Rotor-Gene 3000, Rotor-Gene 6000, and Rotor-Gene Q

Real-Time PCR Detection Systems

(Corbett Research, Qiagen GmbH)

ge**neri bio**tech

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1. Real-time PCR cycler settings

1.1. Temperature profile setup

To set the temperature profile, follow the manufacturer's instructions for use (IFU).

Do not use Long Range or Touchdown functions with GENERI BIOTECH's kits.

1.2. Fluorescent channel setup

Set the fluorescent channels according to the manufacturer's IFU. Fluorophores mentioned in GENERI BIOTECH's kit manuals correspond to the channels listed in the table below:

Fluorophore	Acquiring Channel	
	RG 3000	RG 6000 / Q
FAM/SYBR	FAM	Green
HEX	JOE	Yellow
ROX	ROX	Orange
Cy5	Cy5	Red

1.3. Gain setup

Perform the Gain Calibration when setting up a new analysis with reactions that have not previously been run on the Rotor-Gene machine. Choose **Manual Calibration** at 60 degrees Celsius, following the manufacturer's IFU as well as the rules given in the GENERI BIOTECH's kit manuals. Note the appropriate Gain for each fluorophore and kit.

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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.

On the toolbar, click the **Analysis** button (**1**), select the **Quantitation** tab and choose the channel/channels you want to analyse. Click the **Show** button to start the data analysis. If several windows are opened simultaneously, they can be organized by clicking the **Arrange** button (**2**).

In the analysis window, leave the **Dynamic Tube** and **Slope Correct** options (**3**) selected. If necessary, use the **Outlier Removal** option (**4**) to correct or exclude non-specific signals. (The Outlier Removal option is also called More Settings/Quant. Settings in older software versions.) Clicking the **Log/Linear Scale** at the bottom of the window (**5**) changes the scale from a linear scale to a log scale and vice versa. To set the **Threshold** (**6**), click on the icon, then click and hold on the graph and drag the line to the desired level (alternatively, enter a log value). When setting a threshold manually, it should be positioned in the exponential phase of the amplification curve. Use the **Eliminate Cycles before** (**7**) icon (or, enter a Ct value) to eliminate the noise at the beginning of the run.

