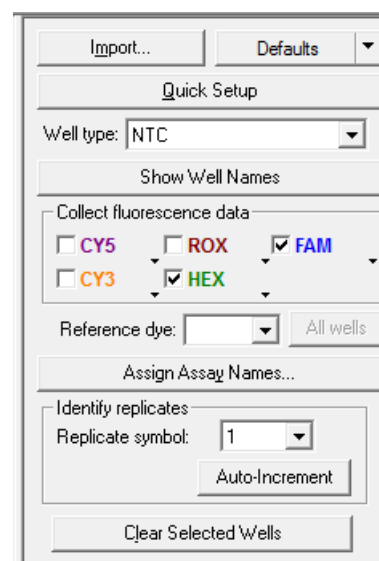


QuickGuide: RealFast™ Variant Detection on Agilent Mx3500P

Setup for Variant Detection Assays:

- Launch the **MxPro** Software (QuickGuide is based on version 4.10) and select **Quantitative PCR (Multiple Standards)** from the pop-up window and press **OK**.

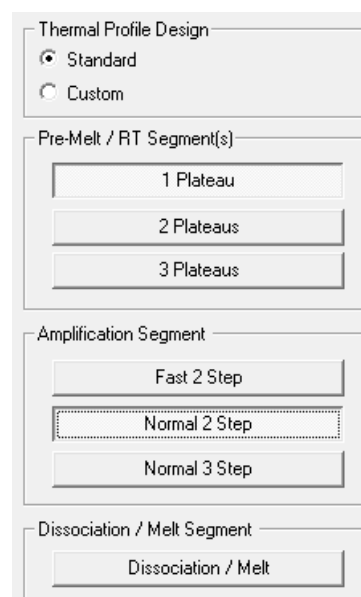
- Within the **Plate Setup** define your **No Template Control (NTC)**.
 - Select well(s) within the plate by mouse click or ctrl-click.
 - Choose **NTC** within the menu for the **Well type** (top-right).
 - Tick the box for **FAM** and **HEX** within the **Collect fluorescence data** field.
 - Click on the **Assign Assay Names** button. In the **Well Information** window, you can give a name to samples (e.g. NTC).



- Define your **Samples**.
 - Select wells for all your unknown samples by ctrl+mouse click or click+drag.
 - Choose **Unknown** within the menu for the **Well type**.
 - Tick the box for **FAM** and **HEX** within the **Collect fluorescence data** field.
 - Click on individual well in the **Plate Setup** to enter the sample name in the **Assign Assay Names** menu.
- Define your **Positive Control** and **Negative Control** for the targeted variant.
 - Select a well by mouse click
 - Choose **FAM Positive Control** within the menu for the **Well type**.
 - Choose **HEX Negative Control** within the menu for the **Well type**.
 - Tick the box for **FAM** and **HEX** within the **Collect fluorescence data** field.
 - Click on the **Assign Assay Names** button and give a name to the controls.

- Click on the **Next** button (bottom-right).

- Setup the PCR program.
 - In **Amplification Segment** select **Normal 2 Step**.
 - Adjust the **Thermal Profile** by clicking on the respective numbers in the graph:

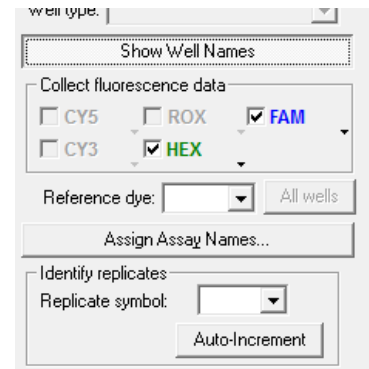
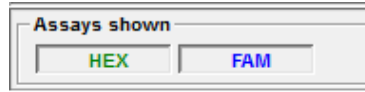


	Cycles	Temperature(°C)	Duration (mm:ss)	Data Collection
Segment 1	1	95°C	03:00	none
Segment 2	40	95°C	00:15	none
		60°C	01:00	Endpoint

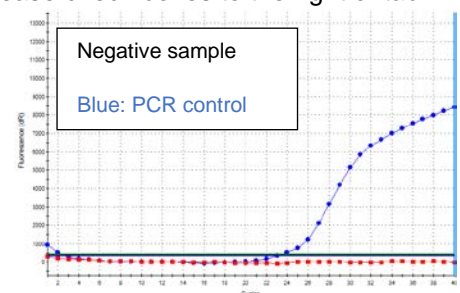
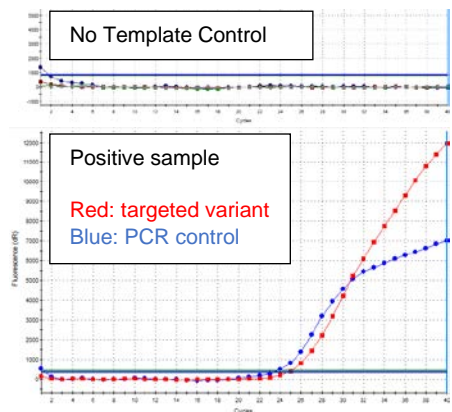
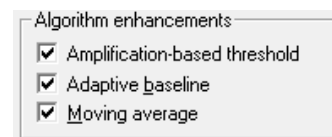
- Save your experiment and start the run.

Analysis of Variant Detection Assays:

- Launch the **MxPro** Software and load the file containing your data with **File > Open**. The software displays the **Plate Setup** window.
 - Make sure that buttons for **HEX**, **FAM** (or assay names) as well as **Show Well Names** are active.



- Control the settings on the right part of the window. FAM and HEX should be enabled for **fluorescence data collection**.
- Press the **Analysis** button in the top-right corner of the window.
 - Press **All** in the top-left corner of the plate to select / deselect all wells for analysis. Select individual wells by clicking (click-drag, ctrl-click) into the corresponding field of the plate. Select rows or columns by highlighting A-H or 1-12 in the plate.
 - Keep default settings in the **Algorithm enhancements** field (all boxes ticked)
 - Click on the **Results** tab to display your results.
 - Select the **Area to analyze** in the top-right corner. Start with **Amplification plots**.
 - Select the **No Template Control NTC**. No amplification should be visible in both channels.
 - Select the **Negative Control** and move the threshold line for HEX and FAM above the background signal of FAM (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).
 - Review your **Unknown** samples. Samples positive for the targeted variant, as well as the Positive Control will show a signal in the FAM and the HEX channel. Samples negative for the targeted variant, as well as the Negative Control will show a signal in the HEX channel only.
 - Select **Text report**. Double-clicking on a sample in the chart opens the corresponding amplification plot as floating window. In order to customize your text results please check boxes to the right of tab.
- Export your data via **File > Export Text Report** and choose your file format.



» **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 Instructions for Use*. «