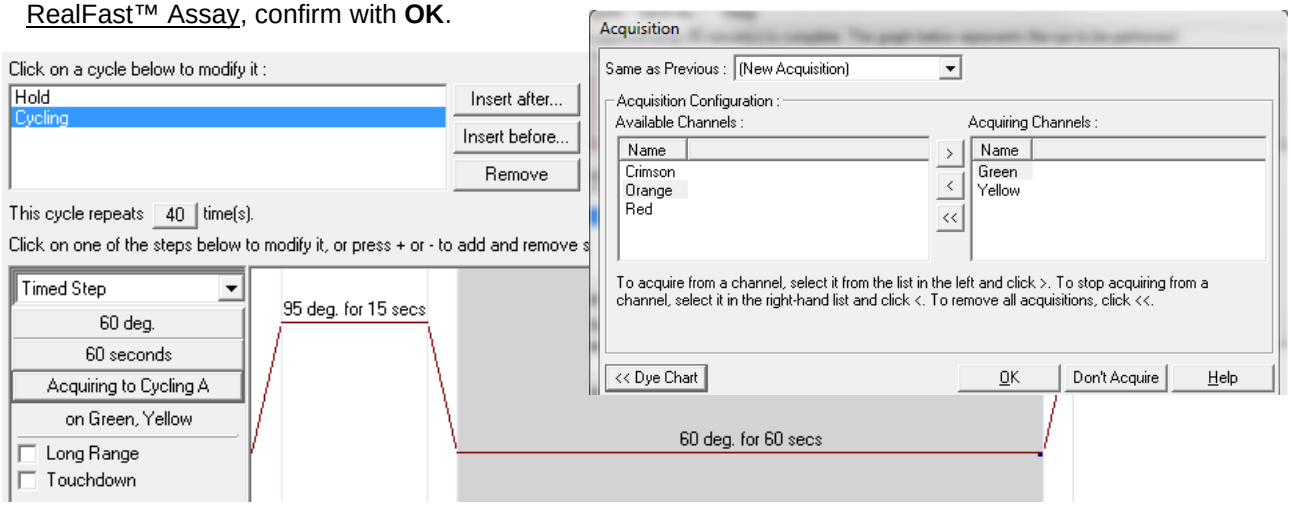
QuickGuide: RealFast™ Variant Detection on Rotor-Gene® 6000

Setup for Variant Detection and Onco RealFast™ 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**.

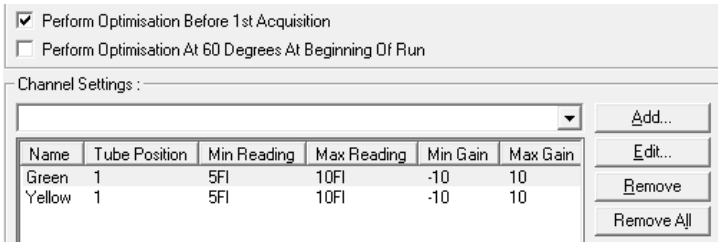
- The **New Run Wizard** window will appear.
  - Select the **Rotor Type** and lock the ring by ticking the box **Locking Ring Attached**. Press **Next.**
  - Define a **Reaction Volume** of **20 µl.** *Optional*: enter an operator name and run specific notes. Press **Next.**
  - Click **Edit Profile …** and enter the PCR program according to the Instructions for Use of your RealFast™ Assay, confirm with **OK**.

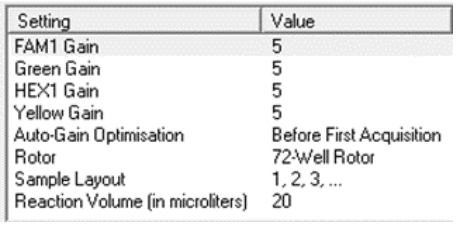


  - Click **Gain Optimisation …**

  - Select **Acquiring Channels** 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.





*» **Note**: In case you conduct 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** (Unknown, NTC, Positive Control and Negative Control) can be defined.
- Press **Finish** and the **Run Progress** window will appear.

Analysis of Variant Detection and Onco RealFast™ Assays:

- 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.

