LightCycler 480 I, LightCycler 480 II, and Cobas z 480

Real-Time PCR Detection Systems

(Roche Diagnostics GmbH)

generi biotech

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1. Real-time PCR cycler settings

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 probes. A suitable detection format is recommended in the GENERI BIOTECH's kit manual, typical GENERI BIOTECH 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
Quantification in four channels	Define a new det. format according IFU.
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 to 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). For a melting target, use a 0.57 °C/s ramp rate with 1 acquisition per °C.

2. Data analysis

2.1. Ct determination – Quantification methods

*The number references in brackets correspond to **Figure A**.*

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 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 a 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 via 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.

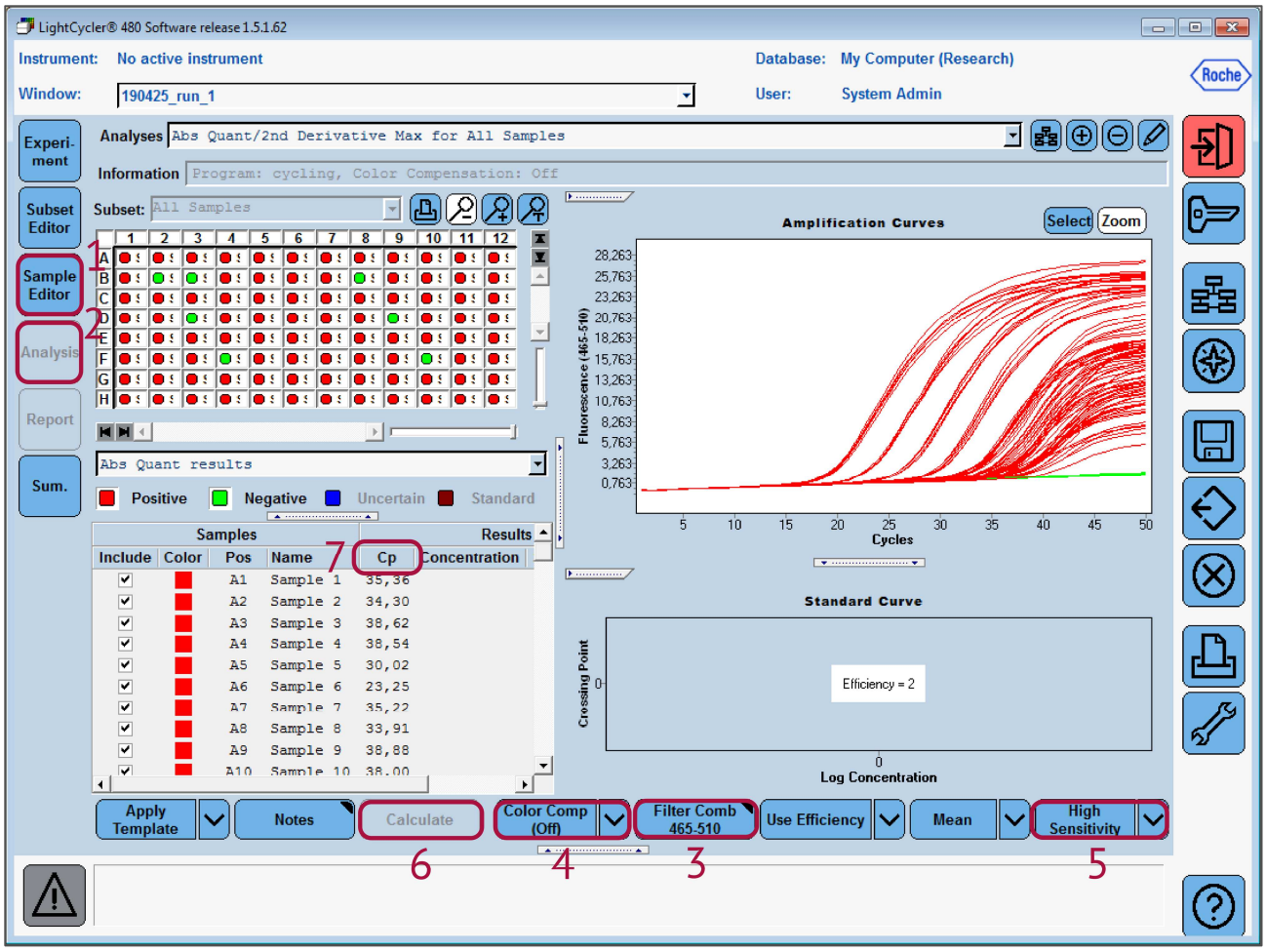

Figure A. Analysis window: Abs Quant/2nd Derivative Max

2.2. Genotype determination – Allelic discrimination method

*The number references in brackets correspond in **Figure B**.*

Allelic discrimination enables assigning the genotype of the DNA samples. In Roche machines, this is referred to as 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 the filter combination for the targets. Make sure to turn the colour compensation off (**10**) and to select **Analysis Mode 1** (**11**). For 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 the analysis settings via any of the multi select buttons, you must always recalculate the analysis (**14**). Use the **Analysis** tool bar (**15**) to switch between subsets.