Determine the Ct values in the **Ct** column (**8**) of the results window.
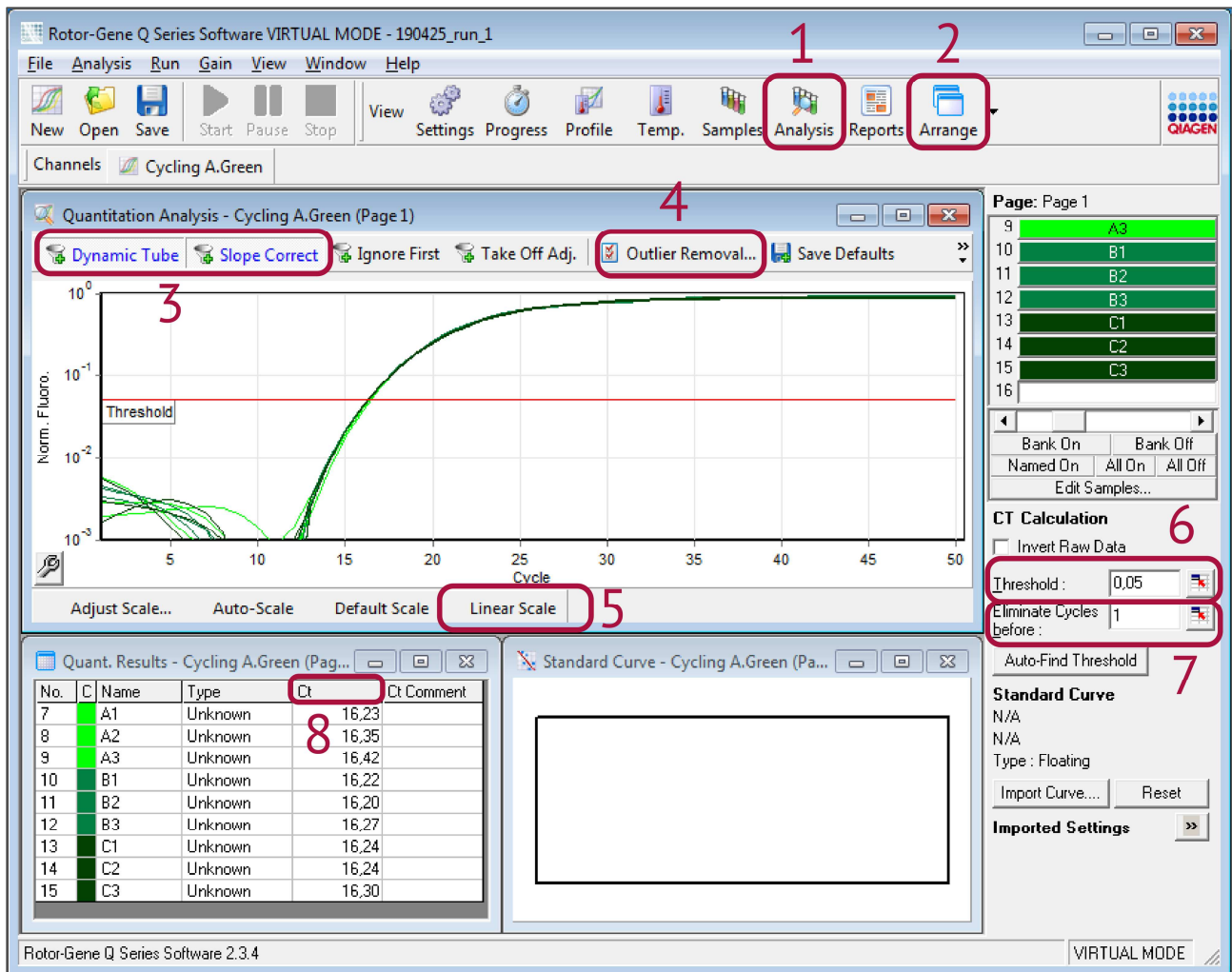
Figure A. Quantitation Analysis window

2.2. Genotype determination – Allelic discrimination method

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

Rotor-Gene cyclers enable genotype determination in two ways: allelic discrimination and scatter graph analysis. Allelic discrimination uses real-time kinetic data from two channels and requires a discrimination threshold to be set. The second method – scatter graph analysis – is based on the relative expression of amplification plots across two channels. Unlike allelic discrimination, genotype is decided based on regions defined from the scatter graph rather than from a single threshold. The second method is recommended for GENERI BIOTECH's kits.

On the toolbar, click the **Analysis** button (**1**), select the **Other** tab and then choose **Scatter Graph Analysis**. (Scatter Graph Analysis is also called Scatterplot Analysis in older software versions.) Drag the mouse pointer over two channels. Once the desired channels have been highlighted, click the **Show** button to start the data analysis. If several windows are opened simultaneously, they can be organized by clicking the **Arrange** button (**2**).

Each assay (i.e., each particular mutation) should be analysed individually. Prior to initialising the analysis, use the right-hand toggle to display samples which belong to a single particular assay. In the analysis window, click **Genotypes** (**3**) to define which genotype is detected in each channel. Leave the **Dynamic Tube** and **Slope Correct** options (**4**) selected. Use the **Outlier Removal** option (**5**) to correct or exclude non-specific signals where necessary. Typically, set the **NTC Threshold** to 5–10% to eliminate the non-specific signal. (The Outlier Removal option is also called More Settings/Quant. Settings in older software versions.) To perform genotyping, define regions by clicking and dragging a selection in the graph window (**6**).

