LigtCycler 480 I, LigtCycler 480 II, and Cobas z 480

Real-Time PCR Detection Systems

(Roche Diagnostics GmbH)

generi biotech

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1. Settings of the real-time PCR cycler

1.1. Detection format setup

Select the detection format in the run settings according to the manufacturer's instructions for use (IFU). The detection format depends on the number of fluorophores and type of the probes. A suitable detection format is recommended in the GENERI BIOTECH's kit manual, typical GENERI BIOTECH´s formats are listed in the table below:

Method	Detection Format
Quantification in one channel	Mono Color Hydrolysis Probe / UPL Probe
Quantification in two channels	Dual Color Hydrolysis Probe / UPL Probe
Allelic discrimination	Dual Color Hydrolysis Probe / UPL Probe
Melting temperature analysis in one channel	Mono Color Hydrolysis Probe / UPL Probe
Melting temperature analysis in two channels	Dual Color Hydrolysis Probe / UPL Probe

1.2. Fluorescent channel setup

To set fluorescent filters, follow the manufacturer's IFU. You can choose from default Filter Combination names or create your own combination. Fluorophores mentioned in the GENERI BIOTECH's kit manuals correspond with the excitation/emission wavelengths (nm) listed in the table below:

Fluorophore	LC 480 I		LC 480 II		Cobas z 480	
	Excitation	Emission	Excitation	Emission	Excitation	Emission
FAM/SYBR	483	533	465	510	465	510
HEX	523	568	533	580	540	580
ROX	558	610	533	610	540	610
Cy5	615	670	618	660	610	670

1.3. Temperature profile setup

Set the basic temperature profile´s parameters according to the instrument manufacturer's IFU.

Use the maximum ramp rate for each PCR target, i.e., 4.4 °C/s for denaturation and extension steps (heating up) and 2.2 °C/s for annealing and hybridization steps (cooling down). In case of melting target, use 0,57 °C/s ramp rate with 1 acquisition per °C.

2. Data analysis

2.1. Ct determination – Quantification methods

Threshold cycle (Ct) determination is used in detection and quantification methods such as:

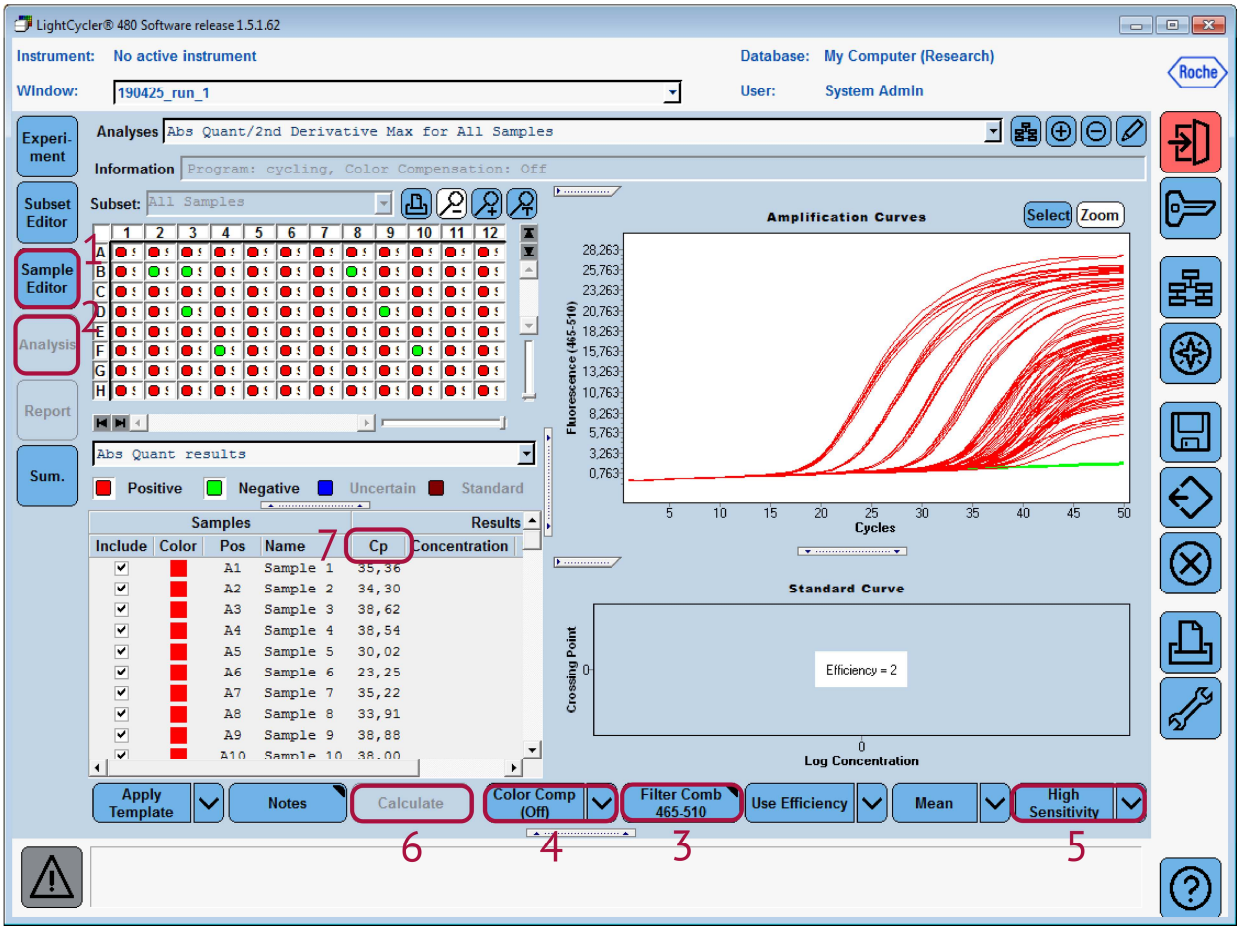
- gene expression studies
- somatic mutation studies
- pathogen nucleic acid studies

