

CFX Opus 96 (BioRad) Manual

Blackhills Diagnostic Resources S.L.U. Camino del Pilón 86, casa 7 local - 50011 Zaragoza, Spain +34 976 094 603 – <u>info@bdrdiagnostics.com</u>



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

Run Setup	×
M Protocol III Plate IN Start Run	
Create New	Express Load
Select Existing	Quick Plate_96 wells_All Channels.pltd \sim
Selected Plate	

Figure 2: Creating a new plate

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

File Edit Settings Editing Tools ? Image: Setting Tools Image: Setting Tools Image: Setting Tools Image: Setting Tools ? Image: Setting Guide Image: Setting Tools Image: Setting	Plate Editor - New				– 🗆 ×
By Bry Loading Guide SYBR/FAM only All Channels FRET	File Edit Settings Editing Tools				?
1 2 3 4 5 6 7 8 9 10 11 12 Select Fluorophores		SYBR/FAM only All Channels	希 Well Groups 🗖 Trace Styles	🔍 Spreadsheet View/Importer 🛛 🕌 Setu	p Wizard 🤱 User Preferences
	1 2 3 4	5 6 7	8 9 10	11 12	Select Fluorophores

Figure 3: Plate Editor

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

Channel	Fluorophore	Selected	Color
1	FAM	~	
	SYBR		
2	HEX	>	
	TET		
	Cal Gold 540		
	VIC		
3	ROX		
	Texas Red		
	Cal Red 610		
4	Cy5		
	Quasar 670		
5	Quasar 705		
	Fig	gure 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*.

Select Fluorophores	Experiment Settings ×					
0 I T	Targets	Samples and Bio	logical Groups			
Sample Type		Name 🛆	Full Name	Reference	Select To Remove	-
Load SYBR <none> <</none>	1	Actin	Actin		~	
Sample Names	2	B-GLOBIN	B-GLOBIN			
	3	DQA1*03	DQA1*03			≡
Load <none> <</none>	- 4	DQA1*05	DQA1*05			
Pialanian Graun	5	DQB1*02	DQB1*02			
Biological Group	6	DQB1*03:02	DQB1*03:02			
Load cnone>	7	GAPDH	GAPDH		v	
Show Biological Groups	8	NODQB1*02	NODQB1*02			
Replicate #	New		Add			Remove checked item(s)
Load 🗌 1		Show Analysis Sett	ings			
Technical Replicates	Exclude	the following sampl	- le types from Gene	Expression analy	sis:	
Show Technical Replicates			Negative Control	Positive Con	trol 🗌 Standar	d
Experiment Settings						
Clear Replicate #						OK Cancel
Clear Wells						

Figure 5: Experiments Settings

Kit	Exact sp	elling	Kit	Exact sp	elling
	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	2) 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.

Select	t Fluorophores
Sample Type	Unknown 🗸
Target Names Load ☑ FAM Load ☑ HEX	<pre><none> </none></pre> +
Sample Names Load <pre> Content</pre>	~ +

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.

Run Setup	
Protocol III Plate > Start Run	
Create New	Express Load
Select Existing	2-Step_Amp+Melt.prcl ~
Selected Protocol	
2-Step_Amp+Melt.prcl	Edit Selected

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.

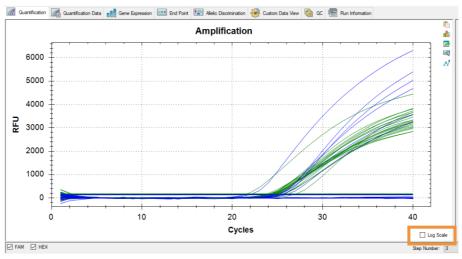


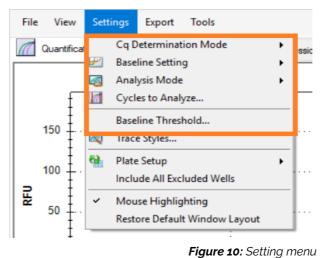
Figure 9: Amplification plot showing the linear and log scale option

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

- <u>Cq Determination Mode</u>: Single Threshold.
- <u>Baseline Setting:</u> Baseline Subtracted Curve Fit.



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



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.

Baselir	ne Threshold			×
	ne Cycles to Calculated			
🖲 Us	er Defined	Во	ld indicates a ch	anged value.
	Well △	Fluor 👌		iseline \land 📥
1	A01	FAM	3	18 ≣
2	A02	FAM	3	18
3	A03	FAM	3	18
4	A04	FAM	3	18
5	A05	FAM	3	18
6	A06	FAM	3	18
7	A07	FAM	3	18
8	A08	FAM	3	18
9	A09	FAM	3	18
10	A10	FAM	3	18
11	A11	FAM	3	18
12	A12	FAM	3	18
13	B01	FAM	3	18
14	B02	FAM	3	18 🖵
	All Selected Row	vs: Begin: 3	😫 End:	18 🜲
	Re	eset All User Defir	ned Values	

Figure 11: Baseline settings

BDR

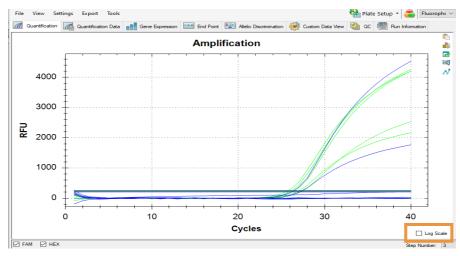


Figure 12: Amplification plot showing the threshold line above the background noise

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).

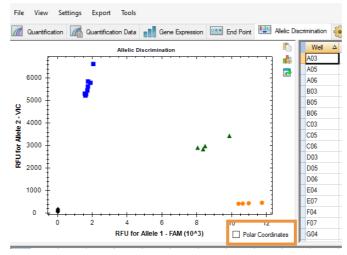


Figure 13: Allelic discrimination plot

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.

SDR

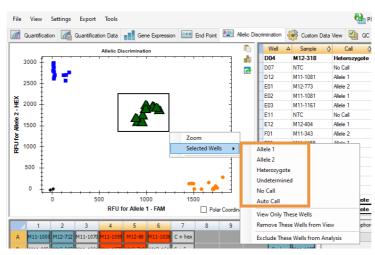


Figure 14: Assigning calls tin the allelic discrimination plot

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.

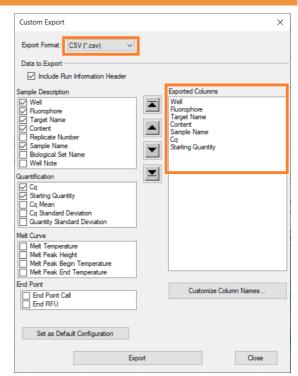


Figure 15: Custom export

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.