

QuickGuide: RealFast[™] Genotyping on Rotor-Gene[®] 6000

Setup for Genotyping Assays:

- Launch the Rotor-Gene® 6000 software (QuickGuide is based on version 1.7).
- 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:

For **singleplex** RealFast[™] Assays use:

	Cycles	Temperature [C°]	Duration [mm:ss]	Acquiring
Hold	-	95	03:00	-
		95	00:15	-
Cycling	40	36-well rotor: 56	01:00	Acquiring to Cycling A
		72-well rotor: 60	01.00	on Green and Yellow

Click on a cycle below to modify it :		Acquisition
Hold	Insert after	Same as Previous : (New Acquisition)
Cycling	lucest before	Acquisition Configuration :
	Insert before	Available channels : Acquiring channels :
	Remove	Crimson
This cycle repeats 40 time(s).		Orange < Yellow Red <<
Click on one of the steps below to modify it, or press + or - to	add and remove :	
Timed Step 95 deg. for 15 secs		To acquire from a channel, select it from the list in the left and click >. To stop acquiring from a channel, select it in the right-hand list and click <. To remove all acquisitions, click <<.
60 seconds		
Acquiring to Cycling A on Green, Yellow Long Range Touchdown		60 deg. for 60 secs

For multiplex RealFast[™] Assays use:

	Cycles	Temperature [C°]	Duration [mm:ss]	Acquiring
Hold	-	95	03:00	-
		95	00:15	-
Cycling	40	36- well & 72-well rotor: 60	01:00	Acquiring to Cycling A on Green Yellow Red Orange

Red

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.

Perform Optimisation Before 1st Acquisition Ferform Optimisation At 60 Degrees At Beginning Of Run Channel Settings • Add. Edit. Name Tube Position Min Reading Max Reading Min Gain Max Gain 5FI 10FI -10 Green 1 10 **Remove** Yellow 5FI -10 10 10FI Orange 5FI 10FI -10 10 Remove All

10FI

-10

10

5FI

Repeat this procedure with **Orange** and **Red** for **multiplex** RealFast[™] Assays. Tick the box **Perform Optimisation Before 1**st **Acquisition.** Close window.

» **Note**: In case you carry out different RealFast[™] Assays within one run, it is advisable to do gain optimisation individually for each of the assays. Create new channels, e.g. FAM1, HEX1,.....for data acquisition. «

- > 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 (WT) (mostly Cycling A. Yellow), mutant (MUT) (mostly Cycling A. Green) and Heterozygous (Cycling A. Green and Cycling A. Yellow). Confirm with OK.

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

- Press Dynamic Tube. Optional: If necessary, use either Slope Correct or Ignore First for optimization of amplification curves, whatever fits best.
- 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.

» **Note**: With **multiplex** RealFastTM Assays the two markers (corresponding to HEX/FAM and Cy5/ROX, respectively) have to be analyzed **one after the other** ! «

- Repeat the analysis for the other marker by pressing Analysis → Allelic Discrimination. Then select Cycling A. Orange and Cycling A. Red and press
 Show.
- Proceed as described above. For most assays wild type (WT) corresponds to Cycling A. Red and mutant (MUT) to Cycling A. Orange.

» **Note**: For assignment of fluorophores to hydrolysis probes see Instructions for Use of the respective RealFastTM Assay. «

Wild Type

Mutant

Heterozygous



Cycling A.Orange	Cycling A.Red
Cycling A.Orange	

Cycling A.Red

\sim	10	
/	13	
~	10	

Setting	Value
FAM1 Gain	5
Green Gain	5
HEX1 Gain	5
Yellow Gain	5
Auto-Gain Optimisation	Before First Acquisition
Rotor	72-Well Rotor
Sample Layout	1, 2, 3,
Reaction Volume (in microliters)	20

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 (WT) (mostly Cycling A. Yellow), mutant (MUT) (mostly Cycling A.
 Green) and heterozygous (Cycling A. Green and Cycling A. Yellow). Confirm with OK.

⊳	Press Dynamic Tube.
	Optional: If necessary, use either Slope Correct or Ignore
	<i>First</i> for optimization of amplification curves, whatever fits
	best.

- 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.

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

Scatter Analysis Graph - Cycling	A.Green, Cycli Heterozygous
Mutant	Heterozygcus
None	Wild Type Wild Type

- 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.

» **Note**: With **multiplex** RealFastTM Assays the two markers (corresponding to HEX/FAM and Cy5/ROX, respectively) have to be analyzed **one after the other** ! «

- Repeat the analysis for the other marker by pressing Analysis → Allelic Discrimination. Then select Cycling A. Orange and Cycling A. Red and press Show.
- Proceed as described above. For most assays Wild Type corresponds to Cycling A. Red and Mutant to Cycling A. Orange.

Genotype	Reacting Channels	
Wild Type		Cycling A.Red
Heterozygous	Cycling A.Orange	Cycling A.Red
Mutant	Cycling A.Orange	

» **Note**: For assignment of fluorophores to hydrolysis probes see Instructions for Use of the respective RealFastTM Assay. «