Absolute quantification is suitable for most of the GENERI BIOTECH´S applications requiring Ct determination.

In the **Sample Editor** (**1**), select the **Abs Quant** workflow. In the **Analysis window** (**2**), choose **Abs Quant/2nd Derivative Max** from the **Create New Analysis list**.

If you perform a multicolour experiment, use the **Filter Comb** button (**3**) to choose filter combination for the targets you want to analyse. Make sure to turn the colour compensation off (**4**) and to select the **High Sensitivity** algorithm (**5**). After you have changed analysis settings by any of the multi select buttons, you must always recalculate (**6**) the analysis.

Determine the Ct as Cp values in the **Cp** column (**7**) of the result table.


LightCycler® 480 Software release 1.5.1.62
Instrument: No active instrument
Database: My Computer (Research)
WIndow: 190425_run_1
User: System Admin
Roche
Experi-ment
Subset Editor
Sample Editor
Analysis
Report
Sum.
Analyses Abs Quant/2nd Derivative Max for All Samples
Information Program: cycling, Color Compensation: Off
Subset: All Samples
Amplification Curves
Select
Zoom
Fluorescence (465-510)
Cycles
Abs Quant results
Positive
Negative
Uncertain
Standard
Samples
Results
Include
Color
Pos
Name
Cp
Concentration
A1 Sample 1 35,36
A2 Sample 2 34,30
A3 Sample 3 38,62
A4 Sample 4 38,54
A5 Sample 5 30,02
A6 Sample 6 23,25
A7 Sample 7 35,22
A8 Sample 8 33,91
A9 Sample 9 38,88
A10 Sample 10 38,00
Standard Curve
Crossing Point
Efficiency = 2
Log Concentration
Apply Template
Notes
Calculate
Color Comp (Off)
Filter Comb 465-510
Use Efficiency
Mean
High Sensitivity
1
2
3
4
5
6
7