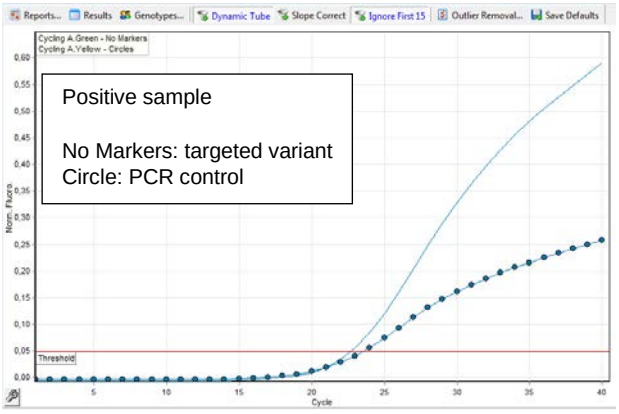
For **Variant Detection** RealFast™ Assays (e.g. HLA-B27, HLA-B57) only:

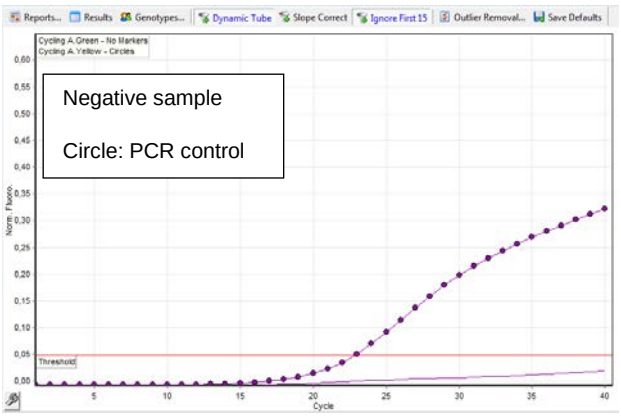
- Press the **Analysis** button from the upper menu and choose **Allelic Discrimination.**
  - Select Cycling A. Green and Cycling A. Yellow and press Show.

- In the **Allelic Discrimination Analysis** window press **Genotypes.**
  - Select the appropriate channels:
    Sample positive for **targeted variant** and **Positive Control:** Cycling A. Green and Yellow
    Sample negative for **targeted variant** and **Negative Control:** Cycling A. Yellow
    Confirm with **OK.**
  - Press **Dynamic Tube.**
    *Optional: If necessary, use either **Slope Correct** or **Ignor 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 > a red threshold line will appear. Adjust the threshold above the background signals of the Negative Control by left-mouse click *(e.g. set the threshold value for the FAM channel just above the background fluorescent signal generated by the HLA-B27 Negative Control. Set the threshold value for the HEX channel at the onset of the exponential phase of the amplification curve).*

- Results are shown in the **Allelic Discrimination Results** window.





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

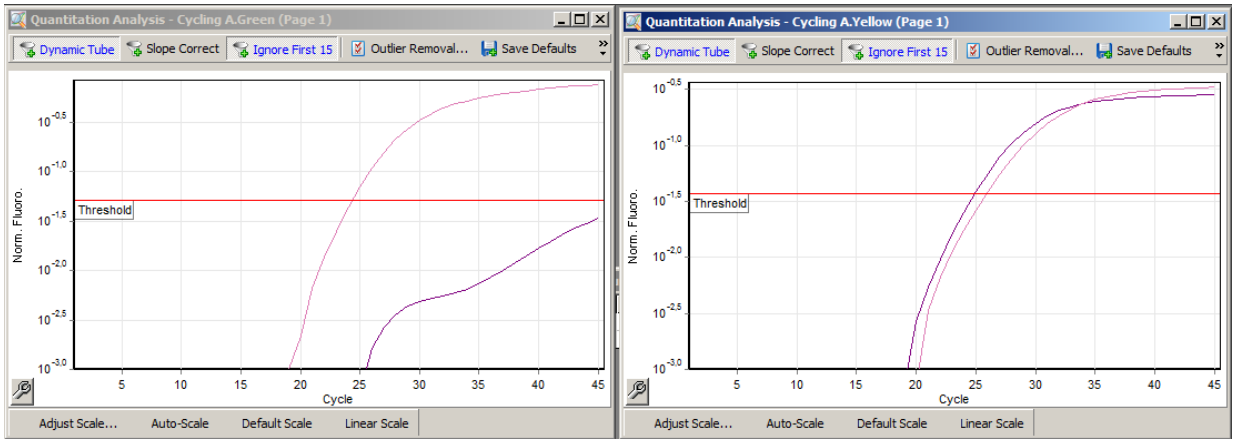
- To export your data go to **File > Save As > Excel Analysis Sheet** (*.csv), name the file and Excel will automatically open data from the analysis window.

