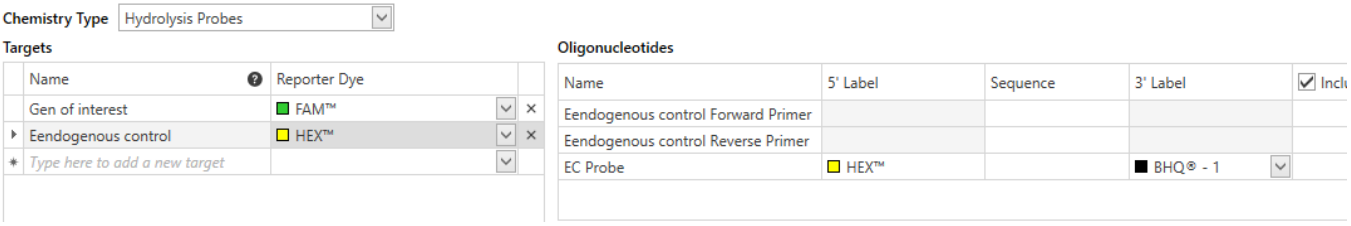
QuickGuide: RealFast™ CNV on MIC qPCR Cycler

Setup for Relative Quantitation 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 first target, which is the gene of interest, and choose your **Reporter Dye** (e.g. FAM)
  - In **Oligonucleotides** define the **3´ Label** for “gene of interest”-probe (e.g. BHQ-1).
  - In the **Targets** field, klick in the field under the first target (gene of interest) and name your second target, which is the endogenous control. Choose your **Reporter Dye** (e.g. HEX)
  - In **Oligonucleotides** define the **3´ Label** for the “endogenous control”-probe (e.g. BHQ-1).

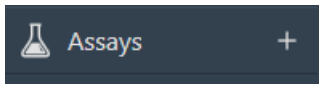


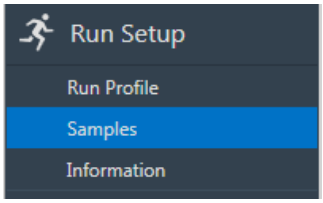
- Within the **Assay Setup** choose **Profile**.
  - Define in **Temperature Control** the mode **Fast TAQ (v3)** and in **Volume** 20 µl.
  - Setup the **PCR program**: 10 min **Hold** at 95°C followed by **Cycling** (40 cycles) for 15 sec at 95°C and 60 sec at 60°C. Data acquisition should be by default at the end of the 60°C step (camera symbol is black).

- **Safe** your assay!

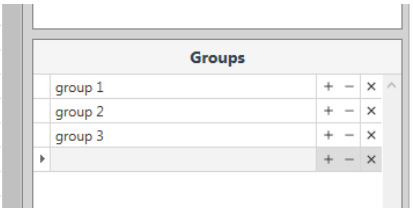
Setup a new run:

- Define a new run by **New** > **Run** in the menu bar.
  - In the menu on the left side look for **Assays** by clicking on “**+**”. Via **Shared Shortcuts** you can choose your safed assays.
- Within the **Run Setup** choose **Samples** and name your samples.
  - Make replicates of 3 for each sample, the NTC and the control.
  - In the drop-down menu for **Type** select **Unknown** for your samples and **NTC** for the negative control.
  - Define **Groups** in the field on the right side and assign them to the corresponding replicates.





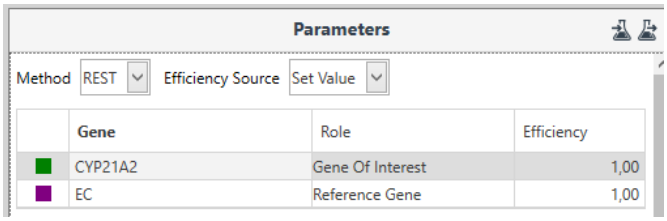
	Colour	Name	Type	Groups	Assay
1		Sample 1	Unknown	group 1	My CNV Assay
2		Sample 1	Unknown	group 1	My CNV Assay
3		Sample 1	Unknown	group 1	My CNV Assay
4		Sample 2	Unknown	group 2	My CNV Assay



  - In the **Samples** window select the correct assay from the **Assay** drop down menu.
  - Save the run setup, load your PCR tubes and start the run.

Analysis of Relative Quantitation Assays:

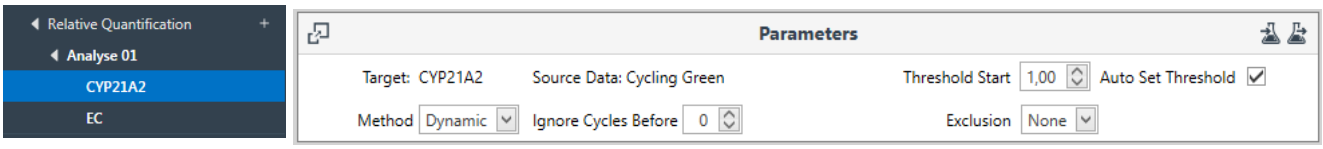
- Open the micPCR software.
- Click on the **Open** icon and select the data file you wish to analyze.
- In the **Analysis** section on the left side add a new **Relative Quantification** analysis by clicking "**+**". Optionally you can name the analysis.



- Within **Parameters** select **Set Value** as **Efficiency Source** and **REST** as **Method** from the drop down menu. Control within **Role** that your gene of interest is also correctly assigned as **Gene Of Interest**. The endogenous control of the assay has the role of a **Reference Gene**.
- Assign **Roles** to **Groups**: The NTC (negative control template) has the role **(None)**. The assay calibrator should have the role **Control**. Your unknown samples should be assigned to **Treatment**.

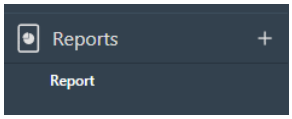
Group	Role
NTC	(None)
Calibrator	Control
BK102	Treatment
BK103	Treatment
BK111	Treatment
BK112	Treatment

- Within Relative Quantitation on the left side click on your gene of interest (here *CYP21A2*).
  - Select **Dynamic** mode as **Method**.
  - Set **Exclusion** to **None**
  - Control the standard deviation (σ) of your replicates. The value should be as low as possible. If needed exclude obvious outliers.



- Within Relative Quantitation on the left side click on your endogenous control (EC).
  - Select **Dynamic** mode as **Method**.
  - Set **Exclusion** to **None**
  - Control the standard deviation (σ) of your replicates. The value should be as low as possible. If needed exclude obvious outliers.

- Select again the analysis overview and review your **Results** in the **Expression Ratio** column. Refer to the product description for interpretation of your results. There you will find a table with expression ratios corresponding to the copy number of your gene of interest.



- You can create a customized report by clicking on "**+**" in the **Reports** section on the left.