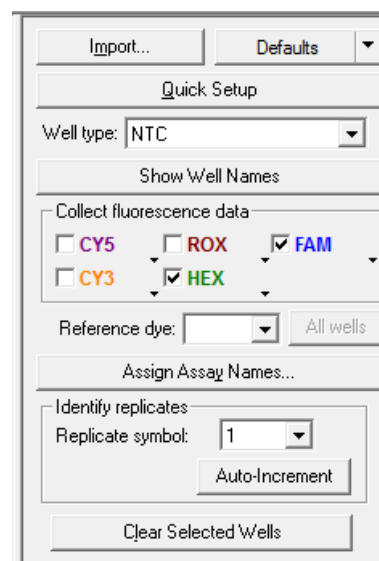


## QuickGuide: RealFast™ Genotyping on Agilent Mx3005P

### Setup for Genotyping Assays:

- Launch the **MxPro software** (QuickGuide is based on version 4.10) and select **Allele Discrimination / SNP's Realtime** 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.
  - 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").

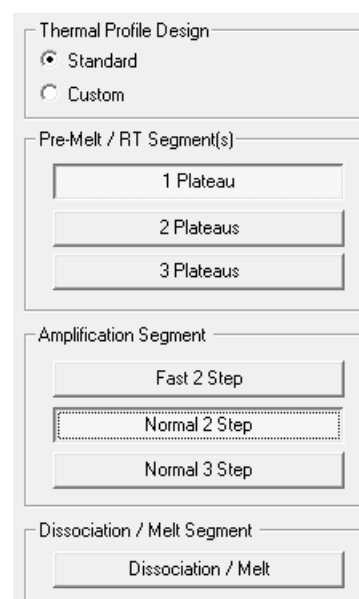


- Define your **HEX Positive Control**.
  - Select a well by mouse click.
  - Choose **HEX Positive Control** within the menu for the **Well type**.
  - Tick the box for **FAM** and **HEX** within the **Collect fluorescence data** field.
  - Click on the **Assign Assay Names ...** button and give a name to the HEX Positive Control (e.g. "WT-CTRL").
- Define your **FAM Positive Control**.
  - Select a well by mouse click
  - Choose **FAM Positive 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 FAM Positive Control (e.g. "MUT-CTRL").

- 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.

- 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

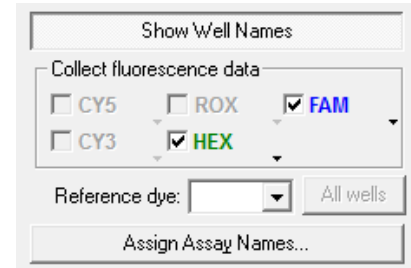
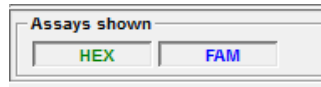
- Save your experiment and start the run.

## Analysis of Genotyping 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.

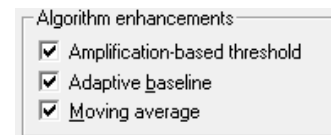


- Control the settings on the right part of the window. FAM and HEX should be enabled for **fluorescence data collection**.

- 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)

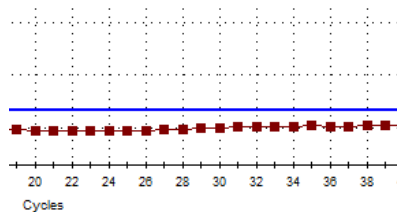


- Click on the **Results** tab to display your results.

- Select the **Area to analyze** in the top-right corner. Start with **Amplification plots**.

- Select the **HEX Positive Control** and the assay **HEX** (or corresponding name) from the menu on the right side. An amplification curve should be visible.

- Select the **HEX Positive Control** and the assay **FAM** (or corresponding name). In the amplification plot no amplification is visible and the threshold for FAM should be above the background signal. If not simply click on the threshold line in the plot and move it above the background signal.



- Select the **FAM Positive Control** and the assay **FAM** (or corresponding name). An amplification curve should be visible.

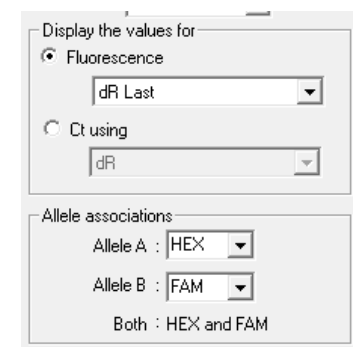
Rep...	Assay	Type	Ct
1	HEX	NTC	No Ct
1	FAM	NTC	No Ct
2	HEX	HEX Po...	21.84
2	FAM	HEX Po...	No Ct
3	HEX	FAM Po...	No Ct
3	FAM	FAM Po...	22.66
4	HEX	Unknown	22.60
4	FAM	Unknown	23.57
5	HEX	Unknown	24.39
5	FAM	Unknown	25.82

- Select the **FAM Positive Control** and the assay **HEX** (or corresponding name). No amplification is visible and the threshold for HEX should be above the background signal. If not, proceed as described before and move the threshold line above the background signal.

- Select the No Template Control **NTC**. No amplification should be visible in both channels.

- Review your **Unknown** samples and check if homozygotes match with the criteria explained for the **Positive Controls**.

- Select **Dual Color Scatterplot** in **Area to analyze** in the top-right corner. Set the **Display** to **Fluorescence** and choose **dRLast** in the dropdown menu. In **Allele associations** assign fluorescence labels to alleles according to your assay. Press the **Rename Alleles/Genotypes** button for user-defined allele/genotype names (wild type, mutant, heterozygous). Homozygotes will be clustered top-left or bottom-right, the NTC bottom-left and heterozygotes in the center.



- 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.