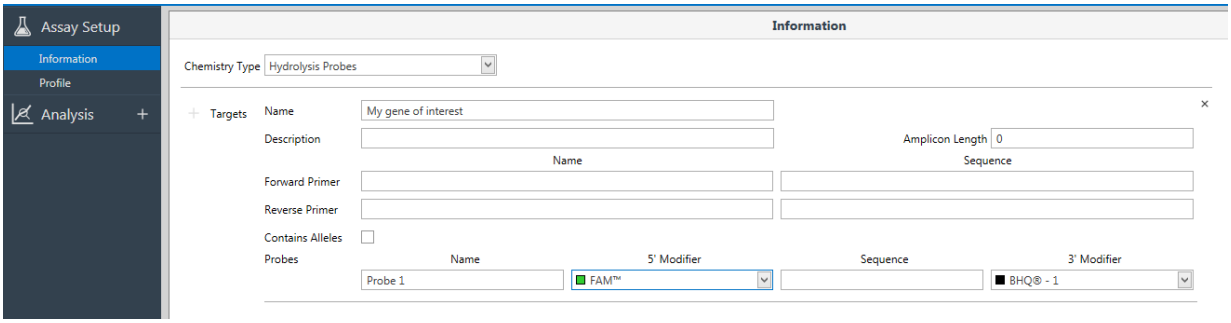
QuickGuide: RealFast™ CNV on MIC qPCR Cycler

Setup for Relative Quantitation 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 of interest) and optionally provide a **Description**.
  - Name your **Probes** and define the **5´**and **3´Modifier** in the corresponding menu (e.g. 5´-FAM and 3´-BHQ-1).
  - Klick on "**+**" next to **Targets** and define your second target (e.g. the endogenous control) and probe (e.g. 5´-HEX and 3´-BHQ-1).

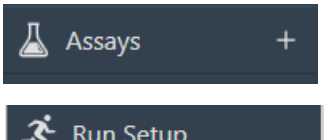


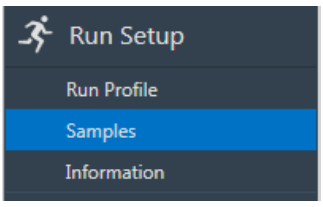
- Within the **Assay Setup** choose **Profile**.
  - Define in **Temperature Control** the mode **Fast TAQ (v3)** and in **Volume** 20µl.
  - Setup the **PCR program**: **10 min** activation at **95°C** followed by 40 times **15 sec** at **95°C** and **60 sec** at **60°C**. Data acquisition should be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

- **Safe** your assay!

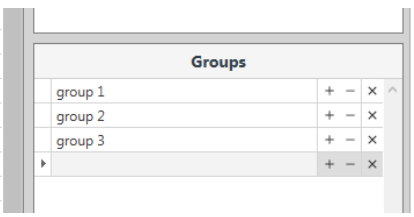
Setup a new run:

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





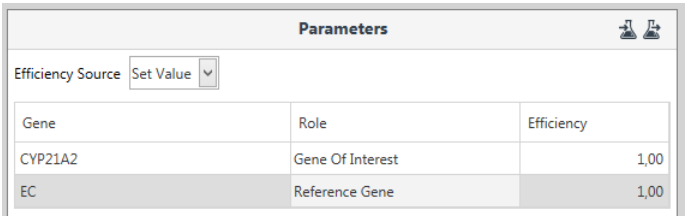
	Colour	Name	Type	Groups	Assay
1		Sample 1	Unknown	group 1	My CNV Assay
2		Sample 1	Unknown	group 1	My CNV Assay
3		Sample 1	Unknown	group 1	My CNV Assay
4		Sample 2	Unknown	group 2	My CNV Assay



  - 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:

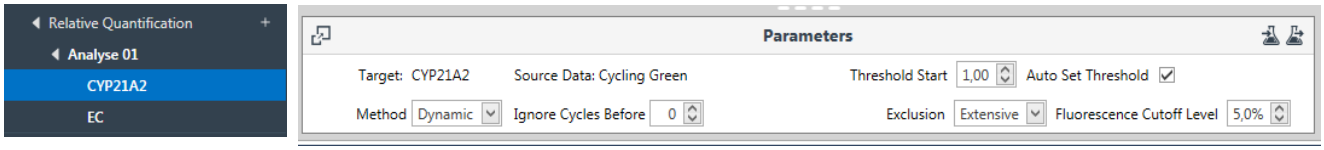
- Open the micPCR software.
- Click on the **Open** icon and select the data file you wish to analyze.
- In the **Analysis** section on the left side add a new **Relative Quantification** analysis by clicking "**+**". Optionally you can name the analysis.



- Within **Parameters** select **Set Value** 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´s 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**.
  - 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**.
  - 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.