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

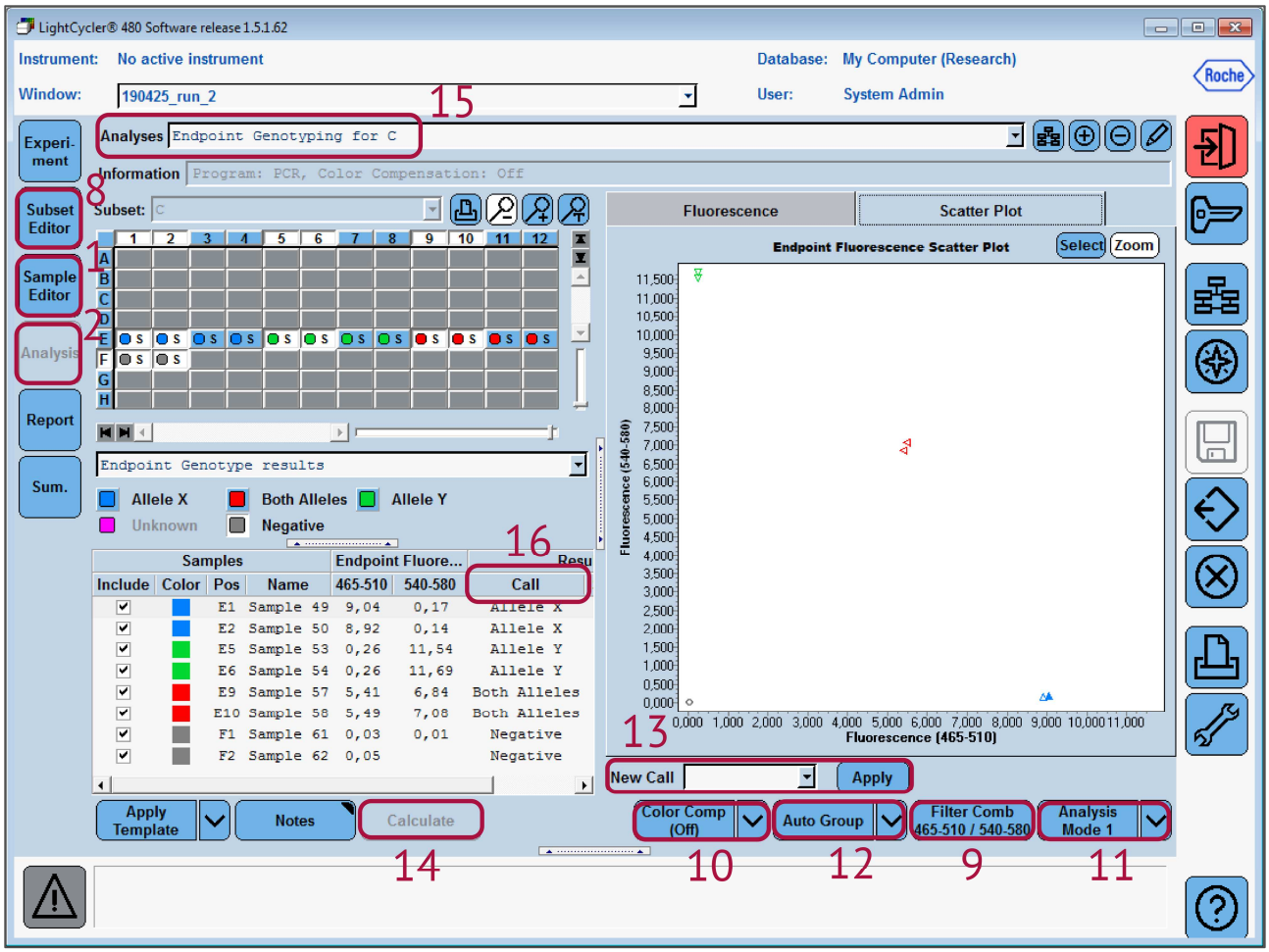

Figure B. Analysis window: Endpoint Genotyping

2.3. Melting temperature determination – Dissociation curve method

*The number references in brackets correspond to **Figure C**.*

For GENERI BIOTECH´s genotyping kits, the fluorescence is lowest at the beginning of the dissociation stage of the 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 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 via any of the multi select buttons, you must always recalculate the analysis (**22**).

