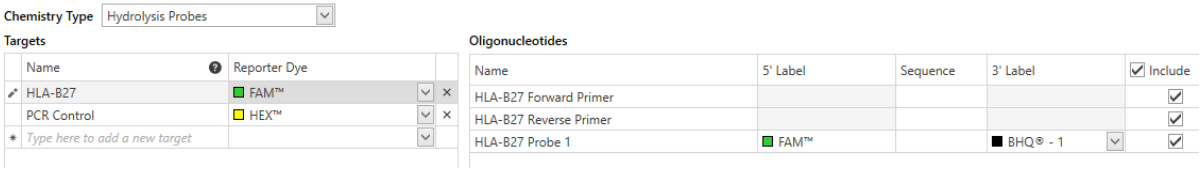
# QuickGuide: RealFast™ Variant Detection on MIC qPCR Cycler

**Setup for Variant Detection RealFast™ Assays:**

- Open the micPCR software (QuickGuide is based on version 2.10.3).

Definition of a new assay:

- Define a new assay by **New** > **Assay** in the menu bar.
- Within the **Assay Setup** choose **Information**.
  - Set the **Chemistry Type** to **Hydrolysis Probes**.
  - In **Targets** name your targets, which are the gene of interest and the endogenous control, and choose your **Reporter Dye** (e.g. FAM and HEX)
  - In the field **Oligonucleotides** define the **5´**and **3´Modifier** for both **Probes**. Typically, the gene/allele of interest is labeled with 5´-FAM and 3´-BHQ1 and the endogenous control with 5´-HEX and 3´-BHQ1.



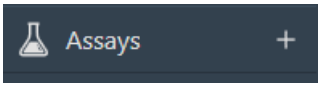

*» **Note**: For assignment of fluorophores to hydrolysis probes see Instructions for Use of the respective RealFast™ Assay. «*

- Within the **Assay Setup** choose **Profile**.
  - Define in **Temperature Control** the mode **Fast TAQ (v3)** and in **Volume** 20 µl.
  - Setup the **PCR program** according to the Instructions for Use of your RealFast™ Assay.
    Data acquisition should be by default at the end of the 60°C step (camera symbol is black).

- **Safe** your assay!

Setup a new run:

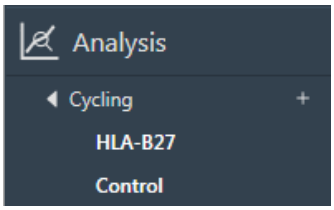
- Define a new run by **New** > **Run** in the menu bar.
  - In the menu on the left side choose **Assays** and click on "**+**" next to it. Via the **Shortcut Library** you can choose one of your previously designed assays for variant detection.




- Within **Run Setup** choose **Samples** and name your samples.
  - In the field **Type** choose **NTC** for your No Template Conbtrol (NTC), **Positive Control** for your positive control, **Negative Control** for your negative control, and **Unknown** for the samples you wish to analyze.
  - In the field **Assay** choose the correct variant detection assay

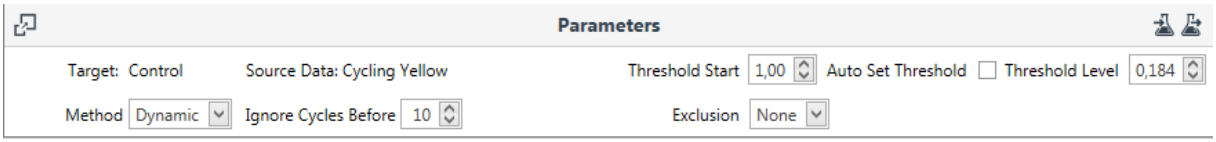
- Save the run setup, load your PCR tubes and start the run.

## Analysis of Variant Detection RealFast™ Assays:



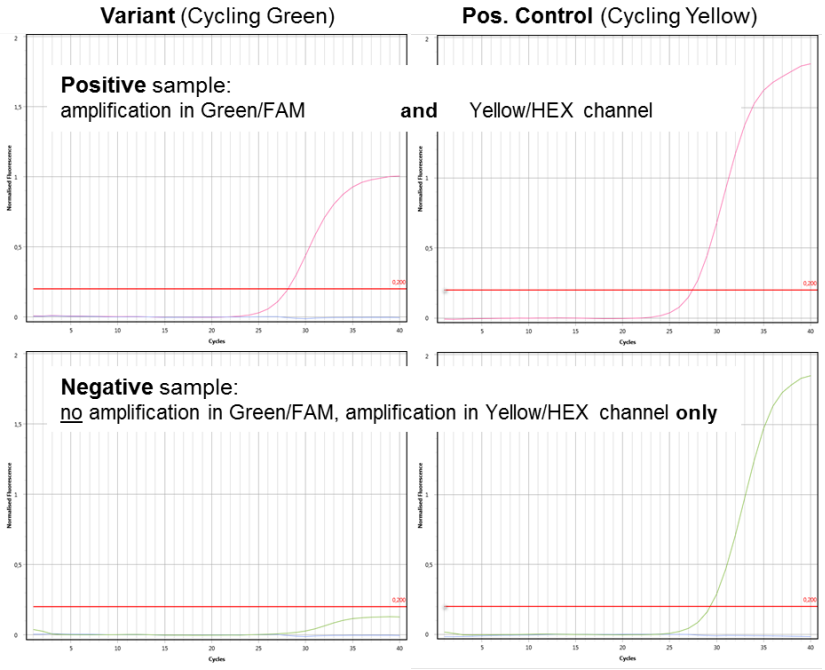

- Open the micPCR software.
- Click on the **Open** icon and select the data file you wish to analyze.
- Add a new **Cycling** analysis by clicking "**+**" in the **Analysis** section. In the drop-down menu you can select the targets, which you previously designed.

  - Select the target corresponding to the internal **PCR control** in the menu on the left.
  - Set **Ignore first cycles** to "10" within **Parameters** (optional).
    Choose **Dynamic** as **Method**. The **Exclusion** method should be **None**.
    Set the threshold value for the HEX channel at the onset of the exponential phase of the amplification curve.




  - Verify that all samples and the **Positive Control** show an amplification curve in the Yellow/HEX channel. Mouseover a sample in the **Samples** section highlights the corresponding amplification curve.

  - Select the target corresponding to your **gene of interest** in the menu on the left.
  - Set **Ignore first cycles** to "10" within **Parameters** (optional).
    Choose **Dynamic** as **Method**. The **Exclusion** method should be **None**.
    Set the threshold value for the FAM channel just above the background fluorescent signal generated by the Negative Control (e.g. HLA-B27 Negative Control).
  - In the **Cycling Analysis** window, only samples which are positive for the variant will show an amplification curve in the Green/FAM channel. Move the mouse over a curve to see the sample name.




- Optionally generate a report by clicking "**+**" next to **Reports** on the left.