

QuantStudio™ 5 Dx Manual

AMPLIFICATION CURVES ANALYSIS

CREATE THE TEMPLATE

Open the software and select *Create 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.

Set up the experiment properties as shown in Figure 1. In *Experiment type* select *Standard Curve*, and then click *Next*.

In the *Experiment Method* tab, set up the protocol. When done, click *Next*.

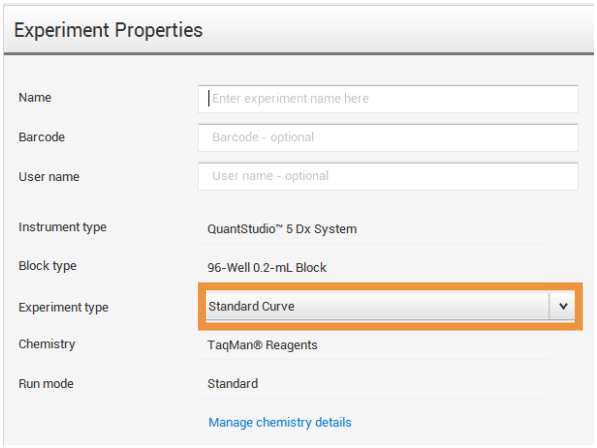

***Figure 1**: Experiment properties*

Then, follow the next steps in the *Plate* tab:

1. Click on *Advanced Setup*. Add new targets, name the genes and select the reporter as specified in Figure 3. For multi-reaction kits, add the target genes of all reactions.
2. Click on *Quick Setup*. Select *None* as passive reference in *Plate Attributes*.
3. Last, click on the top right *Save* button and save the template

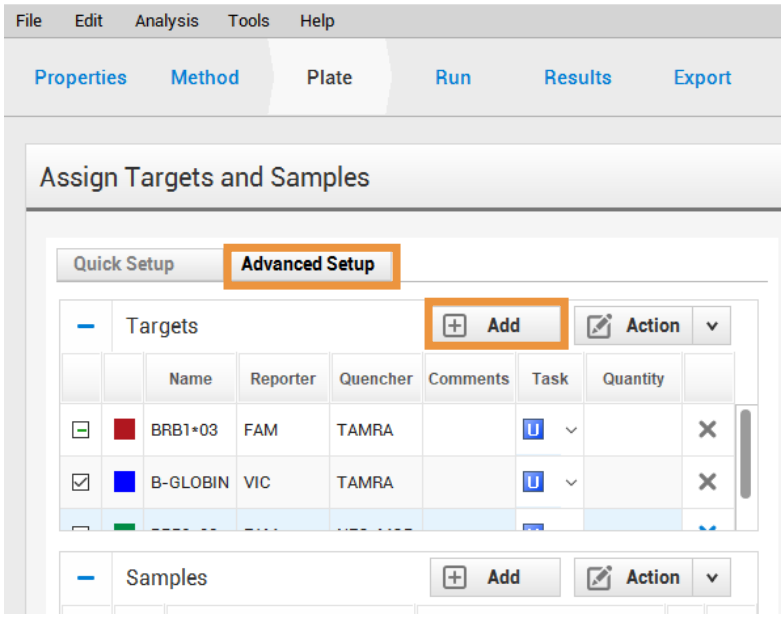

***Figure 2**: Advanced plate setup*

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 3**: 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 3).

SET UP THE EXPERIMENT FROM DESKTOP SOFTWARE

Start the software and create a new experiment from template as shown in Figure 4.

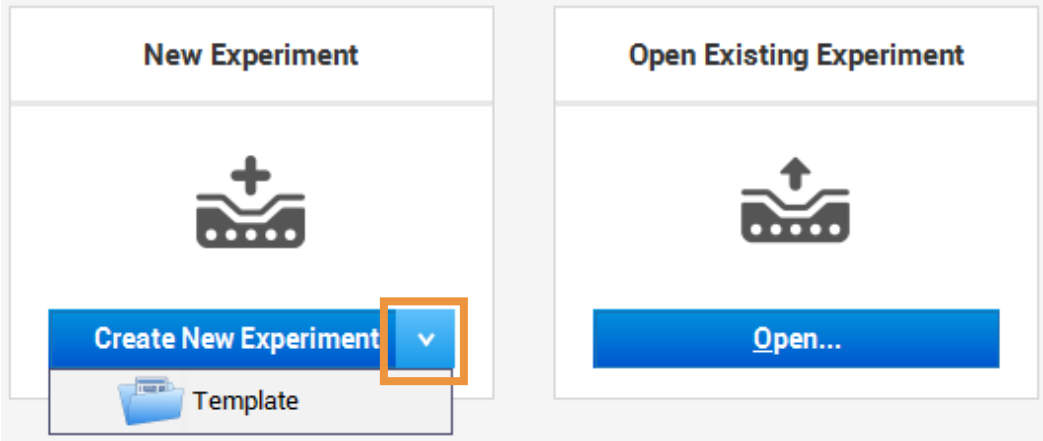

***Figure 4**: Creating a new experiment from template*

In the *Plate* tab select *Advanced setup* and add samples. Assign samples and targets to the corresponding wells in the plate.

