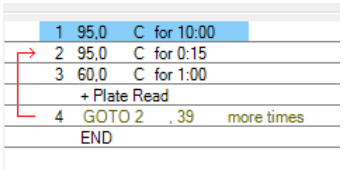
QuickGuide: RealFast™ CNV on Bio-Rad CFX96

Setup for Relative Quantitation Assays:

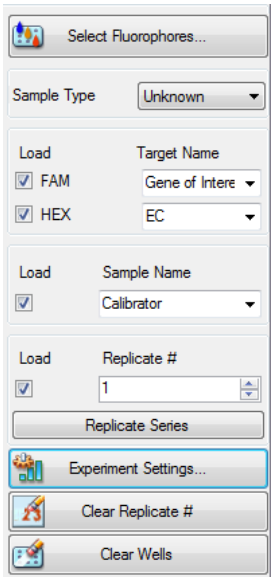
- Open the Bio-Rad CFX Maestro software (QuickGuide is based on version 2.2).

- In the Startup Wizard select instrument **CFX96** and run type **User-defined.**

- In the Run Setup select **Create New** within the **Protocol Tab**. The **Protocol Editor** opens.
  - Select a sample volume of 20 µl and setup the **PCR program**: 10 min at 95°C followed by 40 times 15 sec at 95°C and 60 sec at 60°C. Make sure that the **Plate Read** is added to the 60°C step. Press **OK** and save the protocol file. Press **Next**.



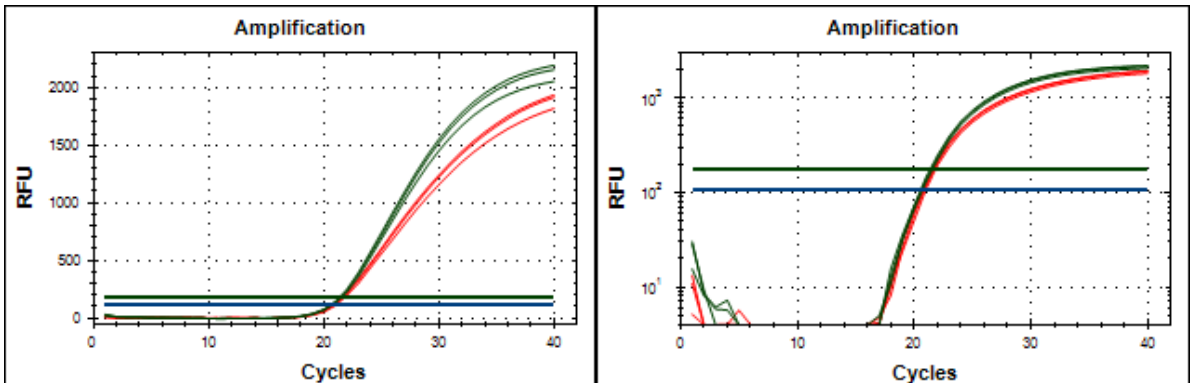
- In the tab called **Plate** select **Create New**. The **Plate Editor** opens.
  - Select **Settings > Plate Type** and choose the correct type of plate: Plate Size and Plate Type.
  - Select scan mode **All Channels.**
  - Click **Select Fluorophores** and select FAM and HEX.
  - Select wells by clicking in the well selector and choose the sample type (**NTC** or **Unknown**); a minimal setup should contain triplicates of a negative control template, the calibrator and an unknown sample.
  - Click **Load** check boxes to load fluorophores **FAM** and **HEX**
  - Type target names (FAM: gene of interest; HEX: EC / endogenous control) and press **Enter**. The name is now available from the drop down menu.
  - Type sample name and press **Enter.**
  - Click check box to load **Replicate** number.
  - Open **Experiment Settings** and select the endogenous control **EC** as **Reference** in the **Targets** tab.
  - In the **Samples and Biological Groups** tab select the supplied Calibrator sample as **Control**. Press **OK**.
  - Choose **Well Groups** in case you are running several assays at the same time
  - Press **OK** and save the plate file.



- Press **Next,** load your PCR tubes and start the run.

Analysis of Relative Quantitation Assays:

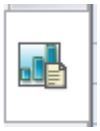
- Open the data file: **File > Open > Data File**
- Within the **Quantification** tab select your samples and click the check box for **HEX** in the amplification chart.
  - Right-click on the threshold line and select **Show Threshold Values**.
  - Set the threshold according to the settings in the Assay Description: **Settings > Baseline Threshold > Auto Calculated**
  - Follow the same principle and review the threshold for FAM.
- Select both fluorophores and review the results by clicking on individual wells in the well selector.



Example: Amplification Plot of the Calibrator sample – linear and log graph type (red: gene of interest, green: EC)

- Click on the **Gene Expression** tab. In the **Bar Chart** select a Mode from the drop down menu to the right of the chart. Select **Normalized Expression (ΔΔCq)**, **Relative To Control** (Graph Data) and **Calibrator** (Control). Review **Experiments Settings** for correct assignment of reference (EC) and control (ViennaLab Calibrator). For details right-click on the **Graph Expression Data Table** select **Show Details**.

- In the **Well Results** Tab you can find the **Expression** column.



The ratio for the **Calibrator** (Control Sample) is set to "1“ and values for your samples are relative to the **Calibrator**. Refer to the product description for interpretation of your results.

- To open a report, select **Tools > Reports** and adjust the report according to your needs.