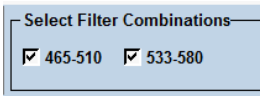
QuickGuide: RealFast™ CNV on LightCycler® 480II

Setup for Relative Quantitation Assays:

- Open the **LightCycler® 480 software** (QuickGuide is based on version 1.5.1) and login with your username and password. The **Overview** window appears.
  - 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
  - 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	00:10:00	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 programs in the **Program Name** window with "**+**" and edit **Cycles** and **Analysis Mode**. To edit **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 > Rel Quant**
  - **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 the first replicate (triplett) by ctrl+mouse click
  - **Edit Rel Quant Properties** field: Type **NTC** in the **Sample Name** field. Check **Negative Control**. Press **Make Replicates**.
  - In the chart choose **Target Negative** (in the **Combined Sample and Target** Type column) for the **Filter Combination** FAM and **Ref Negative** for HEX.
  - Name your targets in the **Target Name** column: FAM = **Gene of Interest**, HEX = **EC** (Endogenous control), once per replicate.

Pos	Filter combination	Color	Repl Of	Sample Name	Combined Sample and Target Type	Concentration	Target Name
A1	FAM (483-533		A1	NTC	Target Negative		Gene of Inte
A1	VIC /HEX / Y		A1	NTC	Ref Negative		EC

- Define your **Calibrator**:
  - **Select Samples** field: select a replicate (triplett) by ctrl+mouse click
  - **Edit Rel Quant Properties** field: Type **Calibrator** in the **Sample Name** field. Check **Positive Control/Calibrator**. Press **Make Replicates**.
  - In the chart choose **Target PosCalibrator** (in the **Combined Sample and Target** Type column) for the **Filter Combination** FAM and **Ref PosCalibrator** for HEX.
  - Name your targets in the **Target Name** column: FAM = **Gene of Interest**, HEX = **EC** (Endogenous control), once per replicate.

Pos	Filter combination	Color	Repl Of	Sample Name	Combined Sample and Target Type	Concentration	Target Name
A4	FAM (483-533		A4	Calibrator	Target PosCalibrato		Gene of Inte
A4	VIC /HEX / Y		A4	Calibrator	Ref PosCalibrator		EC

- Define your **Samples**:
  - **Select Samples** field: select a replicate (triplett) by ctrl+mouse click
  - **Edit Rel Quant Properties** field: Type your sample name in the **Sample Name**. Check **Unknown**. Press **Make Replicates**.
  - In the chart choose **Target Unknown** for the **Filter Combination** FAM and **Ref Unknown** for HEX.
  - Name your targets in the **Target Name** column: FAM = **Gene of Interest**, HEX = **EC** (Endogenous control), once per replicate.

Pos	Filter combination	Color	Repl Of	Sample Name	Combined Sample and Target Type	Concentration	Target Name
A7	FAM (483-533		A7	Sample	Target Unknown		Gene of Inte
A7	VIC /HEX / Y		A7	Sample	Ref Unknown		EC

  - Load your samples and start the experiment

Analysis of Relative Quantitation Assays:

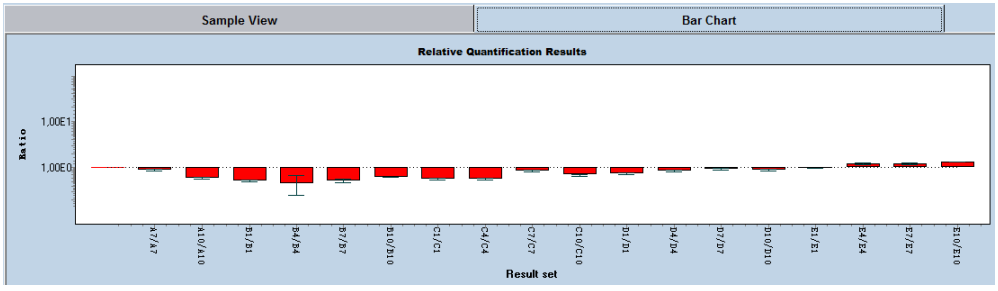
- Open the **LightCycler® 480 software** and login with your username and password. The **Overview** window appears.
- Click on  or choose **Navigator** in the flip-window on the top left.
  - Choose an experiment from the data bank or
  - import an experiment located outside the data bank by pressing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

either **Advanced Relative Quantification** for CAH RealFast™ CNV Assay
or **Basic Relative Quantification** for CYP2D6 RealFast™ CNV Assay.

