QuickGuide: RealFast™ Genotyping for LightCycler® 480II

Setup for Genotyping Assays:

- Open the **LightCycler® 480 software** and login with your username and password.
  - Choose **New Experiment**.

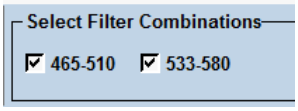
- Define your PCR program in the **Run Protocol** tab.
  - Select **Dual Color Hydrolysis Probe / UPL Probe** as Detection Format.
  - Select a reaction volume of **20 µl** and setup the **Program**:

Program Name	Cycles	Analysis Mode	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)
pre-incubation	1	None	95°C	none	00:03:00	4.4
amplification	40	Quantification	95°C	none	00:00:15	4.4
			60°C	single	00:01:00	2.2
cooling	1	None	40°C	none	00:00:30	2.2

  - Add program steps in the **Programs** window with "**+**" and edit **Cycles** and **Analysis Mode**. To edit **Target (°C)**, **Acquisition Mode** and **Hold**, click on the corresponding step in the **Program Name** window and change parameters or add steps ("**+**") in the **Temperature Targets** window.

- Click on **Subset Editor** button on the left side of the window.
  - Press "**+**" to create a new subset and rename your subset.
  - Select wells in the grid and press **Apply**.

- Click on **Sample Editor** button on the left side of the window.
  - **Select Workflow > Endpt Geno**.
  - **Select Filter Combinations > 456-510** nm (FAM) and **533-580** nm (HEX).
  - Choose your **Subset** of Samples.



- Define your **No Template Control (NTC)**:
  - **Select Samples** field: select well by mouse-click or two wells by ctrl+mouse click.
  - **Edit Endpt Geno Properties** field: Type **NTC** in the **Sample Name** field and press **Enter**. Choose **Negative Control** as **Sample Type**.

Pos	Color	Repl Of	Sample Name	EndPt Sample Type	EndPt Genotype
A1			NTC	Negative Con	
A2			NTC	Negative C	

- Define your **Positive Controls** as Standard (*alternatively: Define your Positive Controls as Unknown*):
  - **Select Samples** field: select a well by mouse click.
  - **Edit Endpt Geno Properties** field: Type the name of your **Positive Control** for **HEX** in the **Sample Name** field. Choose **Standard**.
  - In the chart (EndPt Genotype) type in the genotype corresponding to your **Positive Control HEX**. This will be in most cases **wild type (WT)** for the fluorophore HEX.

Pos	Color	Repl Of	Sample Name	EndPt Sample Type	EndPt Genotype
B3			Pos Ctrl HEX	Standard	WT
B4			Pos Ctrl FAM	Standard	MUT

  - Repeat the steps above with your **Positive Control FAM**. The genotype will be in most cases **mutant (MUT)** for the fluorophore FAM.
- Define your **Samples:**
  - **Select Samples** field: select a well by mouse click.
  - **Edit Endpt Geno Properties** field: Type the name of your first sample in the corresponding field and press **Enter**. Check **Unknown**.
  - Define the rest of your samples.

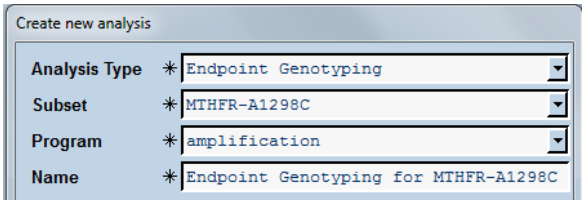
- **Save** or **export** your experiment by pressing the corresponding button:
- Load your samples and start the experiment.

Analysis of Genotyping Assays:

- Open the **LightCycler® 480 software** and login with your username and password.
  The **Overview** window appears.
- Click on  or choose **Navigator** in the flip-window on the top left.
  - Choose an experiment from the data bank, or
  - import an experiment located outside the data bank by pressing **Import.**

- After the file is loaded the **Summary** window of your experiment is displayed.

- Press the **Analysis** button to reach the analysis window.
  - Within the **Create New Analysis** field choose **Endpoint Genotyping.**
  - A pop-up window will be launched. If applicable select a **Subset** of samples or analyze **All Samples** in case your plate contains only one type of assay. Give a **Name** to your analysis. Press the **OK** button.



  - Assign **Allele X** to **FAM** (465-510) and **Allele Y** to **HEX** (533-580) and press **OK**.
  - A Scatter Plot with the signals for HEX (y-axis) and FAM (x-axis) is displayed.
    *Optional: Press **Color Comp** and choose **In Use** or – if available – **In Database** for color compensation of FAM (510) and VIC (580). Press the **OK** button.*
  - Press the **Calculate** button and review your results in the chart bottom-left. The column **Results > Call** displays the genotype of your samples.
    In the **Scatter Plot** points for homozygous samples for the allele Y (HEX) group along the vertical axis, homozygous samples for allele X (FAM) along the horizontal axis. Heterozygotes will generate a cluster in the middle.

- **Save** or **Export** your data by pressing the corresponding button.  or 

- After saving your data you can costumize and generate a report via the **Report** button.