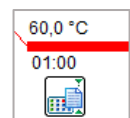


QuickGuide: RealFast™ CNV on StepOne™

Setup of Relative Quantitation Assays:

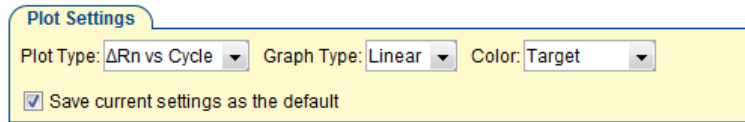
- Open the StepOne Software and click **Advanced Setup**.
 - In **Setup > Experiment Properties** select:
 - Instrument: **StepOne™** (48 Wells)
 - Type of experiment: **Quantitation – Comparative C_T (ΔΔC_T)**
 - Reagents: **TaqMan® Reagents**
 - Ramp speed: **Standard**
 - In **Setup > Plate Setup** go to **Define Targets and Samples**:
 - **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 corresponding button.
 - Type **EC** (endogenous control) as **Target Name** and choose **VIC** as **Reporter** and **NFQ-MGB** as **Quencher**.
- | Target Name | Reporter | Quencher | Color |
|-------------------------|----------|----------|-------|
| Gene of interest | FAM | NFQ-MGB | Red |
| EC (endogenous control) | VIC | NFQ-MGB | Blue |
- **Define Samples** in the corresponding field:
 - Type **Calibrator** in the field for **Sample Names**. This represents the positive control which is included in the assay kit.
 - **Add New Sample(s)** by pushing the corresponding button and rename the field(s) according to the sample(s) you want to analyze.
- In **Setup > Plate Setup** go to **Assign Targets and Samples**
 - Define the **Negative Control Template**:
 - Select a replicate of three wells by ctrl-click.
 - Within the field **Assign Target(s) to the selected wells** check boxes for the gene of interest (e.g. CYP21A2) and **EC**.
Click on the button **N** (Negative Control) in **Task**.
 - Define your **Calibrator**:
 - Select a replicate of three wells by ctrl-click.
 - Within the field **Assign Target(s) to the selected wells** check boxes for the gene of interest (e.g. CYP21A2) and **EC**. Click on the button **U** (Unknown) in **Task**.
 - Check the box for the **Calibrator** within the field **Assign Sample(s) to the selected wells**.
 - Within the field **Select relative quantitation settings** choose **Calibrator** as your **Reference Sample** and **EC** as **Endogenous Control**.
 - Within the field called **Select the dye to use as the passive reference** select **ROX**.
 - Define your **Samples**:
 - Select a replicate of three wells by ctrl-click.
 - Within the field **Assign Target(s) to the selected wells** check boxes for the gene of interest (e.g. CYP21A2) and **EC**. Click on the button **U** (Unknown) in **Task**.
 - Check the box for the **Sample** you wish to assign within the field called **Assign Sample(s) to the selected wells**.
- | Assign | Target | Task |
|-------------------------------------|---------------------|---|
| <input checked="" type="checkbox"/> | Gene of interest | <input type="checkbox"/> U <input type="checkbox"/> N |
| <input checked="" type="checkbox"/> | EC (endogenous ...) | <input type="checkbox"/> U <input type="checkbox"/> N |
- * Mixed U Unknown N Negative Control
- In **Setup > Run Method** go to **Graphical View**
 - Select a reaction volume of **20 µl**
 - Define your PCR program:
 - *optional: include Pre-PCR Read*
 - Holding Stage: **10 min at 95°C**
 - Cycling Stage: **40 cycles 15 sec at 95°C and 1 min at 60°C**. Make sure **Data Collection On** is enabled



Analysis of Relative Quantitation Assays:

After completing a run or after opening a genotyping data file the software displays the Experiment Menu **Analysis**:

- Results automatically appear in the **Amplification Plot**.
 - Adjust the **Plot Settings** to ΔRn vs Cycle (Plot Type), **Linear** or **Log** (Graph Type), **Target** (Color)

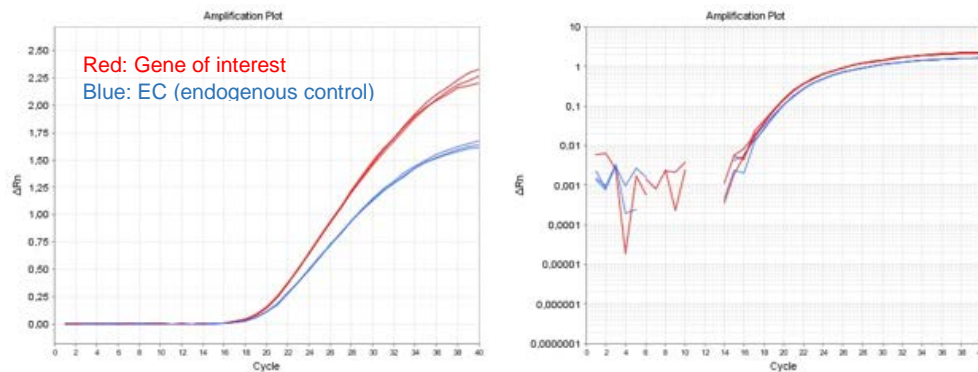


Plot Settings

Plot Type: ΔRn vs Cycle Graph Type: Linear Color: Target

Save current settings as the default

- Tick the box for **Show Threshold** in the **Options** field.
- Press the **Analysis Settings** Button and go to **C_T Settings**.
 - Adjust the **Threshold** according to the settings in the **Assay Description** and press the button Apply Analysis Settings.
- 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.

- Go to **View Well Table**.
 - Press the **Show in Table** button and customize the table.
 - Review the **Relative Quantities (RQ)** and define the CNV status of your samples according to the Assay Description.
 - Go to **Gene Expression** (left) and select **RQ vs Sample** in the **Plot settings**. The relative quantities of each sample are displayed as bar chart.
- To print a report click **Print Report** in the upper menu bar:
 - Select data for the report according to your needs.