No.	Name	Genotype	Cycling A.Green	Cycling A.Yellow
49	NTC	NTC	No Reaction	No Reaction
50	NTC	NTC	No Reaction	No Reaction
52	HLA-B5701 Pos.Ctrl.	HLA-B5701 positiv	Reaction	Reaction
53	P1	HLA-B5701 positiv	Reaction	Reaction
54	P2	HLA-B5701 positiv	Reaction	Reaction
55	P3	HLA-B5701 positiv	Reaction	Reaction
57	1	HLA-B5701 negativ	No Reaction	Reaction
58	2	HLA-B5701 negativ	No Reaction	Reaction
59	3	HLA-B5701 negativ	No Reaction	Reaction

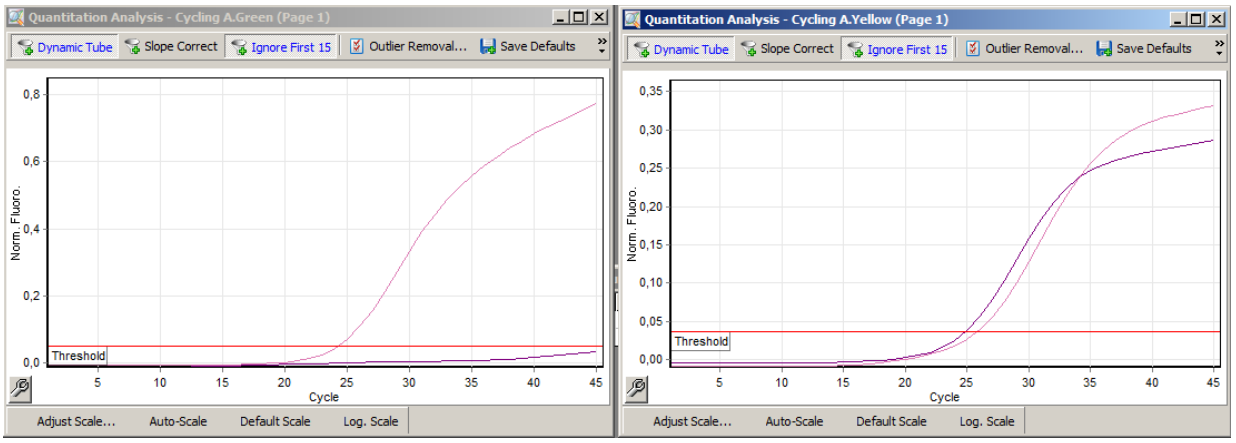
For **Onco** RealFast™ Assays (e.g. EGFR T790M) only:

- Press the **Analysis** button from the upper menu and choose **Quantitation Analysis.**
  - ➢ Select Cycling A. Green and press Show, select Cycling A. Yellow and press Show.
  - ➢ Press **Dynamic Tube.**
    *Optional: If necessary, use either **Slope Correct** or **Ignor First 15** for optimization of amplification curves, whatever fits best.*

- Set the **Discrimination Threshold** as following:
  - ➢ Select **Log. Scale** in the **Quantitation Analysis** window**.** Click on the threshold button in the lower right panel > a red threshold line will appear. Set the threshold in the exponential phase of the amplification curves.



*Optional: check the correct threshold setting also in the **Linear Scale** view of the **Quantitation Analysis** window.*



  - ➢ C_t values of FAM and HEX signals are displayed in the **Quant. Results** windows – **Cycling A. Green** and **Cycling A Yellow**, respectively.

*» **Note**: For **Onco** RealFast™ Assays (e.g. EGFR T790M), it is **mandatory** to do the analysis according to the section Data Analysis / Interpretation of Results in the respective Instructions for Use. «*

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

- To export your data go to **File > Save As > Excel Analysis Sheet** (*.csv), name the file and Excel will automatically open data from the analysis window.