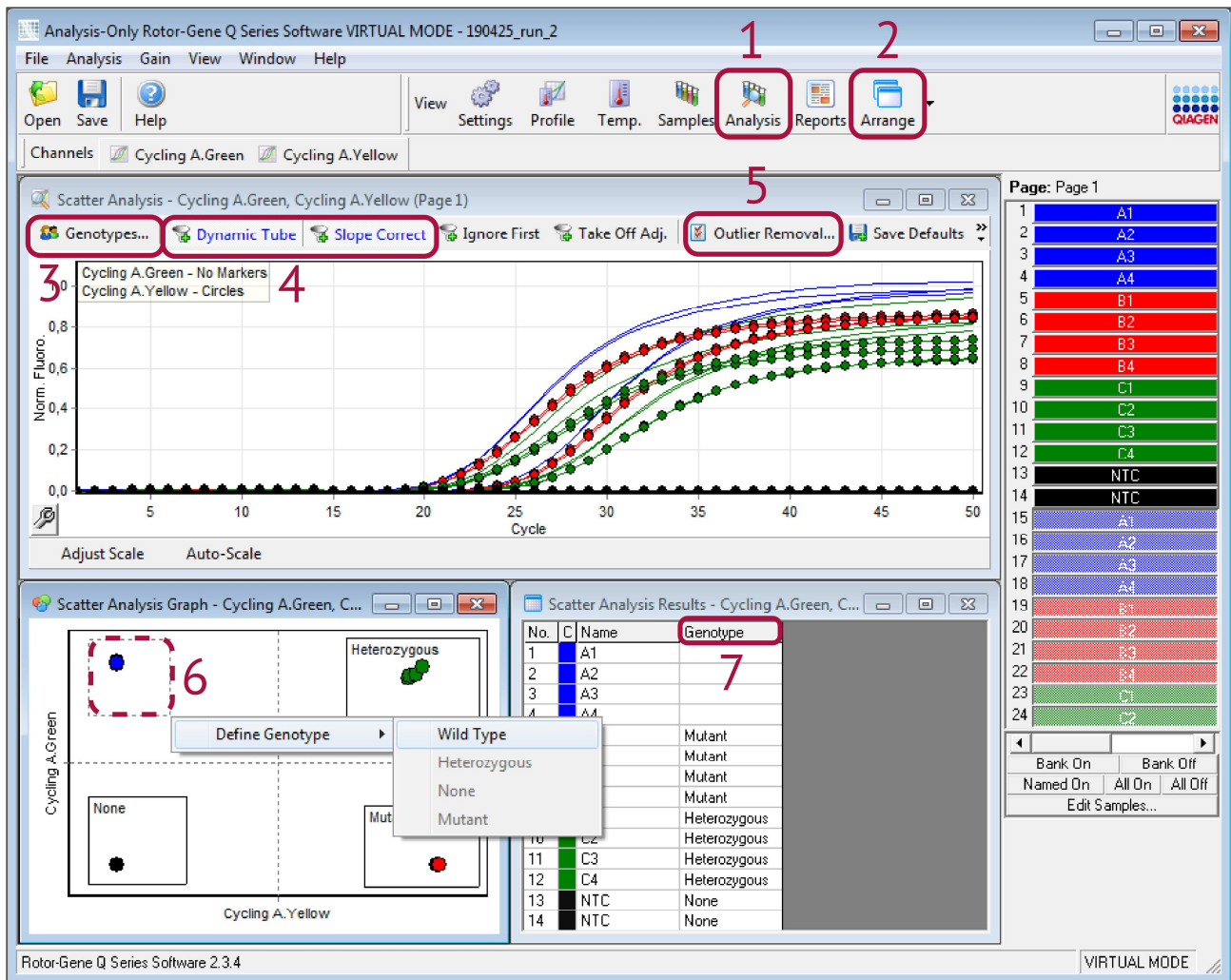Determine the genotype identity in the **Genotype** column (**7**) of the results window.

Figure B. Scatter Analysis window

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.)

On the toolbar, click the **Analysis** button (**1**), select the **Melt** tab and choose the channel/channels you want to analyse. Click the **Show** button to start the data analysis. If several windows are opened simultaneously, they can be organized by clicking the **Arrange** button (**2**).

Before defining peaks, deselect the **Flip sign of dF/dT** checkbox (**3**) to obtain correct data. To set the **Peak Bins**, use the **New** button (**4**) and follow the manufacturer's IFU. If necessary, set the **Threshold** and/or **Temperature Threshold** (**5**) to eliminate the noise. Bins can then be assigned to genotypes using the **Genotypes** command (**6**).

