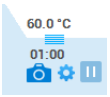
QuickGuide: RealFast™ Variant Detection on AB QuantStudio 5

Setup for Variant Detection and Onco RealFast™ Assays:

- Open the QuantStudio Design & Analysis Software (QuickGuide is based on version 1.5.2) and click **Create New Experiment**.

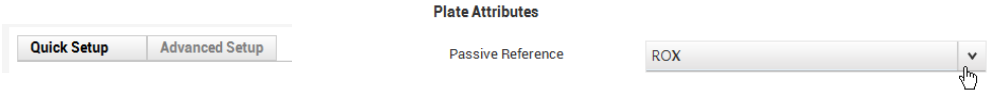
- In **Experiment Properties** define
  Name: Name of experiment
  Instrument type: **QuantStudio 5 System**
  Block type: **96-Well 0.2 ml Block**
  Experiment type: **Standard Curve**
  Chemistry: **TaqMan® Reagents**
  Run mode: **Standard**

- In **Method** select a sample volume of **20 µl** and setup the **PCR program** according to the Instructions for Use (IFU) of your RealFast™ Assay.
  - Make sure **Data Collection On** is enabled.



- In **Plate → Quick Setup → Plate Attributes** define **None** as **Passive Reference** dye for Variant Detection RealFast™ Assays (e.g. HLA-B27, HLA-B5701, other HLA-Assays) only.

  Select **ROX** as passive **reference dye** for Onco RealFast™ Assays (e.g. EGFR T790M) only.



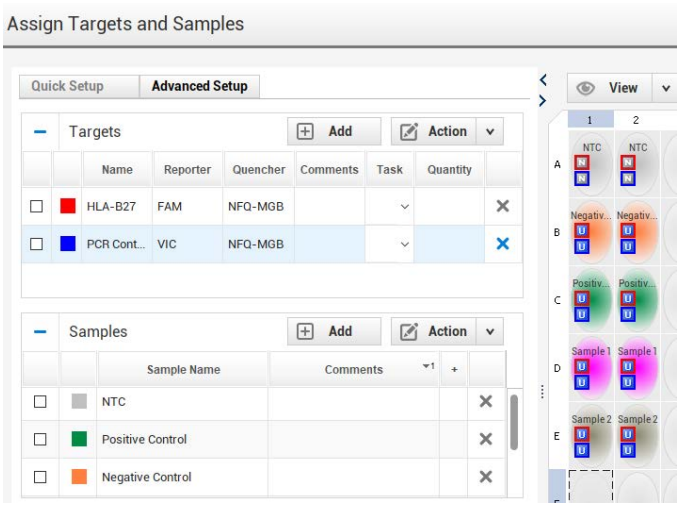
- In **Plate → Advanced Setup** assign **Targets** and **Samples** to selected wells.
  - Select **Targets** and edit and enter assays. Keep default settings for reporter dyes:
    Target 1 = FAM (corresponds to FAM-labeled probe). Quencher = NFQ-MGB
    Target 2 = VIC (corresponds to HEX-labeled probe). Quencher = NFQ-MGB

*» **Note**: In case you want to run several **different** RealFast™ Assays in parallel, add targets for each of the markers to be analyzed. For assignment of fluorophores to hydrolysis probes see Instructions for Use of the respective RealFast™ Assay. «*

  - Click **Add** repeatedly to enter all your samples and controls. Assign **Targets** and **Samples** and select the total number of wells by click+drag in the plate layout.

  - Define your **No Template Control** (NTC). Select a replicate (2 wells) in the plate layout by click+drag. Select "**N**" for both targets in the **Task** field.

  - Define your **Samples** and **Controls** (e.g. HLA-B27 Negative Control, HLA-B27 Positive Control). Select well(s) in the plate layout. Assign sample(s) to selected well(s). Select "**U**" for both targets in the **Task** field.



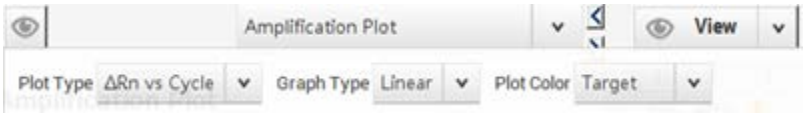
  - **Save** the experiment.

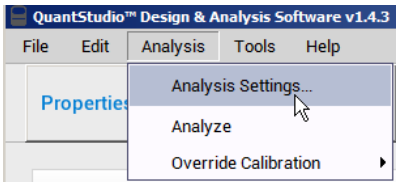
  - Load your PCR tubes / plate into the QuantStudio™ instrument and press **START RUN** button to start the run.

Analysis of Variant Detection and Onco RealFast™ Assays:

After completing a run or after opening an **Existing Experiment** the software displays the **Results** tab.

- The **Amplification Plot** appears automatically**.**
- If you have **different** RealFast™ Assays in one run, select the appropriate target by clicking **Action** and using the dropdown menu.
- Press the button **Show Plot Settings** that is indicated by an eye-symbol and adjust the parameters:
  - Plot Type: **ΔRn Vs. Cycle**
  - Graph Type: **Linear**
  - Plot Color: **Target**
  - Move to **Analysis Settings.**





For **Variant Detection** RealFast™ Assays (e.g. HLA-B27, HLA-B5701, other HLA-Assays) only:

- Disable **Default Settings**, **Automatic Threshold** and **Automated Baseline**. Select the individual targets and adjust the **Threshold** according to the IFU. **Baseline Start Cycle** should be set to "3" and **Baseline End Cycle** to "15". Adjust the threshold manually. *Set the threshold value for the FAM channel just above the background fluorescent signal generated by the e.g. HLA-B27 Negative Control. Set the threshold value for the HEX channel at the onset of the exponential phase of the amplification curve.*

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

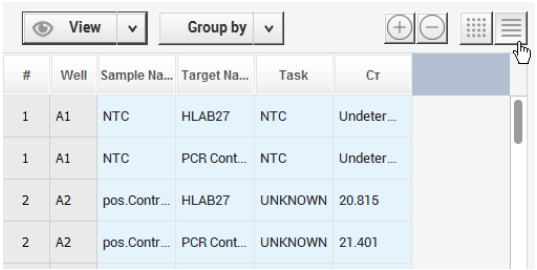
- Enable **Automatic Threshold** and **Automated Baseline**.

*» **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 IFU. «*

- Confirm the analysis settings by pressing the **Apply** button.
- Review your samples by selecting individual wells in the plate layout.

*» **Note:** Threshold settings of individual targets can be alternatively adjusted in the **Show Plot Settings** window. «*

- To show results as table click the **Well Table** icon in the upper right corner. Adjust the table according to your needs by selecting/deselecting the listed features in the **View** dropdown menu.
- Verify absence of any contamination in the **Negative Control**. No amplification should be visible.



- To print a report, select **File > Print Report** in the upper menu bar:
  - Select data for the report according to your needs.
  - If you wish to export your data go to the **Export** tab and choose the file type and content