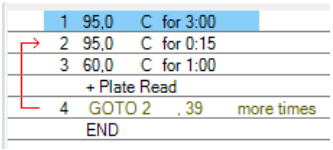
QuickGuide: RealFast™ Variant Detection on Bio-Rad CFX96

Setup for Variant Detection RealFast™ 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 set up the PCR program according to the Instructions for Use of your RealFast™ Assay:
  - For Variant Detection RealFast™ Assays (e.g. HLA-B27):
    3 min at 95°C followed by 40 times 15 sec at 95°C and 60 sec at 60°C.

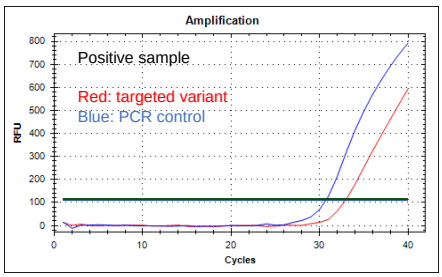


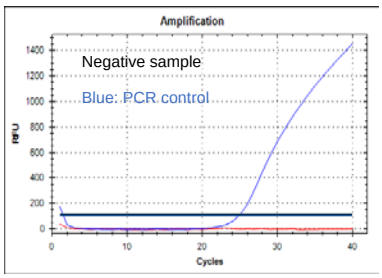
  - Press "OK“ and save the protocol file. Press "Next“.

- 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** for the No Template Control and **Unknown** for your samples, as well as for Controls.
  - In the field **Target Names** check boxes to load fluorophores **FAM** and **HEX.**
  - Type target names (FAM = Variant; HEX = PCR control) 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 Variant Detection RealFast™ Assays:

- Open the data file: **File > Open > Data File.**
- Select the **Quantification** tab
  - Select the well group (top right) in case you were running several assays at the same time.
  - Set the threshold according to the Instructions for Use of the respective RealFast™ Assay *(e.g. set the threshold value for the FAM channel just above the background fluorescent signal generated by the HLA-B27 Negative Control. Set the threshold value for the HEX channel at the onset of the exponential phase of the amplifcation curve)*.
  - Review your samples individually. Samples positive for the targeted variant (e.g. HLA-B27), as well as the Positive Control will show a strong fluorescent signal in both, the **FAM** and the **HEX** channels. Samples negative for the targeted variant, as well as the Negative Control will show a signal in the **HEX** channel only (PCR control).





- To open a report, select **Tools > Reports** or click the **Reports** button on the toolbar in the **Data Analysis** window. Adjust the report according to your needs.