CFX Opus 96 (BioRad) Manual

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Rev. 01 - 2022/06/10

CFX Opus 96 Manual

CREATE THE TEMPLATE

First, select the instrument in *Run setup* in the Startup Wizard. The first time carrying out an analysis, you should create a new Run Setup. To perform further analysis of results with the Genvinset® Report Viewer software, it is important to correctly create the setup before the run.

Click on *File>New>Protocol*. Set up the amplification program. Click on *OK* and save the protocol file.

In the *Run Setup* window click on *Plate* and then select *Create New...* A new window will open.

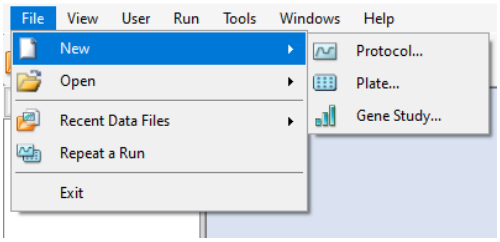

***Figure 1**: Creating a new protocol*

***Figure 2**: Creating a new plate*

1. At the top of the Plate Editor window, select *All Channels*, as shown in Figure 3.

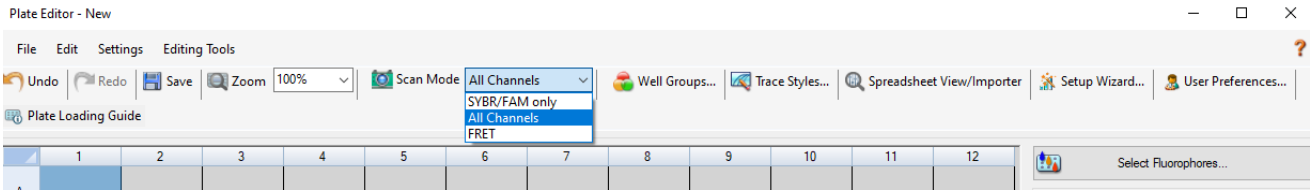

***Figure 3**: Plate Editor*

2. Click on *Select Fluorophores*, select FAM and HEX in the pop-up window and then click on *OK*.

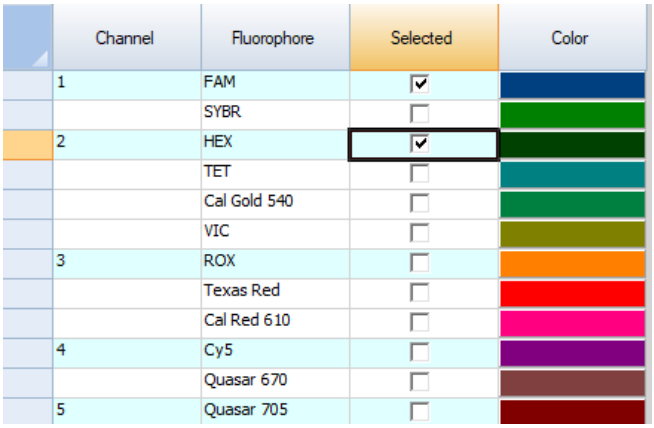

***Figure 4:** Select Fluorophores*

3. At the bottom right of the Plate Editor window, click on *Experiments Settings* and a new window appears (Figure 5). Add new target names, naming the genes as specified in Figure 6. For multi-reaction kits, add the target genes of all reactions.

   You may remove Actin and GAPDH from the target names, checking the box to remove and clicking on *Remove checked item(s)*. CFX Maestro software excludes the sample type NTC (no template control) from gene expression analysis. When done, click *OK*.