Finally, 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.

Once the amplification is over, the amplification curves appear in the *Results* tab. If no data are displayed, click Analyze. The amplification Plot is displayed for the selected wells in the *Plate Layout*.

PLOT CONFIGURATION

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

1. In the Results tab, select *Amplification Plot* from the dropdown list.
2. Click to configure the plot, then make the following selections:

- *Plot Type:* Select *ΔRn vs Cycle.*
- *Graph Type:* Linear
- *Plot Color*: Target.
- Select *Show: Threshold.*
- Select *Show: Baseline*

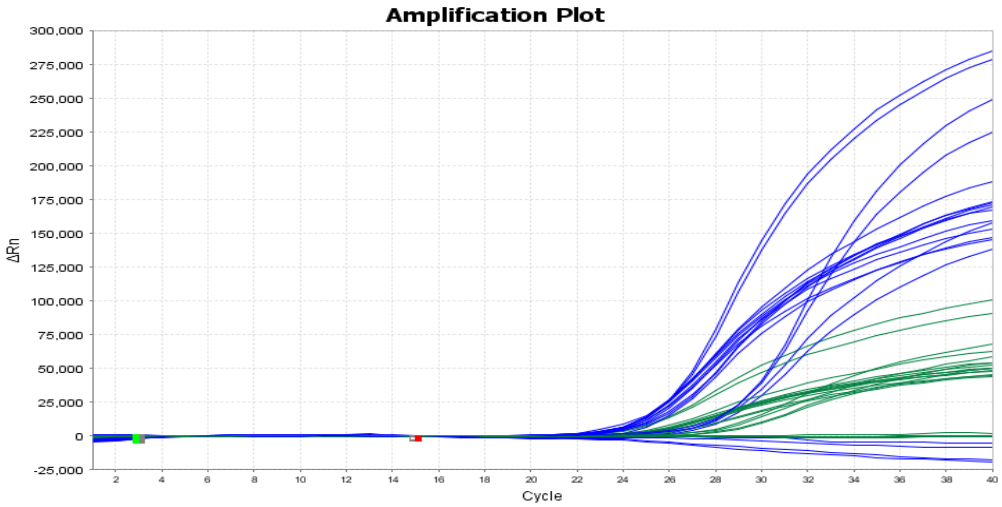

***Figure 5**: Amplification plot*

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.

In the *Results* tab, click  (in top-right corner). For each target gene, follow the next steps:

1. Deselect *C_T Settings to Use: Default Settings*.
2. Deselect *Automatic Threshold*. Type out the preferred threshold.
3. Deselect *Automatic Baseline*. Set 3 as the Baseline Start Cycle and 18 as the Baseline End Cycle.
4. Click *Apply*.

Last, click on *Analyze* to reanalyse to experiment with the new settings.

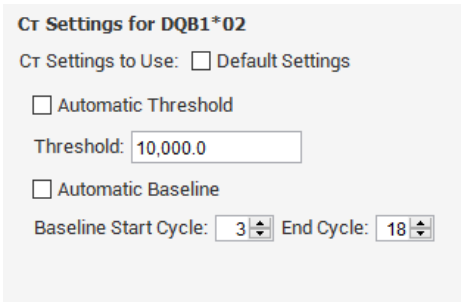

***Figure 6**: C_T settings*

EXPORT THE FILE

The process to obtain a file to export to the Genvinset® Report Viewer's software is the following:

In the *Export* tab, choose the Excel (*.xls or *.xlsx) exporting format. Then click *Export* (top-right corner).

