QuickGuide: RealFast™ Genotyping for Rotor-Gene® 6000

Setup for Genotyping Assays:

- Launch the **Rotor-Gene® 6000 software**.
- From the upper menu press **New.**
  - ➢ Within the New Run window select the **Advanced** tab.
  - ➢ Choose **Two Step** and press **New**.

- The **New Run Wizard** window will appear.
  - ➢ Select the **Rotor Type** and tick 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 ….**, enter the following PCR program and confirm with **OK**:

	Cycles	Temperature [C°]	Duration [mm:ss]	Acquiring
Hold	-	95	03:00	-
Cycling	40	95	00:15	-
		56	01:00	Acquiring to Cycling A on **Green** and **Yellow**
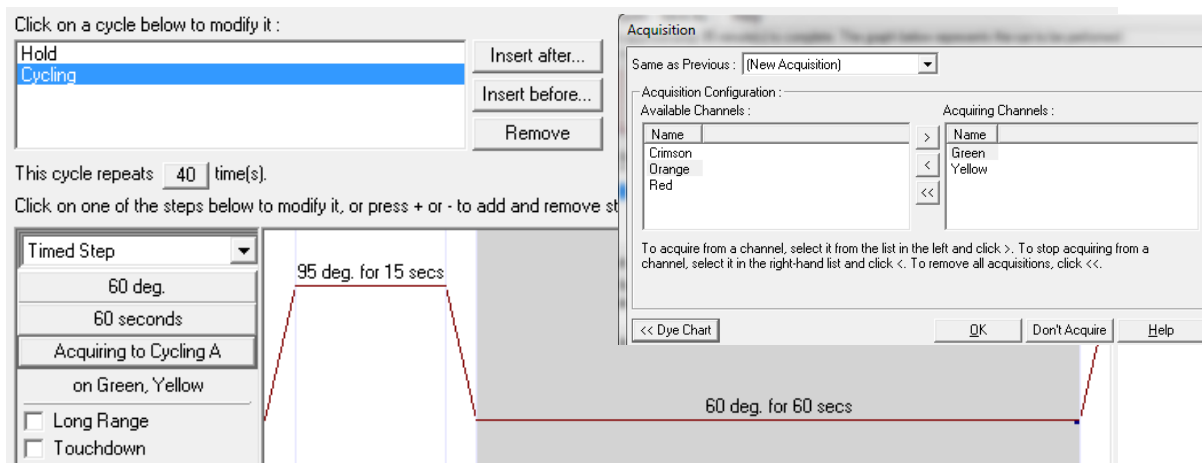
  - ➢ Click **Gain Optimisation ....**
  - ➢ Select **Green** from the drop down menu **Channel Settings.** Press **Add ....**
    Keep the default values in **Auto-Gain Optimisation Channel Settings** and confirm.
    Select **Yellow** from the drop down menu **Channel Settings.** Press **Add ....** Keep the default values in **Auto-Gain Optimisation Channel Settings** and confirm.
    Tick the box **Perform Optimisation Before 1ˢᵗ Acquisition.** Close window.
 - ➢ Proceed with **Next.**
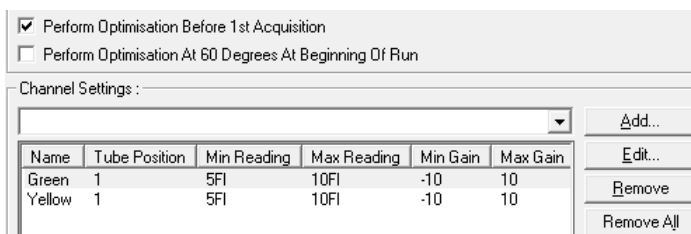
- Press **Start Run**.
- Define a file name and press **Save**.
- The **New Run Wizard** window will pop up, where the sample **Names** and **Types** (NTC, Unknown and Positive Controls) can be defined.
- Press **Finish** and the **Run Progress** window will appear.

Analysis of Genotyping Assays using Allelic Discrimination:

- Launch the **Rotor-Gene® 6000 software**.
- In the upper menu press **Open** and load your genotyping data file.
- The software displays the **Raw Channel (Cycling A. Green)** window.

- Press the **Analysis** button from the upper menu and choose **Allelic Discrimination**.
  - ➤ Select **Cycling A. Green** and **Cycling A. Yellow** and press **Show**.

- In the **Allelic Discrimination Analysis** window press **Genotypes.**
  - ➤ According to your assay, select the appropriate channels for **Wild Type** (mostly **Cycling A. Yellow**), **Mutant** (mostly **Cycling A. Green**) and **Heterozygous** (**Cycling A. Green** and **Cycling A. Yellow**). Confirm with **OK.**
  - ➤ Press **Dynamic Tube** and set **Ignore First** to **15 Cycles**.

Genotype	Reacting Channels	
Wild Type		Cycling A.Yellow
Heterozygous	Cycling A.Green	Cycling A.Yellow
Mutant	Cycling A.Green	

- Set the **Discrimination Threshold** as following:
  - ➤ Click on the threshold button ⬛ in the lower right panel. In the analysis graph click and drag the red threshold line above the background signals of the positive controls.
- Results are shown in the **Allelic Discrimination Results** window.
- To generate a report press **Reports** form the upper menu and select the relevant report in the **Report Browser** to be shown and printed.
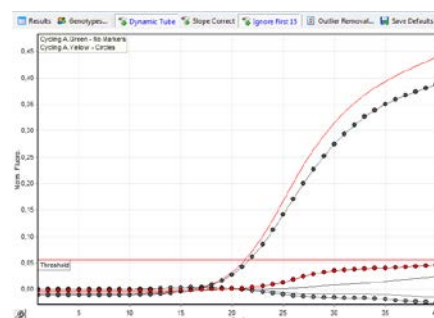
Analysis of Genotyping Assays using Scatter Plot:

- Launch the **Rotor-Gene® 6000 software**.
- In the upper menu press **Open** and load your genotyping data file.
- The software displays the **Raw Channel (Cycling A. Green)** window.

- Press the **Analysis** button from the upper menu and choose **Scatter Graph Analysis**.
  - ➤ Select **Cycling A. Green** and **Cycling A. Yellow** and press **Show**.

- In the **Scatter Analysis** window press **Genotypes.**
  - ➤ According to your assay, select the appropriate channels for **Wild Type** (mostly **Cycling A. Yellow**), **Mutant** (mostly **Cycling A. Green**) and **Heterozygous** (**Cycling A. Green** and **Cycling A. Yellow**). Confirm with **OK.**
  - ➤ Press **Dynamic Tube** and set **Ignore First** to **15 Cycles**.

Genotype	Reacting Channels	
Wild Type		Cycling A.Yellow
Heterozygous	Cycling A.Green	Cycling A.Yellow
Mutant	Cycling A.Green	

- Data points are shown in the **Scatter Analysis Graph** window.
  - ➤ Draw a rectangle around the data points in each quarter > define the corresponding genotypes as **Wild Type**, **Mutant** and **Heterozygous**, respectively, and the No-Template Control as **None**.

- Samples and their corresponding genotypes are shown in the **Scatter Analysis Result** window.

- To generate a report press **Reports** form the upper menu and select the relevant report in the **Report Browser** to be shown and printed.