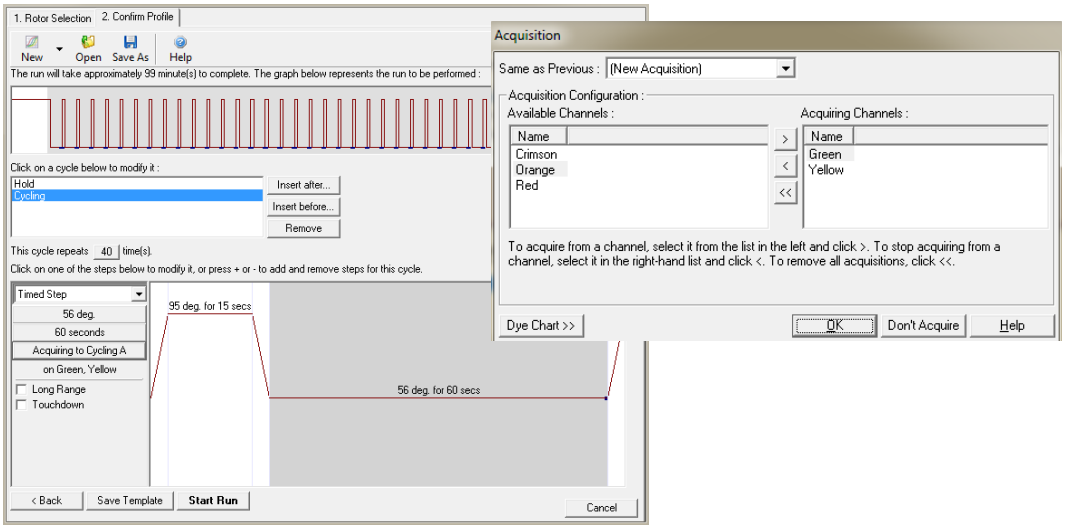
QuickGuide: RealFast™ CNV on Rotor-Gene® 6000

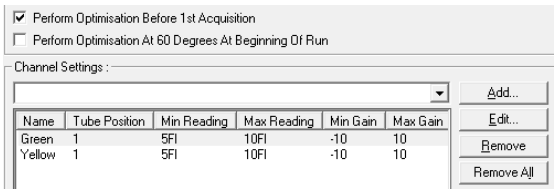
Set up of Relative Quantitation Assay:

- Launch the **Rotor-Gene® 6000** Software (QuickGuide is based on version 1.7).
- From the upper menu press **New** and select the **Advanced** mode. Choose **Two Step** by double clicking.
- The **New Run Wizard** window will appear.
  - Load samples
  - Select the **Rotor Type** and lock the ring by ticking the box **Locking Ring Attached**. Press **Next.**
  - Define a Reaction Volume of **20 µl.** *Optional*: *enter an operator name and run specific notes.* Press **Next.**
  - Click **Edit Profile …** and enter the following PCR program, confirm with **OK**:

	Cycles	Temperature [C°]	Duration [mm:ss]	Acquiring
Hold	-	95	10:00	-
Cycling	40	95	00:15	-
		36-well rotor: **56** 72-well rotor: **60**	01:00	Acquiring to Cycling A on **Green** and **Yellow**



  - Click **Gain Optimisation …**
  - From the drop down menu **Channel Settings** select **Green**. Press **Add …**
    Keep the default values in **Auto-Gain Optimisation Channel Settings** and confirm settings with **OK.**
  - From the drop down menu **Channel Settings** select **Yellow**. Press **Add …**
    Keep the default values in **Auto-Gain Optimisation Channel Settings** and confirm settings with **OK.**
  - Tick the box **Perform Optimisation Before 1st Acquisition.**
    Close window.
  - Proceed with **Next.**

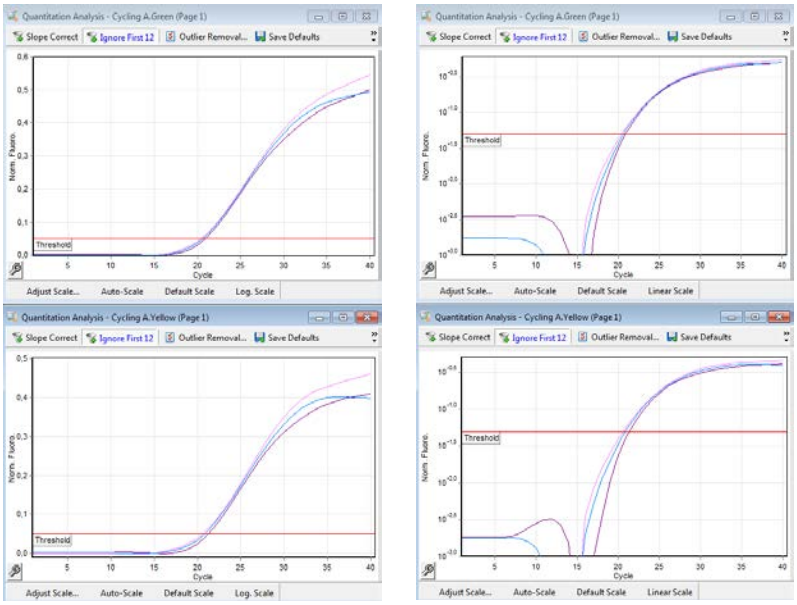


- Press **Start Run**.
- Define a file name and press **Save**.
- The **New Run Wizard** window will pop up, where the sample **Names** and **Types** (Unknown, NTC, Positive Control and Negative Control) can be defined.
- Press **Finish** and the **Run Progress** window will appear.

Analysis of Relative Quantitation Assay:

- Launch the **Rotor-Gene® 6000®** Software.
- In the upper menu press **Open** and load your CNV data file.
- The software displays the **Raw Channel (Cycling A. Green)** window.
- Press the **Analysis** button from the upper menu.
  - Choose **Other** > **Delta Delta CT**.
  - Double click on **New Analysis** and enter an analysis name.

- Tick the box **Validation Run Performed** and confirm with **Yes**.
- Tick the box **Gene of Interest Quantitation**, select **Cycling A. Green** and set **Threshold** according to the settings in the **Assay Description**.
- In the **Quantitation Analysis – Cycling A. Green** window press **Dynamic Tube** and set **Ignore First** to **12** cycles.
- Tick the box **Normaliser Quantitation**, select **Cycling A. Yellow** and set **Threshold** according to the settings in the **Assay Description**.
- In the **Quantitation Analysis – Cycling A. Yellow** window press **Dynamic Tube** and set **Ignore First** to **12** cycles.



Example: Amplification Plot of the Calibrator sample – linear and log graph type

- Tick the box **Calibrator Defined** and select the **2 copies control sample / plasmid (Calibrator)**.
- In the Relative Quant. Analysis window click (right mouse click) on the headline of the table to customize the table columns by selecting the following parameters: **Replicate Name – Norm. CT – Delta CT – DeltaDelta CT – Relative Concentration – Calibrator**.
- To generate a report press **Reports** form the menu and select the relevant report in the **Report Browser** to be shown and printed.
- Export your data via the **Export** button in the **Relative Quant. Analysis** window.