

QuickGuide: RealFast™ Variant Detection on MIC qPCR Cycler

Setup for Variant Detection and Onco 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.

Chemistry Type: Hydrolysis Probes

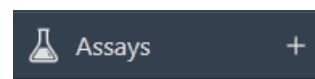
Targets		Oligonucleotides				
Name	Reporter Dye	Name	5' Label	Sequence	3' Label	Include
HLA-B27	FAM™	HLA-B27 Forward Primer				<input checked="" type="checkbox"/>
PCR Control	HEX™	HLA-B27 Reverse Primer				<input checked="" type="checkbox"/>
* Type here to add a new target		HLA-B27 Probe 1	FAM™		BHQ® - 1	<input checked="" type="checkbox"/>

» **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).
- **Save** your assay!

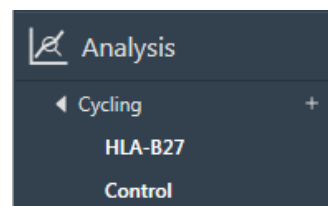
Setup a new run:

- 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 Control (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.

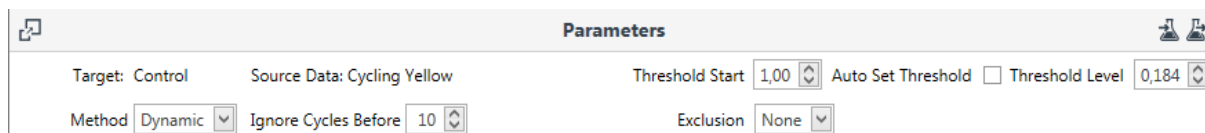


Analysis of Variant Detection and Onco RealFast™ Assays:

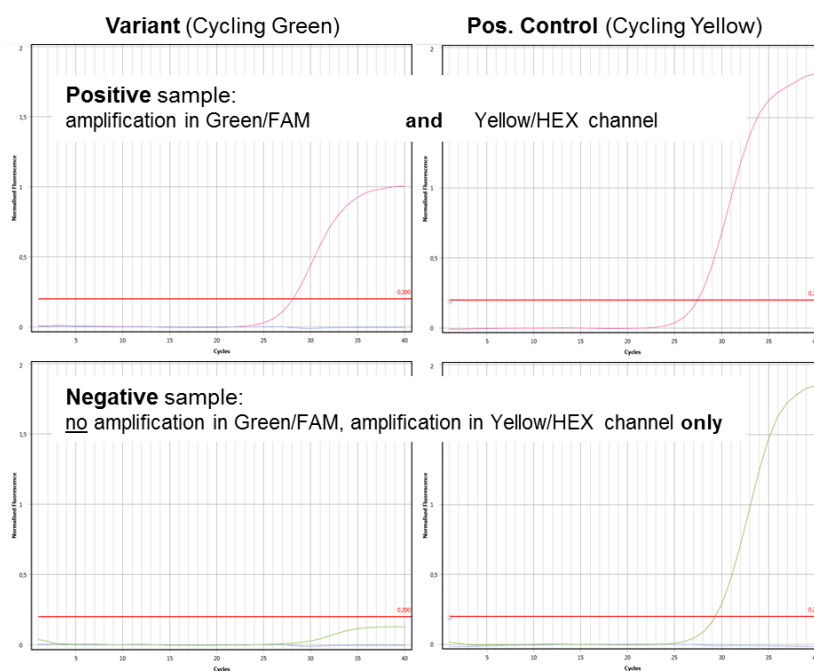
- Open the micPCR software.
- Click on the **Open** icon and select the data file you wish to analyze.
- Add a new **Cycling** analysis by clicking “+” in the **Analysis** section. In the drop-down menu you can select the targets, which you previously designed.



- 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.



» **Note:** For **Onco RealFast™** Assays (e.g. **EGFR T790M**), it is **mandatory** to do the analysis according to the section **Data Analysis / Interpretation of Results** in the respective **Instructions for Use**. «

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