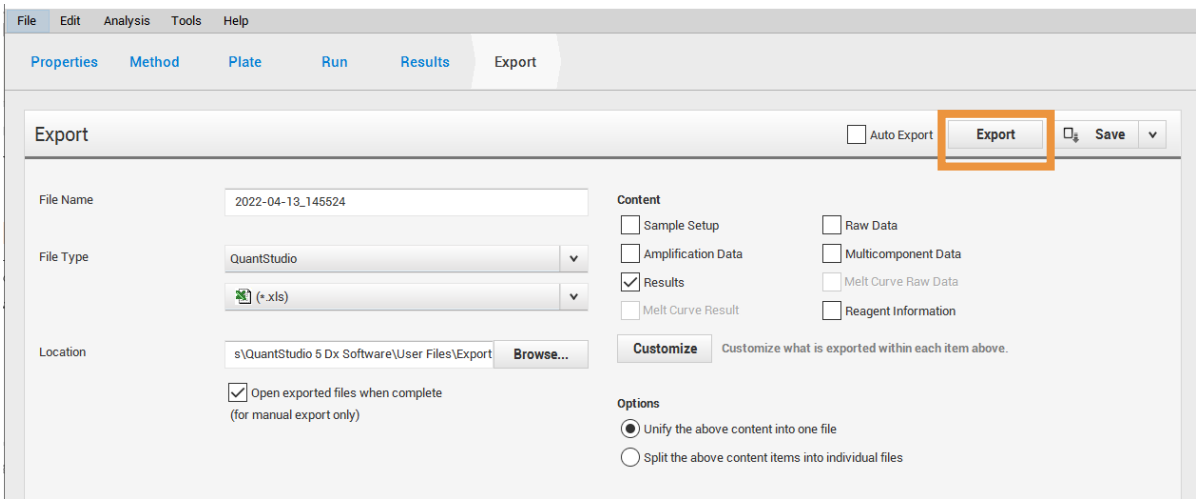

***Figure 7**: Exporting the file*

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.

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 select *Create New Experiment*.
2. Set up the experiment properties as shown in Figure 8. In *Experiment type* select *Genotyping*, and then click *Next*.
3. In the *Experiment Method* tab, set up the protocol. When done, click *Next*.
4. Then, follow the next steps in the *Plate* tab:
   - Click on *Advanced Setup*. Add the corresponding SNPs. Click *Action* > *Edit* to edit the created SNP (Figure 9). Select TAMRA as Quencher.
   - Click on *Quick Setup*. Select *None* as passive reference in *Plate Attributes*.
5. Last, click on the top right *Save* button and save the template.

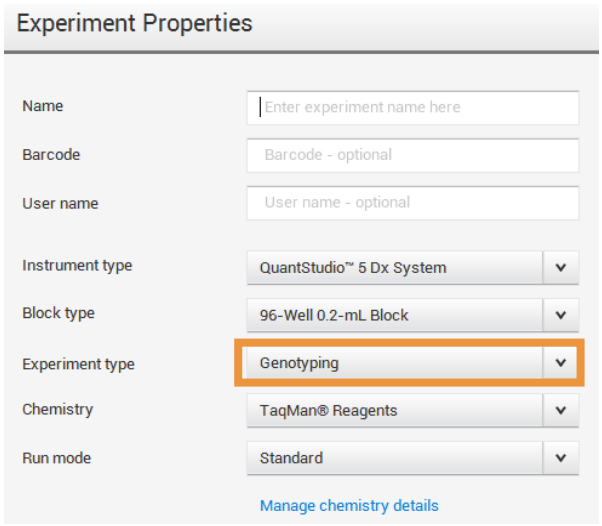

***Figure 8**: Genotyping experiment properties*

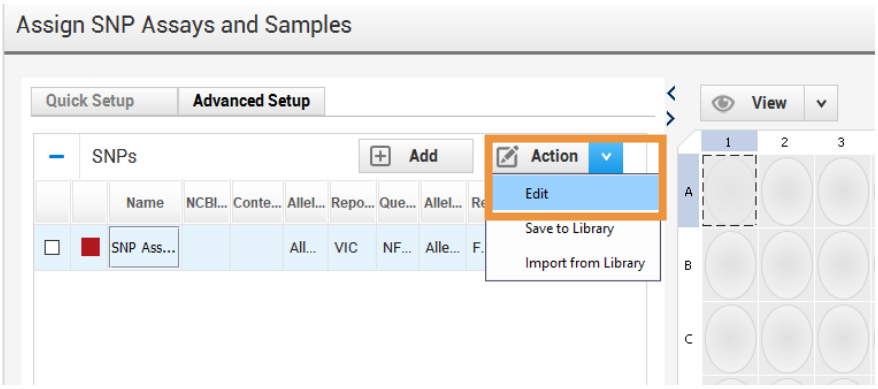

***Figure 9**: Editing the SNPs*

SET UP THE EXPERIMENT FROM DESKTOP SOFTWARE

Start the software and create a new experiment from template as shown in Figure 10.

