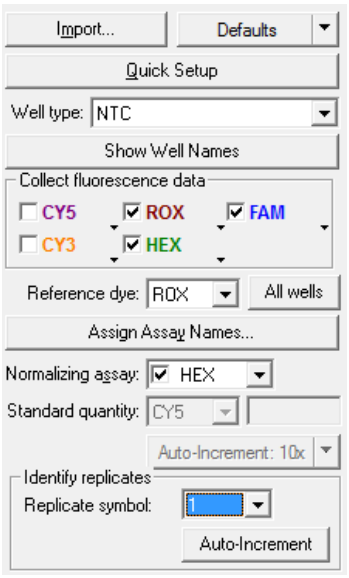
QuickGuide: RealFast™ CNV on Agilent Mx3500P

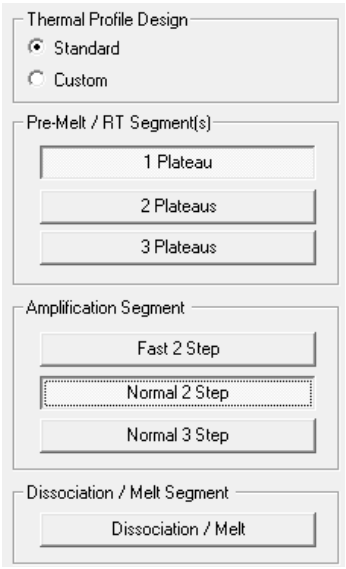
Setup of Relative Quantitation Assays:

- Launch the **MxPro** Software (QuickGuide based on version 4.10) and select **Comparative Quantitation (Calibrator)** from the pop-up window and press **OK**.



- Within the **Plate Setup** define your **No Template Control (NTC)**:
  - Select 3 wells (one replicate) within the plate by ctrl-click (or click-drag).
  - Choose **NTC** within the menu for the **Well type** (top-right).
  - Tick the box for **FAM**, **HEX** and **ROX** within the **Collect fluorescence data** field.
  - Select **ROX** as **Reference Dye.**
  - Click on the **Assign Assay Names** button. In the **Well Information window** you can give a name to your sample (e.g. NTC) and also assign assays within the selected wells (in case of several assays / fluorophores)
  - Select **HEX** as **Normalizing Assay** and tick the corresponding box.
  - **Identify replicates** by setting the **replicate symbol** to "1".

- Define your **Calibrator.**
  - Select 3 wells (one replicate) within the plate by ctrl-click (or click-drag).
  - Choose **Calibrator** within the menu for the **Well type** (top-right).
  - Tick the box for **FAM**, **HEX** and **ROX** within the **Collect fluorescence data** field.
  - Select **ROX** as **Reference Dye.**
  - Click on the **Assign Assay Names** button. Give a name to you sample (e.g. Calibrator).
  - Select **HEX** as **Normalizing Assay** and tick the corresponding box.
  - **Identify replicates** by setting the **replicate symbol** to "2"
- Define your **Samples.**
  - Select 3 wells (one replicate) for each sample within the plate by ctrl-click (or click-drag).
  - Choose **Unknown** within the menu for the **Well type** (top-right).
  - Tick the box for **FAM**, **HEX** and **ROX** within the **Collect fluorescence data** field.
  - Select **ROX** as **Reference Dye.**
  - Select **HEX** as **Normalizing Assay** and tick the corresponding box.
  - **Identify replicates** by setting the **replicate symbol** to "3", "4", "5",… and name your samples within the **Assign Assay Names** field.



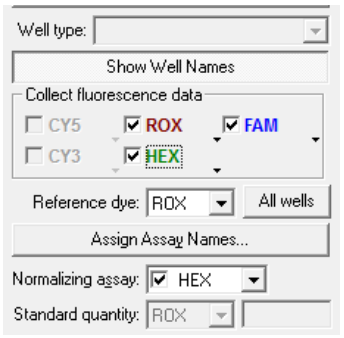
- Click on the **Next** button (bottom-right) to access the **Thermal Profil Setup**.
- Setup the PCR program
  - In **Amplification Segment** select **Normal 2 Step**.
  - Adjust the **Thermal Profile**:

	Cycles	**Temperature** (°C)	**Duration** (mm:ss)	**Data Collection**
Segment 1	1	95°C	10:00	none
Segment 2	40	95°C	00:15	none
		60°C	01:00	Endpoint

- Save your Experiment and start the run

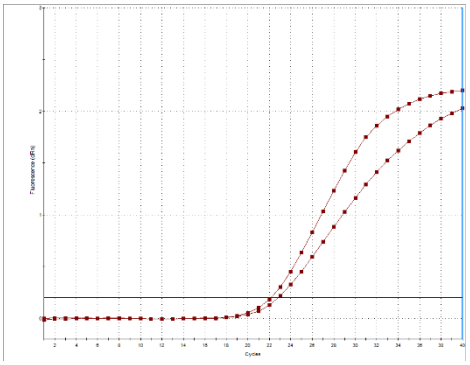
Analysis of Relative Quantitation Assays:

- 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** as well as **Show Well Names** are active.



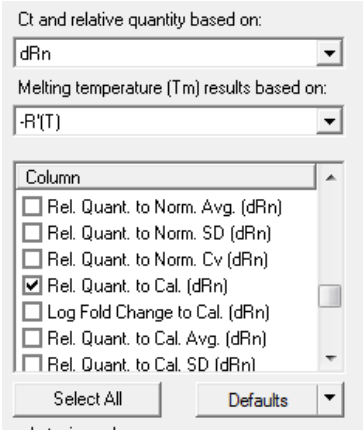
  - Control the settings on right part of the window. ROX, FAM and HEX should be enabled for **fluorescence data collection**, the **Reference Dye** is ROX and the **Normalizing assay** is labeled with HEX.

- 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.
  - Click on the **Results** tab to display your results.
  - Select the **Area to analyze** in the top-right corner. Start with **Amplification plots**. Set the **Threshold fluorescence** for HEX and FAM according to the settings in the **Assay Description**.
  - Review the amplification of your samples by highlighting them in **Select amplification plots to display:**



Rep...	Assay	Type	Ct
1	HEX	NTC	No Ct
1	FAM	NTC	No Ct
2	HEX	Calibrator	22.16
2	FAM	Calibrator	22.85
3	HEX	Unknown	28.66

  - Select **Relative quantity chart**. Here you see the relative quantity of your samples shown as a bar chart and in relation to the **Calibrator**. Mouseover shows hints.
  - Select **Relative quantity plate**. The software displays the plate, the sample name and relative quantities. The ratio for the **Calibrator** is set to "1" and values for your samples are relative to the **Calibrator**. Refer to the product description for interpretation of your results.
  - 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. Select **Rel. Quant. to Cal. (dRn)** to display the relative quantity.



- Export your data via **File > Export Text Report** and choose your file format.