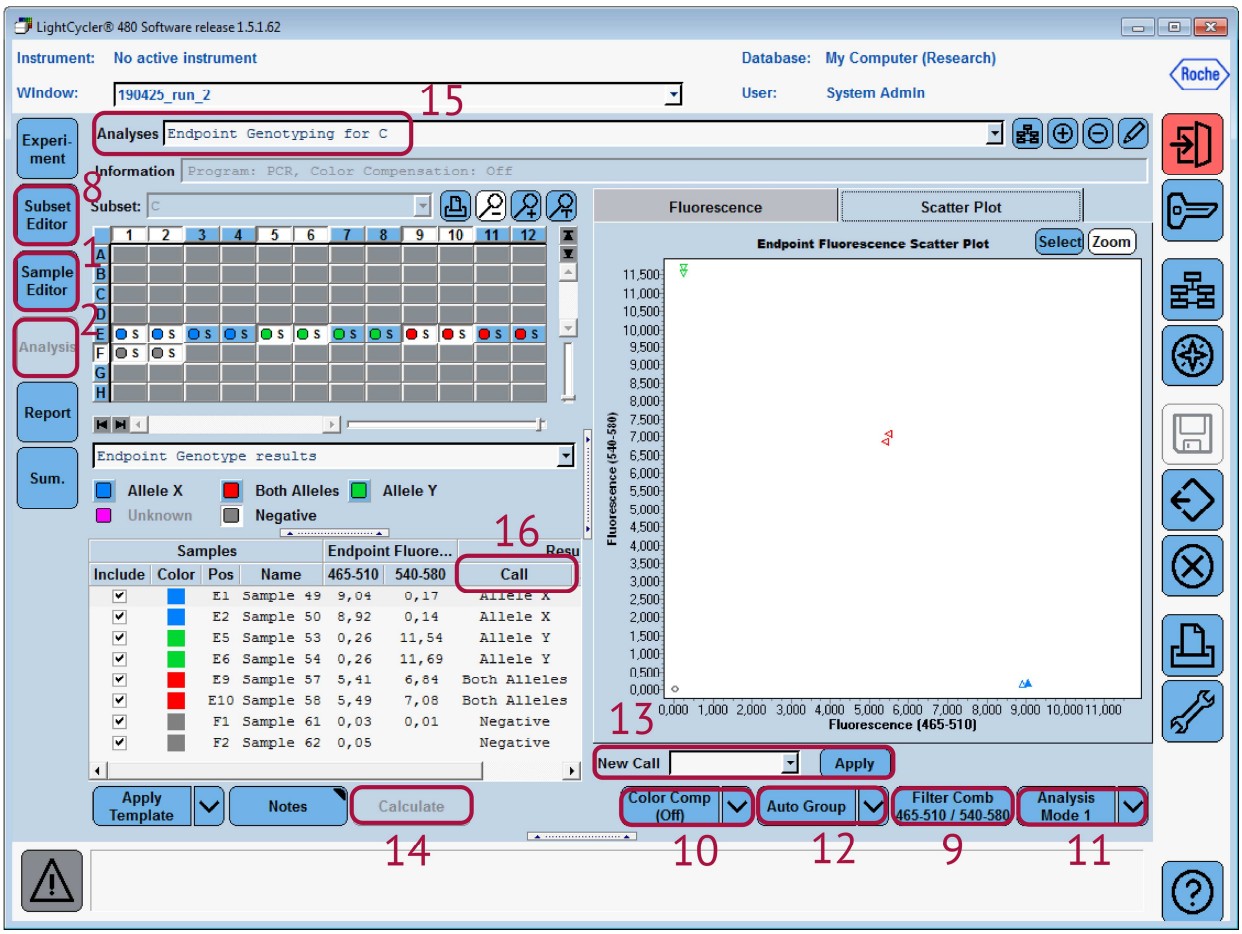
2.2. Genotype determination – Allelic discrimination method

Allelic discrimination allows assigning the genotype of the DNA samples. In Roche machines, it refers as the endpoint genotyping.

Each assay (i.e., each particular mutation) should be analysed as an individual subset. Use **Subset Editor** (**8**) to group samples into subsets. In the **Sample Editor** (**1**), select the **Endpt Geno** workflow. In the **Analysis window** (**2**), choose **Endpoint Genotyping** from the **Create New Analysis** list.

If necessary, use the **Filter Comb** button (**9**) to change filter combination for the targets. Make sure to turn the colour compensation off (**10**) and to select the **Analysis Mode 1** (**11**). For the automated sample grouping, select the **Auto Group** option (**12**). You can manually change the genotype using the **New Call** drop-down list and **Apply** button (**13**). After you have changed analysis settings by any of the multi select buttons, you must always recalculate (**14**) the analysis. Use the **Analysis** tool bar (**15**) to switch between subsets.

Determine the genotype identity in the **Call** column (**16**) of the result table.



