

## QuickGuide: RealFast™ Genotyping on MIC qPCR Cycler

### Setup for Genotyping Assays:

- Open the micPCR software.

#### 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**.
  - Name your target (e.g. the name of the gene or assay) and optionally provide a **Description**.
  - Check the box **Contains Alleles** and click on “+” next to **Probes**.
  - Name your **Probes** and define the **5’** and **3’ Modifier** in the corresponding menu. Choose **5’-FAM** and **3’-BHQ1** for (most) **mutant probes**, and **5’-HEX** and **3’-BHQ1** for (most) **wild type probes**.

Chemistry Type

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+ Targets

Name	<input type="text" value="your target"/>	Amplicon Length	<input type="text" value="0"/>
Description	<input type="text"/>	Name	Sequence
Forward Primer	<input type="text"/>	<input type="text"/>	<input type="text"/>
Reverse Primer	<input type="text"/>	<input type="text"/>	<input type="text"/>

Contains Alleles

+ Probes

Name	5' Modifier	Sequence	3' Modifier
<input type="text" value="mutant probe"/>	<input type="text" value="FAM™"/>	<input type="text"/>	<input type="text" value="BHQ® - 1"/>
<input type="text" value="wild type probe"/>	<input type="text" value="HEX™"/>	<input type="text"/>	<input type="text" value="BHQ® - 1"/>

#### For multiplex RealFast™ Assays:

- Click on “+” next to **Targets** in case you are running a multiplex assay with four probes. Define your second target and your second set of probes according to the instructions of the mpx RealFast Assay kit. Usually **mutant probes** have to be assigned to **5’-ROX** and **3’-BHQ2** and wild type probes to **5’-Cy5** and **3’-BHQ2**.

+ Probes

Name	5' Modifier	Sequence	3' Modifier
<input type="text" value="mutant probe 1"/>	<input type="text" value="FAM™"/>	<input type="text"/>	<input type="text" value="BHQ® - 1"/>
<input type="text" value="wild type probe 1"/>	<input type="text" value="HEX™"/>	<input type="text"/>	<input type="text" value="BHQ® - 1"/>
<input type="text" value="mutant probe 2"/>	<input type="text" value="ROX™"/>	<input type="text"/>	<input type="text" value="BHQ® - 2"/>
<input type="text" value="wild type probe 2"/>	<input type="text" value="Cy™ 5"/>	<input type="text"/>	<input type="text" value="BHQ® - 2"/>

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

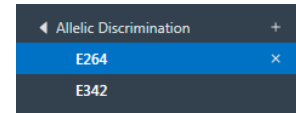
- Within the **Assay Setup** choose **Profile**.
  - Define in **Temperature Control** the mode **Fast TAQ (v3)** and in **Volume** **20µl**.
  - Setup the **PCR program**: **3 min** activation at **95°C** followed by 40 cycles of **15 sec** at **95°C** and **60 sec** at **60°C**. Data acquisition should 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 your assays, e.g. the assay for standard (singleplex) RealFast™ Assays (2 probes) or multiplex RealFast™ Assays (4 probes).
- Within **Run Setup** choose **Samples** and name your samples. Select the correct assay.
  - Save the run setup, load your PCR tubes and start the run.

### Analysis of Genotyping Assays:

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

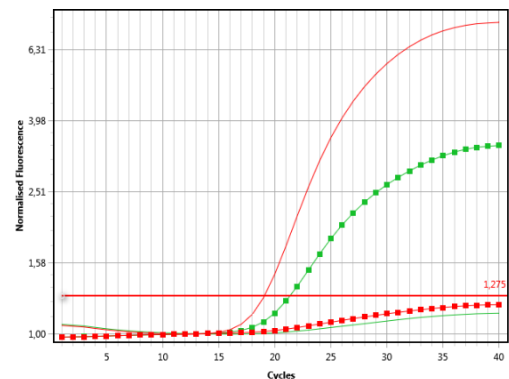


In case of **multiplex** RealFast™ Assays repeat this step with the second target. Optionally, you can name the analysis (double-click).

» **Note:** With **multiplex** RealFast™ Assays the two markers (corresponding to HEX/FAM and Cy5/ROX, respectively) have to be analyzed **one after the other** ! «

- Select the first target in the menu on the left side.
- Deselect all samples in the **Sample** window on the right side, except for the wildtype and mutant control.
- Adjust the threshold in the **Allelic Discrimination** window by moving it above the background signals.
- Move the mouse over a curve to see sample information and name of the probes.
- Within the **Parameters** window setup parameters for genotyping. For **NTC** no box shall be ticked, for **WT** the wild type allele should be selected, for **HET** both alleles should be selected, and for **MUT** only the mutant allele should be selected.
- Set **Ignore first cycles** to “10” within **Parameters**.
- We recommend to individually review all samples in the **Samples** window.

Parameters			
Genotype	E264V-WT	E264V-MUT	
NTC	<input type="checkbox"/>	<input type="checkbox"/>	x
WT	<input checked="" type="checkbox"/>	<input type="checkbox"/>	x
HET	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	x
MUT	<input type="checkbox"/>	<input checked="" type="checkbox"/>	x



- Wild type samples will show one curve corresponding to the wild type probe (mostly HEX) and the curve's plateau significantly exceeds the threshold. Results window: WT
- Mutant samples will show one curve corresponding to the mutant probe (mostly FAM) and the curve's plateau significantly exceeds the threshold. Results window: MUT
- Heterozygous samples show two curves and both plateaus exceed the threshold significantly. Results window: HET
  - If you are running a multiplex assay go to **Allelic Discrimination** on the left and select your second target
  - Repeat steps described above for the ROX and Cy5 probes
  - Optionally generate a report by clicking “+” next to **Reports** on the left.