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

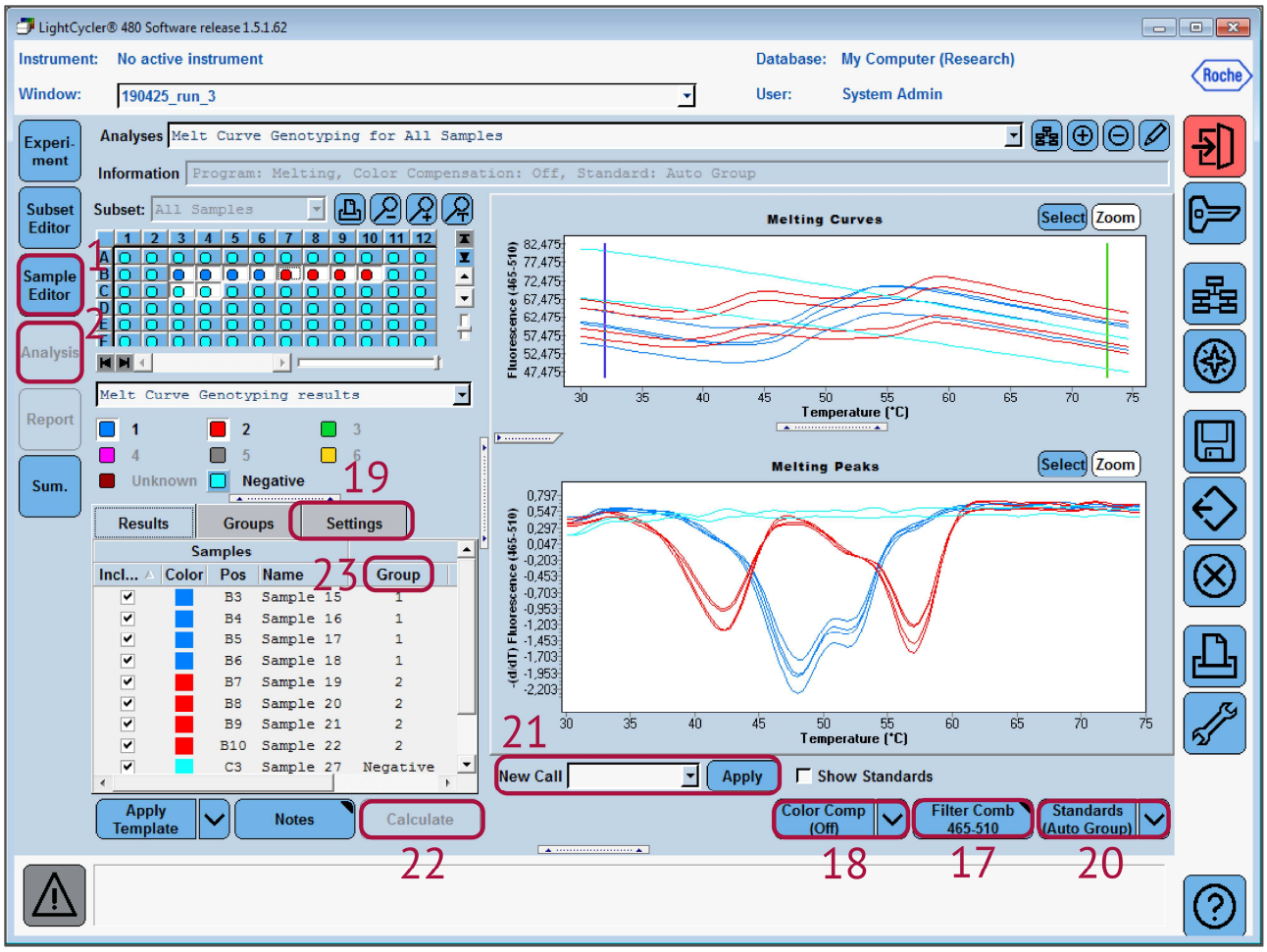

Figure C. Analysis window: Melt Curve Genotyping

3. Contact

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