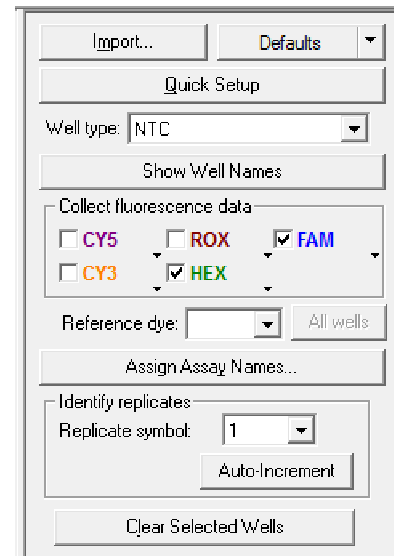
QuickGuide: RealFast™ Variant Detection on Agilent Mx3500P

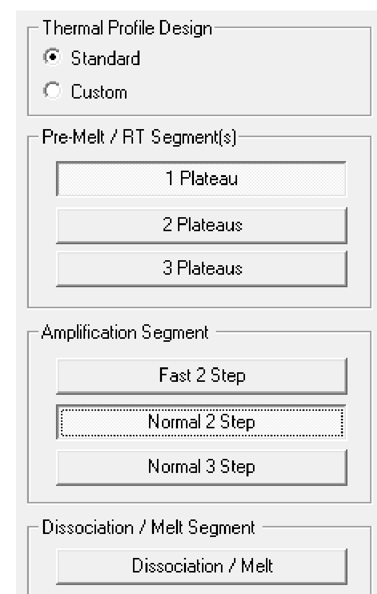
<u>Setup for Variant Detection Assays:</u>

- 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).

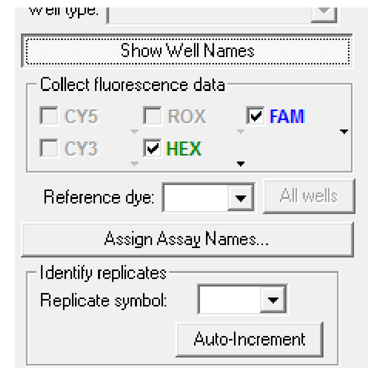
- Setup the PCR program.
  - ➢ In **Amplification Segment** select **Normal 2 Step**.
  - ➢ Adjust the **Thermal Profile** by clicking on the respective numbers in the graph:

	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.

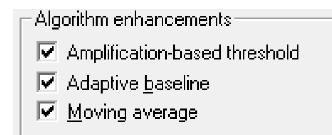
Analysis of Variant Detection Assays:

- 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.

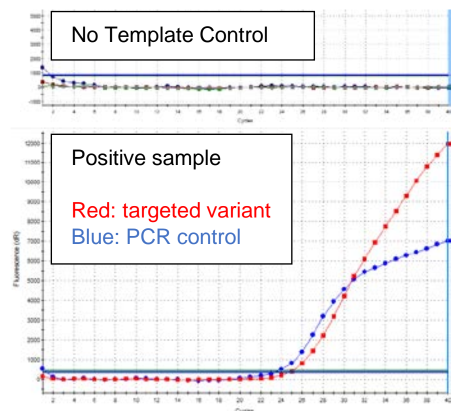


  - Control the settings on the right part of the window. FAM and HEX should be enabled for **fluorescence data collection**.

- 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.