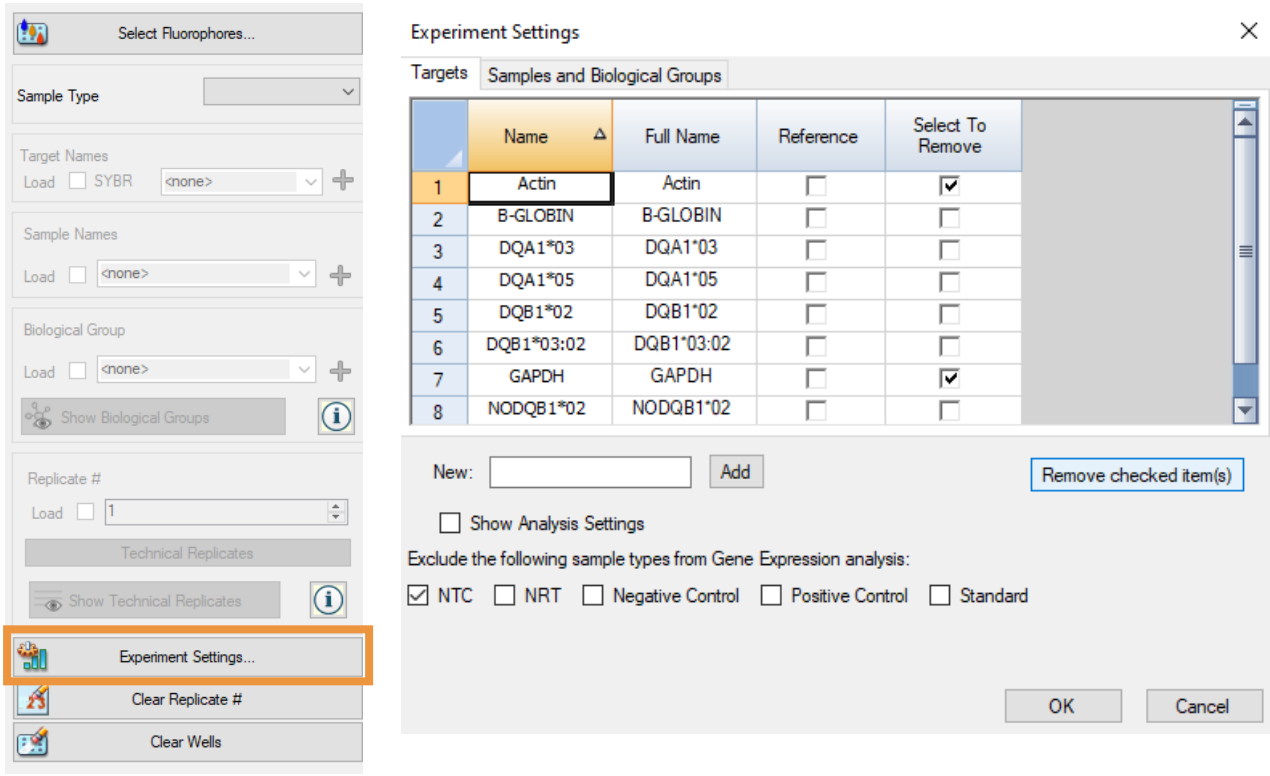

***Figure 5**: Experiments Settings*

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 6**: 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 6).

4. To complete the plate setup, at least one well should be loaded with a Sample Type and the fluorophores. Last, click on *OK* and save the plate file.

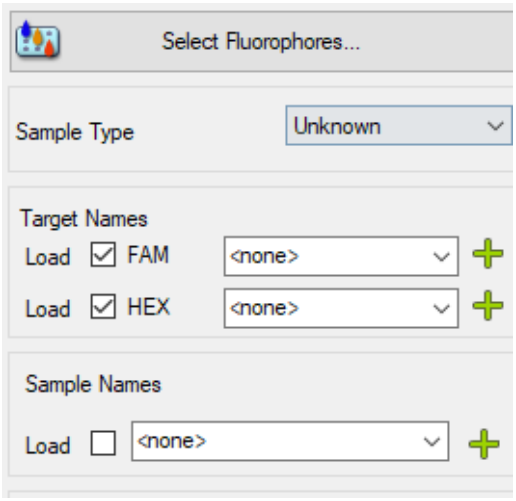

***Figure 7**: Setting up the samples*

SET UP THE EXPERIMENT FROM DESKTOP SOFTWARE

Open the Bio-Rad CFX Maestro software and select the instrument. Go to *View>Run Setup*.