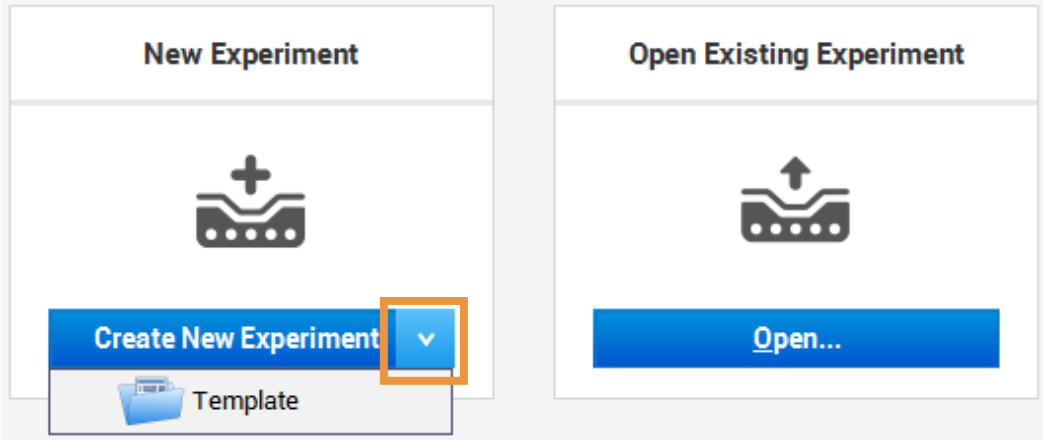

***Figure 10**: Creating a new experiment from template*

In the *Plate* tab select *Advanced setup* and add samples. Assign samples and SNPs to the corresponding wells in the plate.

Finally, 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

1. In the *Results* tab, select *Allelic Discrimination Plot* from the dropdown list.
2. Click to configure the plot:
   - *SNP Assay:* Select the corresponding assay.
   - *Plot Type:* Cartesian.

The Allelic Discrimination Plot is displayed for the selected SNP assay. Once you click anywhere on the graph, the data points in the plot change to the call colours.

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

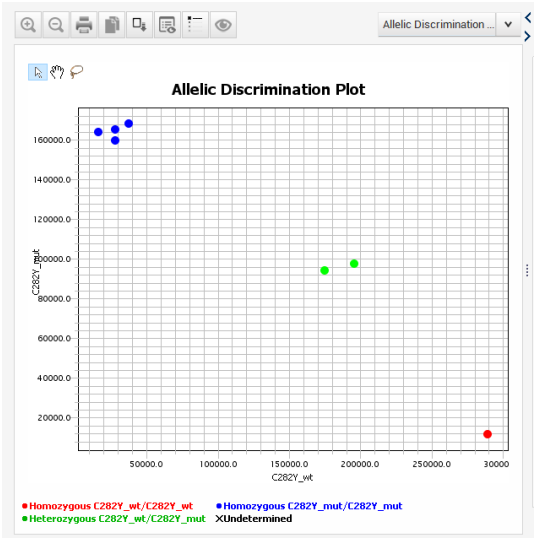

***Figure 11**: Allelic discrimination plot.*

EXPORT THE FILE

On the top-right toolbar, select the *Export* tab. 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 *Export* (top-right corner).

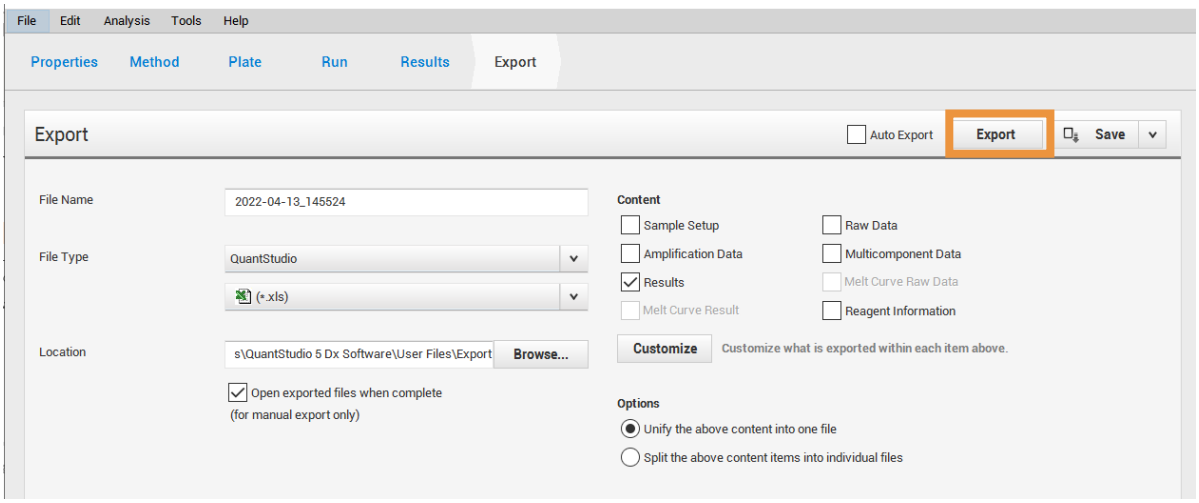

***Figure 12**: Exporting the file*