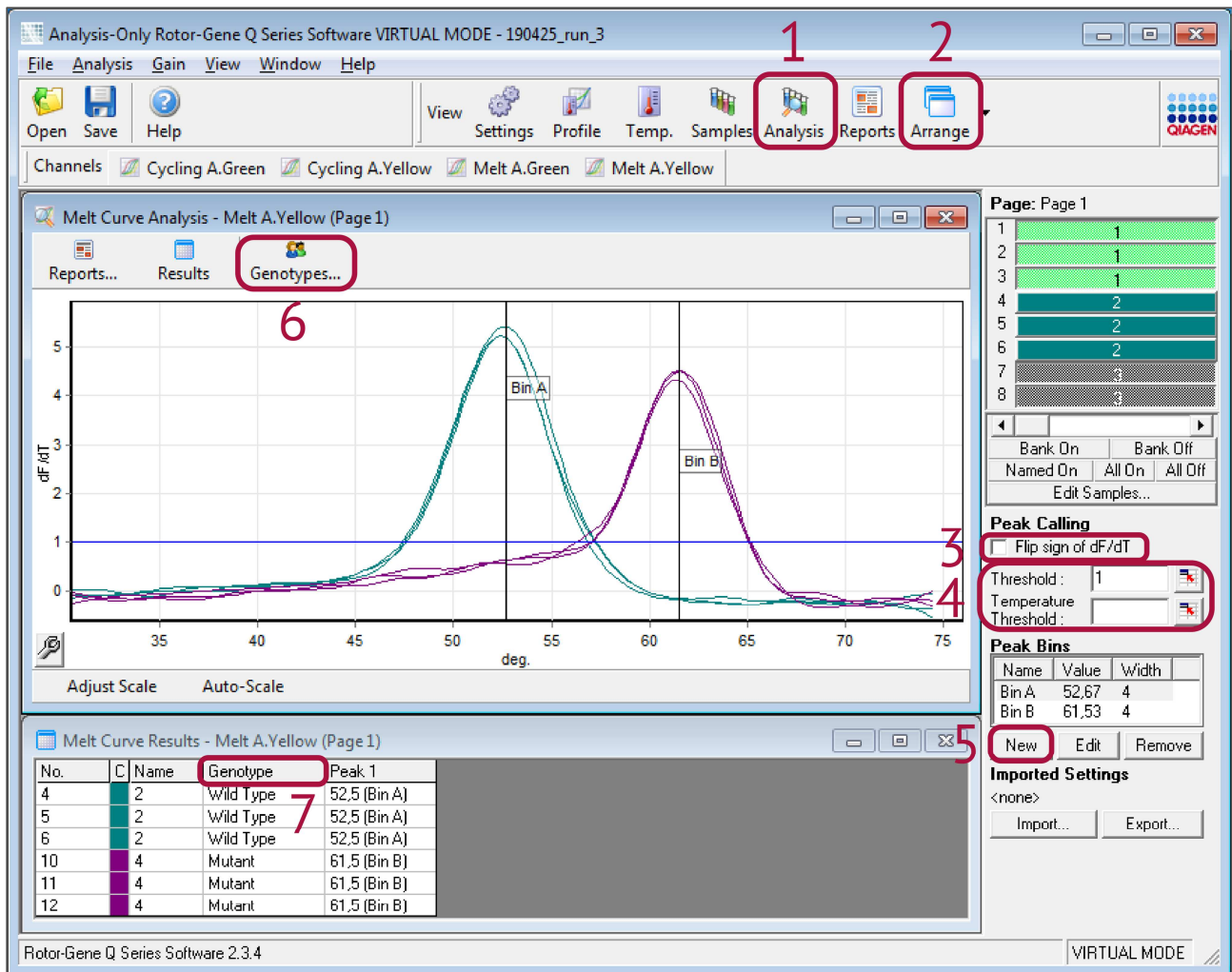Determine the genotype identity in the **Genotype** column (**7**) of the results table.

Figure C. Melt Curve Analysis window

3. Contact

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