1. In the *Run Setup* window, select *Protocol* and click on *Select Existing*…. Select the saved protocol file.

2. Select *Plate* in the Run Setup window and click on *Select Existing*…. Select the saved plate file. Edit the plate by clicking on *Edit Selected…* to add samples and assign the correct target gene to each well.

***Figure 8**: Selecting the plate file*

3. Click on *Next* >> or go to *Start Run* in the *Run Setup* window. Select the checkbox of one or more blocks on which to perform the run. To run the experiment on all blocks, select *All Blocks* located below the Selected Blocks table.
4. 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.

AMPLIFICATION CURVES ANALYSIS

Open the file. Once the amplification is over, the amplification curves appear in the *Quantification* tab In the *Data Analysis* window.

PLOT CONFIGURATION

To select the linear scale, uncheck the box that appears on the right lower side of the amplification window, as shown in Figure 9.

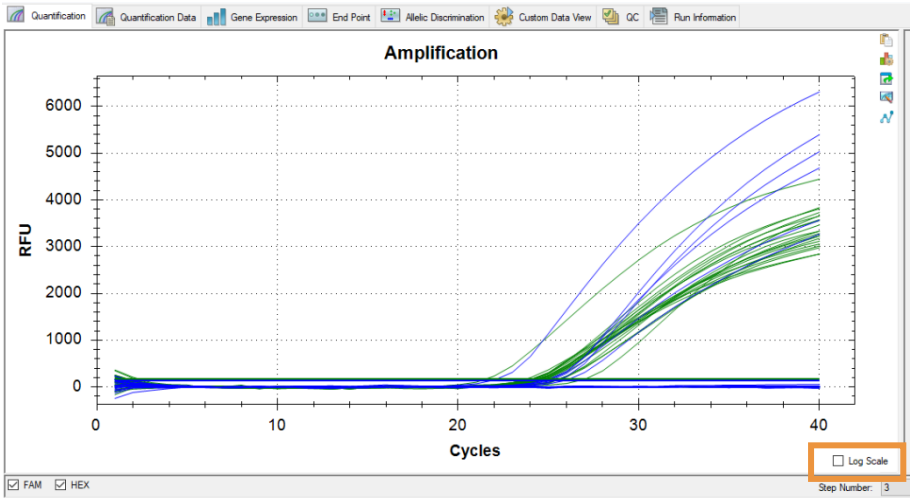

Figure 9: *Amplification plot showing the linear and log scale option*

On the top of the window, click on *Settings*. The following settings may help in the results' visualization and interpretation. Select:

- *Cq Determination Mode: Single Threshold*.
- *Baseline Setting: Baseline Subtracted Curve Fit*.

- *Analysis Mode:* Fluorophore.
- *Cycles to analyse:* By clicking on this tab, a pop-up window will appear. Analyse cycles from 1 to the maximum number of cycles allowed.

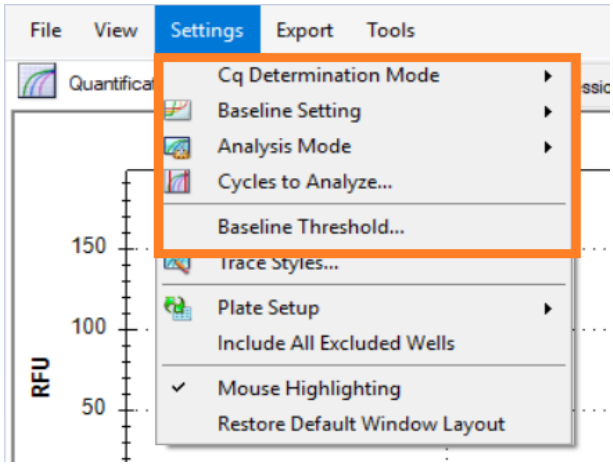

Figure 10: *Setting menu*

SET THE THRESHOLD

On *Settings*, click on *Baseline Threshold.*

This is the menu bar to open the *Baseline Threshold* window. This setting must be done for each channel (FAM and HEX) separately, so only one fluorophore below the graph must be selected.

