

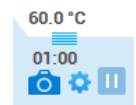
## QuickGuide: RealFast™ CNV on AB QuantStudio 5

### Setup of Relative Quantitation Assays:

- Open the ABI Quantstudio Design & Analysis Software and click **Create New Experiment**.
- In **Properties > Experiment Properties** select:
  - Instrument type: **QuantStudio™ 5 System**
  - Block type: **96-Well 0,1 mL**
  - Experiment type: **Comparative C<sub>T</sub> (ΔΔC<sub>T</sub>)**
  - Chemistry: **TaqMan® Reagents**
  - Run mode: **Standard**

- Go to **Method**

- Select a reaction volume of **20 µl**
- Define your PCR program:
  - Holding Stage: **10 min at 95°C**
  - PCR Stage: **40 cycles 15 sec at 95°C and 1 min at 60°C**. Make sure **Data Collection On** is enabled



- Go to **Plate > Advanced Setup:**


- Define **Targets** in the corresponding field:
  - Provide a name for your gene of interest and choose **FAM** as **Reporter** and **NFQ-MGB** as **Quencher**.
  - Add a new target by pushing the **Add** button.
  - Type **EC** (endogenous control) as target **Name** and choose **VIC** as **Reporter** and **NFQ-MGB** as **Quencher**.
- Define **Samples** in the corresponding field:
  - Type **Calibrator** in the field for **Sample Name**. This represents the positive control which is included in the assay kit.
  - Add new sample(s) by pushing the **Add** button and rename the field(s) according to the sample(s) you want to analyze.
- Define the **Negative Control Template:**
  - Select a replicate of three wells by ctrl-click.
  - Within the field **Targets** check boxes for the gene of interest (e.g. CYP21A2) and **EC**. In the **Task** field choose the icon **N** (Negative Control).
- Define your **Calibrator:**
  - Select a replicate of three wells by ctrl-click.
  - Within the field **Targets** check boxes for the gene of interest (e.g. CYP21A2) and **EC**. In the **Task** field choose the icon **U** (Unknown).
  - Check the box for the **Calibrator** within the field **Samples**.
- Define your **Samples:**
  - Select a replicate of three wells by ctrl-click.
  - Within the field **Targets** check boxes for the gene of interest (e.g. CYP21A2) and **EC**. In the **Task** field choose the icon **U** (Unknown).
  - Within **Samples** push the **Add** button, rename your sample and check the box for the sample you wish to assign to the selected wells.
- Within the tab **Quick Setup** choose **ROX** as **Passive Reference**, **Calibrator** as your **Reference Sample** and **EC** as **Endogenous Control**.
- Load your reaction plate into the QuantStudio 5 instrument and press **START RUN**.

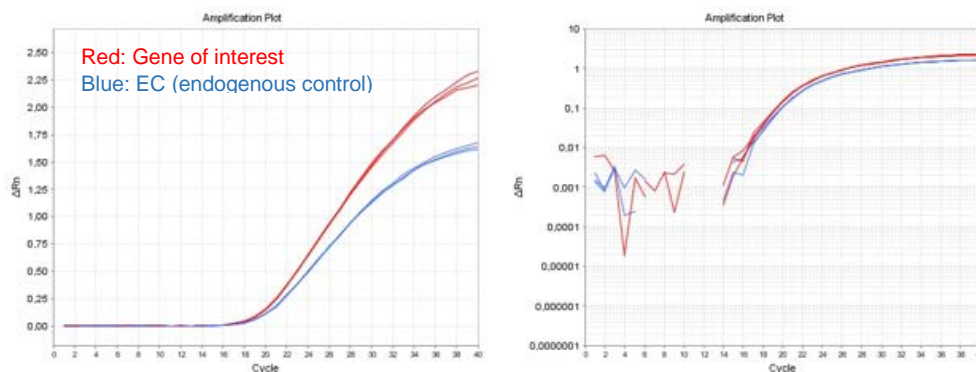
Targets							+ Add	Action
	Name	Reporter	Quencher	Comments	Task			
<input type="checkbox"/>	Gene of interest	FAM	NFQ-MGB				X	
<input type="checkbox"/>	EC	VIC	NFQ-MGB				X	

Targets							+ Add	Action
	Name	Reporter	Quencher	Comments	Task			
<input checked="" type="checkbox"/>	Gene of interest	FAM	NFQ-MGB			N	X	
<input checked="" type="checkbox"/>	EC	VIC	NFQ-MGB			N	X	


## Analysis of Relative Quantitation Assays:

After completing a run open the data file in the ABI Quantstudio Design & Analysis Software:

- Go to the **Results** tab. 
  - Click on the **Show Plot Settings** icon on the left side and adjust the **Plot Settings** to  **$\Delta Rn$  vs Cycle** (Plot Type), **Linear** or **Log** (Graph Type), **Target** (Plot Color)
- Press the **Analysis Settings** Button and go to  **$C_T$  Settings**.
  - Deactivate **Default Settings** and **Automatic Threshold**. Adjust the **Threshold** according to the settings in the **Assay Description**.
  - Make sure that **Algorithm Settings** are adjusted to **Baseline Threshold**, and press the **Apply** button.
- Select individual replicates in the **View Plate Layout** field and review your samples.
  - The interval between the curve for the gene of interest and for the **Endogenous Control (EC)** is related to the copy number variation.



Example: Amplification Plot of the Calibrator sample. Linear (left) and log (right) graph type.

- View the **Well Table** by pressing the corresponding icon 
  - Press the **View** button and customize the table by selecting parameters in the drop-down menu.
  - Review the **Relative Quantities (RQ)** and define the CNV status of your samples according to the Assay Description.
  - Select **Gene Expression** in the drop-down menu (instead of **Amplification Plot**) and select **RQ vs Sample** in the **Show Plot Settings**. The relative quantities of each sample are displayed as bar chart.
- To print a report click select **File > Print Report** in the upper menu bar:
  - Select data for the report according to your needs.
  - If you wish to export your data go to the **Export** tab and choose the file type and content