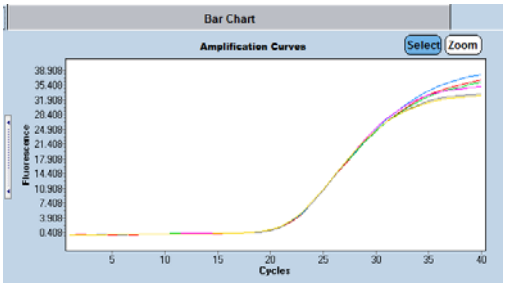
Advanced Relative Quantification for **CAH** RealFast™ CNV Assay:

- Within the **Create New Analysis** field choose **Advanced Relative Quantification**
  - A pop-up window will be launched. If applicable select a **Subset** of samples. Give a **Name** to your analysis. Press the **OK** button.
  - Confirm the default analysis settings by pressing **OK**.
  - A chart containing **Results, Manual Pairing** and **Target Name** is displayed.
  - Press **Color Comp** and choose **In Database** to activate the color compensation for FAM (510) and VIC (580). Press the **OK** button and confirm the **Color Compensation Channels**.
  - Press **Settings** and deselect **Display Target/Reference Ratio**.

- Press the **Calculate** button and review your results. In the Bar Chart, the **Normalized Ratios** of your samples are displayed as columns. 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.



- View individual samples in the **Sample View** by selecting the target and the reference for one sample at the same time. The intervall between the curve for the target and for the reference is related to the copy number variation.

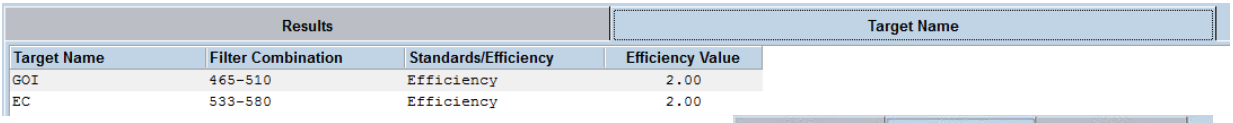


Example: Amplification plot of the calibrator sample

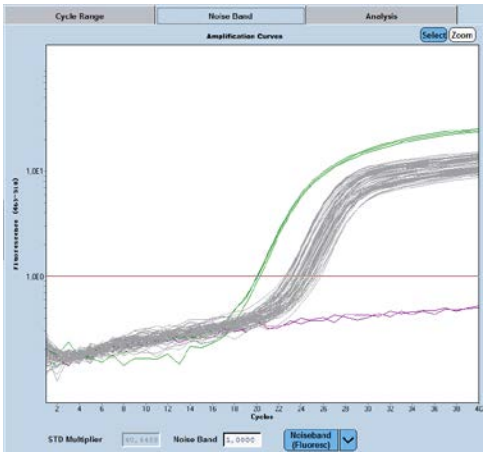
- **Save** or **export** your data by pressing the corresponding button.
- After saving your data you can customize and generate a report via the **Report button**.

Basic Relative Quantification for **CYP2D6** RealFast™ CNV Assay:

- Within the **Create New Analysis** field choose **Basic Relative Quantification.**
- Press **Target Name** in right upper corner and select **GOI** (Gene of Interest).



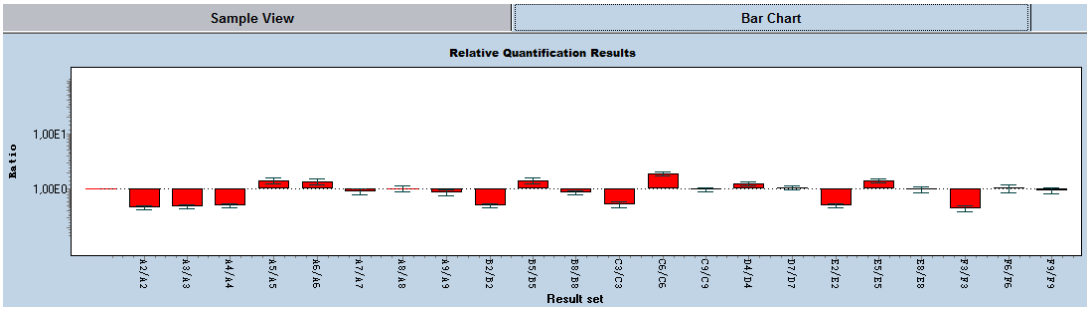
Target Name	Filter Combination	Standards/Efficiency	Efficiency Value
GOI	465-510	Efficiency	2.00
EC	533-580	Efficiency	2.00



  - Press **Show Abs Quant** in the right lower corner.
  - On top of the amplification plot press the tab **Noise Band** and select **Noiseband (Fluoresc)**. Type in the value for the Noise Band of the FAM channel = GOI (refer to the product description).
  - Press **Back to Rel Quant** in the right lower corner and select **EC** (Endogenous control).
  - Press **Show Abs Quant** in the right lower corner, the tab **Noise Band** and select **Noiseband (Fluoresc)**. Type in the value for the Noise Band of the HEX channel = EC (refer to the product description).
  - Press **Back to Rel Quant.**

- Press **Results** in the left upper corner.
  - Press **Color Compensation** and choose **In Database** to activate the color compensation for FAM (510) and VIC (580). Press the **OK** button and confirm the **Color Compensation Channels**.
  - Press **Settings** and disable tick box **Display Target/Reference Ratio**.

- Press the **Calculate** button and review your results.



  - Proceed as described above.