Check *User Defined* and then select all samples. Set 3 as Baseline Begin and 18 as Baseline End (Figure 11).

In the *Single-Threshold* mode, the threshold can be adjusted for each fluorophore by moving it upwards and downwards in the graph.

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.

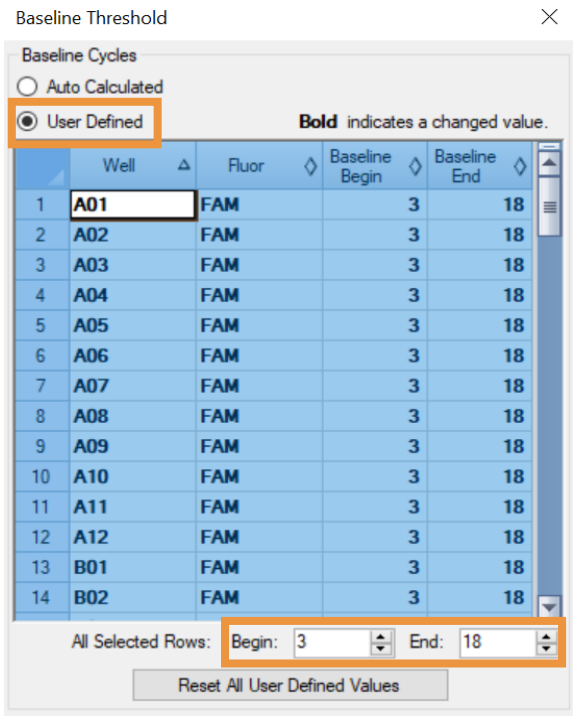

Figure 11*: Baseline settings*

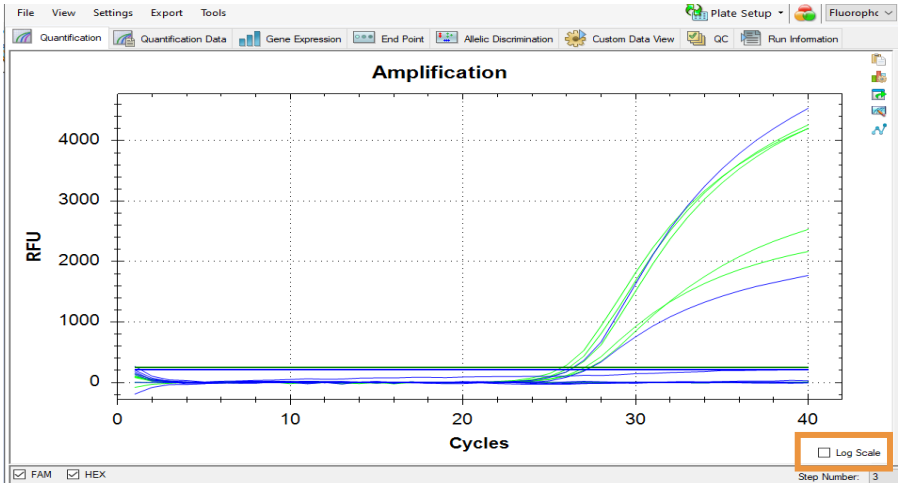

Figure 12: *Amplification plot showing the threshold line above the background noise*

ALLELIC DISCRIMINATION (SCATTER PLOT)

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

SCATTER PLOT

Open the file and select the *Allelic Discrimination* tab. This analysis assigns genotypes to wells with unknown samples.

In the Allelic Discrimination chart, clear the *Polar Coordinates* checkbox to show cartesian coordinates (Figure 13).

