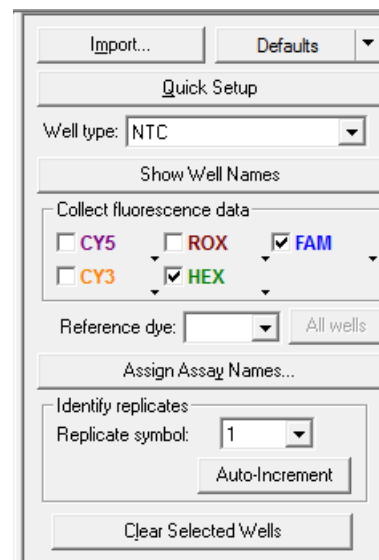


## QuickGuide: RealFast™ Variant Detection on Agilent Mx3500P

### Setup for Variant Detection Assays:

- Launch the **MxPro** Software 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:



Import... Defaults

Quick Setup

Well type: NTC

Show Well Names

Collect fluorescence data

CY5  ROX  FAM

CY3  HEX

Reference dye: All wells

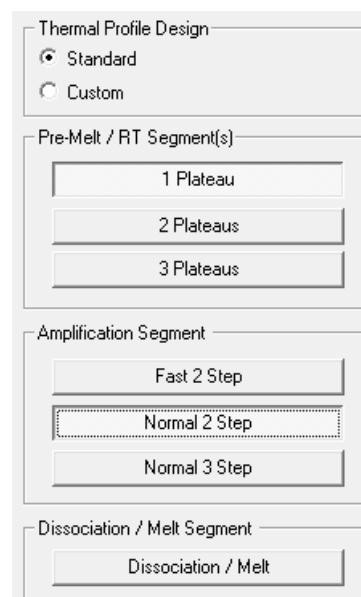
Assign Assay Names...

Identify replicates

Replicate symbol: 1

Auto-Increment

Clear Selected Wells



Thermal Profile Design

Standard

Custom

Pre-Melt / RT Segment(s)

1 Plateau

2 Plateaus

3 Plateaus

Amplification Segment

Fast 2 Step

Normal 2 Step

Normal 3 Step

Dissociation / Melt Segment

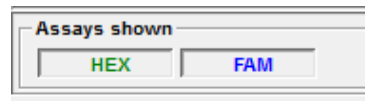
Dissociation / Melt

	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

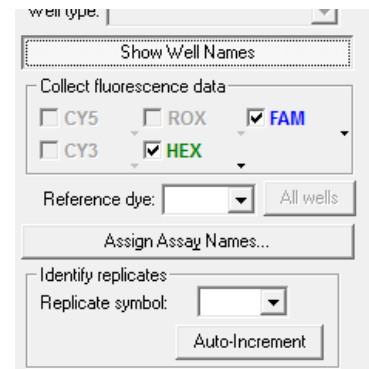
- Save your experiment and start the run.

## Analysis of Variant Detection Assays:

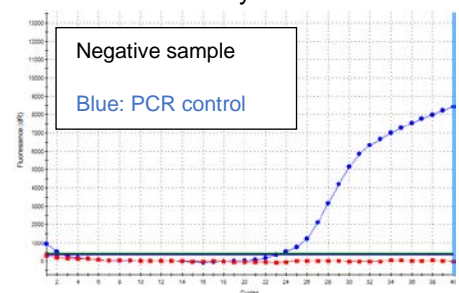
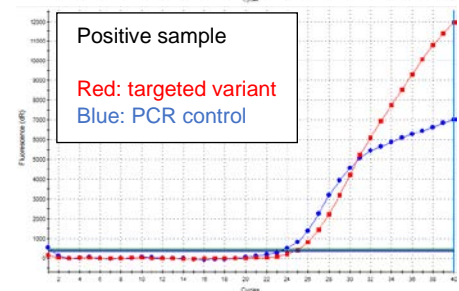
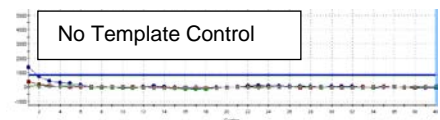
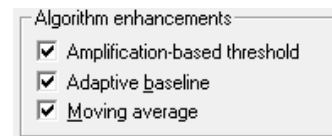
- Launch the **MxPro** Software and load the file containing your data with **File > Open**. The software displays the **Plate Setup** window.
  - Make sure that buttons for **HEX, FAM** (or assay names) as well as **Show Well Names** are active.



- 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.
  - 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*. «