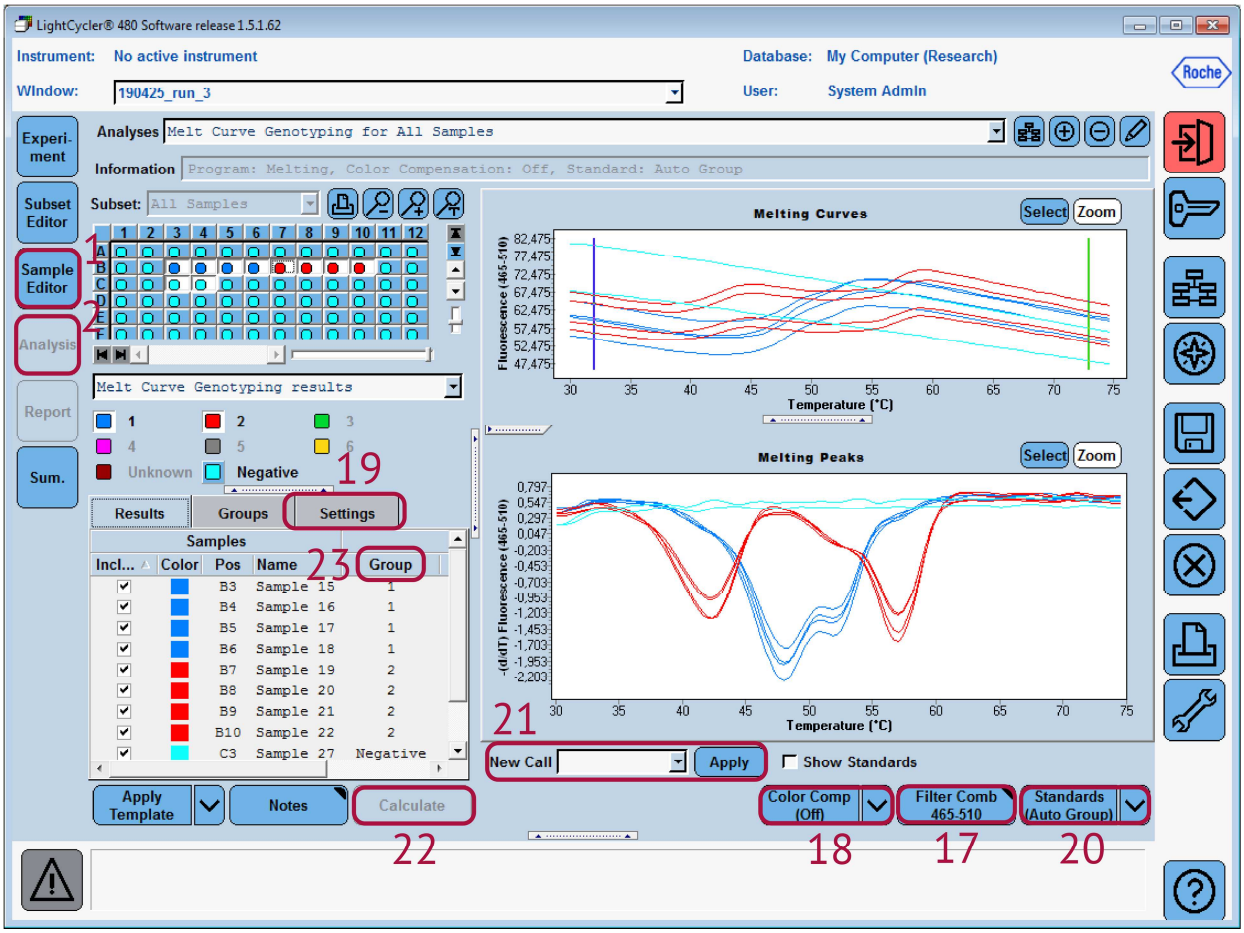
2.3. Melting temperature determination – Dissociation curve method

For the GENERI BIOTECH´s genotyping kits, the fluorescence is lowest at the beginning of the dissociation stage of temperature profile. Fluorescence increases as the temperature rises. The greatest rate of change in fluorescence results in visible negative peaks and represents the Tm of the double-stranded DNA complexes. (Melting temperature analysis results in positive peaks when using non-cleavable hybridization probes or DNA-binding dyes e. g. SYBR Green I.)

In the **Sample Editor** (**1**), select the **Melt Geno** workflow. In the **Analysis window** (**2**), choose **Melt Curve Genotyping** from the **Create New Analysis list**.

If necessary, use the **Filter Comb** button (**17**) to change filter combination for the targets. Make sure to turn the colour compensation off (**18**) and set all fields in the **Settings** tab (**19**) to the default values. For the automated sample grouping, select the **Auto Group** option (**20**). You can manually change the genotype using the **New Call** drop-down list and **Apply** button (**21**). After you have changed analysis settings by any of the multi select buttons, you must always recalculate (**22**) the analysis.

Determine the genotype identity in the **Group** column (**23**) of the result table.



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