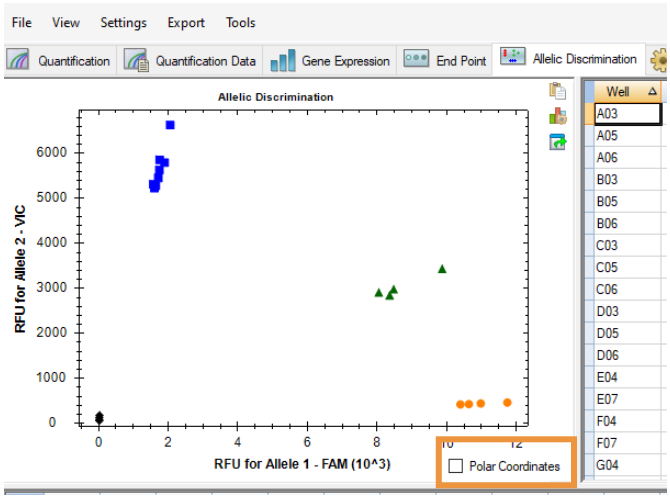

Figure 13*: Allelic discrimination plot*

To change a call, drag across the data point(s) in the Allelic Discrimination chart and choose an option in the Selected Wells list: Allele 1, Allele 2, Heterozygote, Undetermined, No Call, Auto Call.

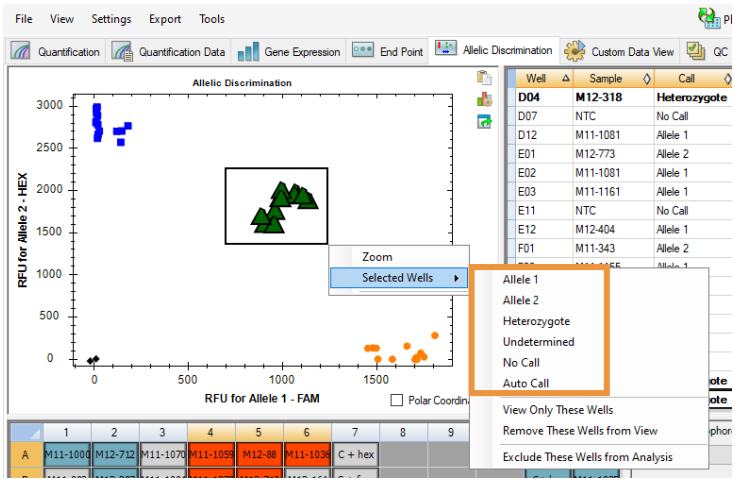

***Figure 14**: Assigning calls tin the allelic discrimination plot*

EXPORT THE FILE

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

Export > *Custom Export*. All the parameters that need to be exported appear selected by default.

Choose the CVS (*.cvs) exporting format.

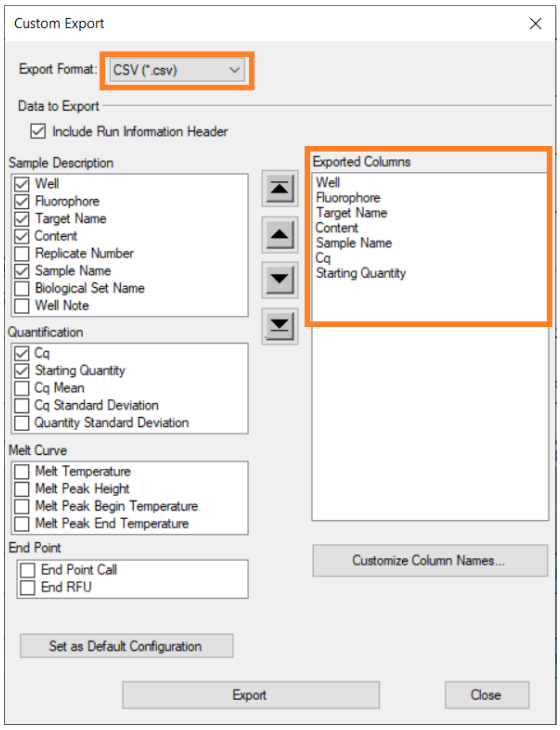

***Figure 15**: Custom export*

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.

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