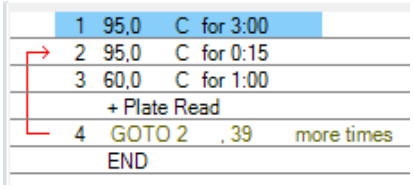
QuickGuide: RealFast™ Genotyping for BioRad CFX96

Setup for Genotyping Assays:

- Open the BioRad CFX Manager.

- 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: **3 min** at **95**°C followed by **40 cycles** of **15 sec** at **95°C** and **1 min** at **60°C**. Press "OK" and save the protocol file. Press "Next".



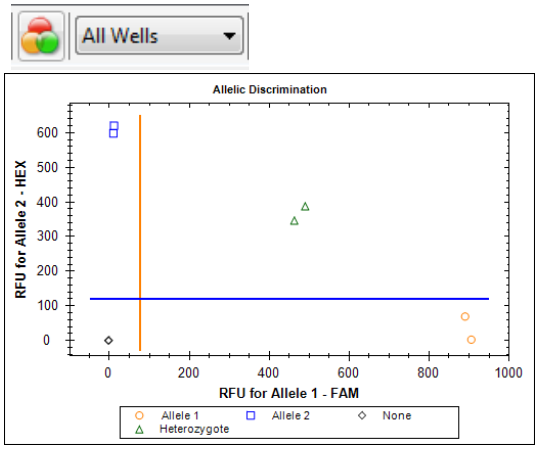
- In the tab called **Plate** select **Create New**. The **Plate Editor** opens.
  - Select **Settings > Plate Type** and choose the correct type of plate.
  - Select Scan Mode **All Channels.**
  - Click **Select Fluorophores** and select **FAM** and **HEX**.
  - Select wells by clicking in the well selector and choose the sample type (**NTC** or **Unknown**); a minimal setup should contain 2 replicates of a No Template Control (NTC), a control homozygous for allele 1 (control 1), a control homozygous for allele 2 (control 2) and an unknown sample.
  - Click **Load** check boxes to load fluorophores **FAM** and **HEX.**
  - Type target names and sample name and press **Enter.**
  - Click check box to load **Replicate** number.
  - Define **Well Groups** in case you are running several assays at the same time.
  - Press **OK** and save the plate file.

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

Analysis of Genotyping Assays: Single Threshold Method:

- Open the data file: **File > Open > Data File.**

- Select the **Allelic Discrimination** tab.
  - Select the well group (top right) in case you were running several assays at the same time.
  - Select **RFU** as **Display Mode.**
  - In **Settings** choose **Cq Determination Mode > Single Threshold.**
  - The software automatically analyses the raw data and generates a scatter plot and a results grid.
  - Adjust the **threshold** by clicking and dragging the vertical and horizontal lines in the scatterplot: Points for allele 1 and allele 2 group along the horizontal and vertical axis, heterozygotes in the middle.



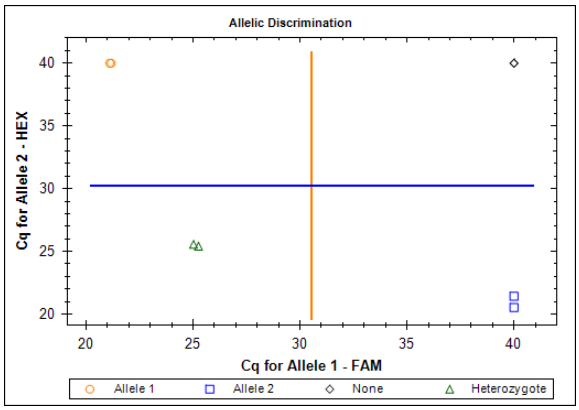
- The correct assignment for each well should be verified in the **Quantification** tab. Each control displays amplification in the respective channel only. For instance, if control 1 shows an amplification in the HEX channel, no amplification in the FAM channel should be visible. If the threshold line is below the background signal of FAM, then manually move the threshold line above the background. Do the same for control 2 and verify that there is an amplification in the FAM channel but no signal in the HEX channel.

- To generate a report, select **Tools > Reports.** In the pop-up window **Report** select **Allelic Discrimination** and adjust the report according to your needs.

Analysis of Genotyping Assays: Regression:

- Open the data file: **File > Open > Data File**

- Select the **Allelic Discrimination** tab.
  - Select the well group (top right) in case you were running several assays at the same time.
  - Select **Cq** as **Display Mode.**
  - In **Settings** choose **Cq Determination Mode > Regression**
  - The software automatically analyses the raw data and generates a scatter plot and a results grid.



- The correct assignment for each well should be verified in the **Quantification** tab.

- To generate a report, select **Tools > Reports.** In the pop-up window **Report** select **Allelic Discrimination** and adjust the report according to your needs.