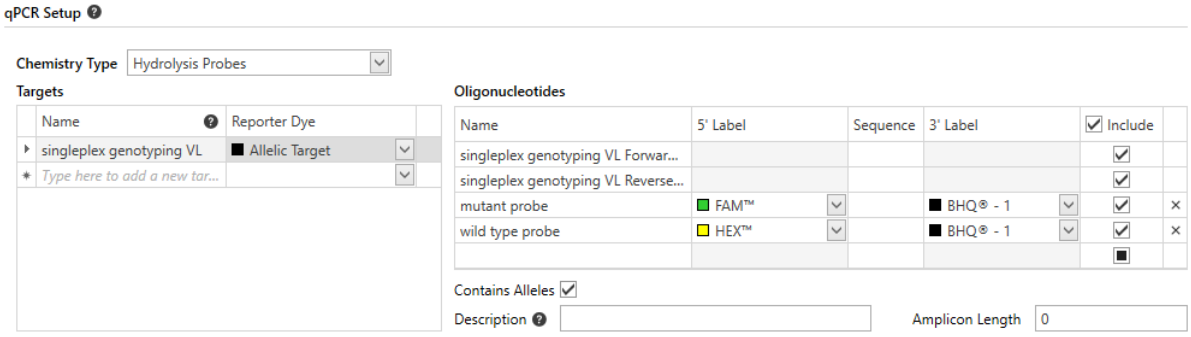
QuickGuide: RealFast™ Genotyping on MIC qPCR Cycler

Setup for Genotyping Assays:

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

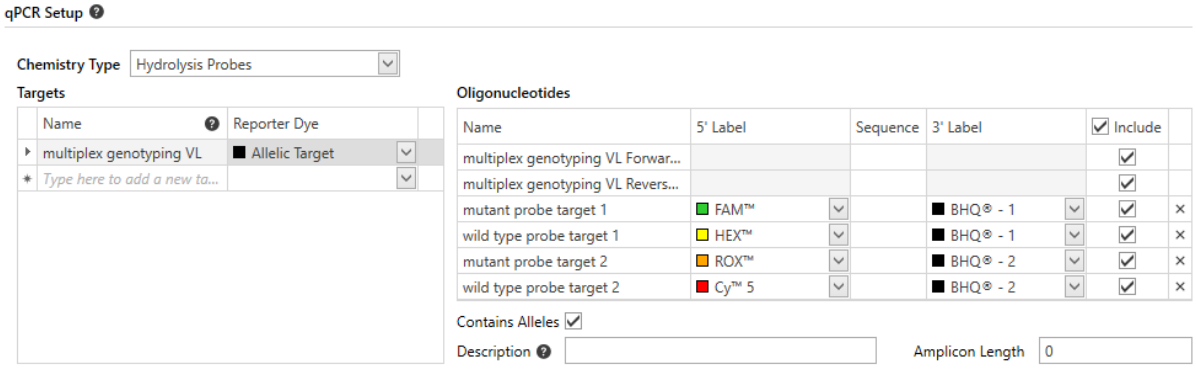
Definition of a new assay:

- Define a new assay by **New** > **Assay** in the menu bar.
- Within the **Assay Setup** choose **Information**.
  - Set the **Chemistry Type** to **Hydrolysis Probes**.
  - 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.



For **multiplex** RealFast™ Assays:

- Klick 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.



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

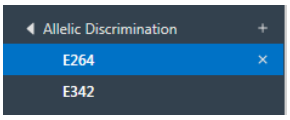
- Within the **Assay Setup** choose **Profile**.
  - Define in **Temperature Control** the mode **Fast TAQ (v3)** and in **Volume** 20 µl.
  - Setup the **PCR program**: **3 min** activation at **95°C** followed by 40 cycles of **15 sec** at **95°C** and **60 sec** at **60°C**. Data acquisition should 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.

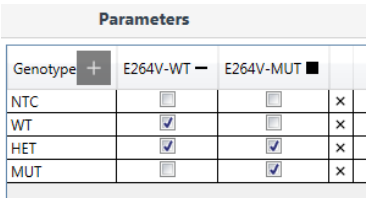
Analysis of Genotyping Assays:



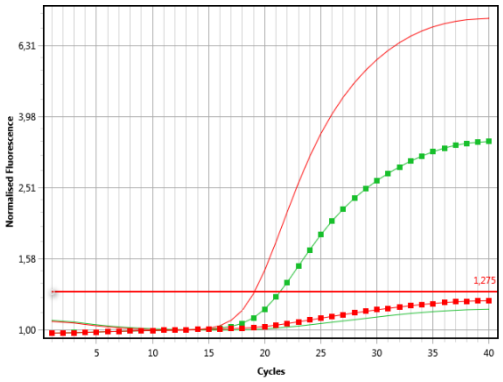
- Open the micPCR software.
- Click on the **Open** icon and select the data file you wish to analyze.
- In the **Analysis** section on the left side, add a new **Allelic Discrimination** analysis by clicking "**+**". In the drop down menu you can see the targets, which you previously designed.

  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.



- 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.