

**CFX96, CFX96 Touch, CFX96 Touch Deep Well,
CFX Connect, and CFX384 Touch**
Real-Time PCR Detection Systems

(Bio-Rad Laboratories, Inc.)

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1. Real-time PCR cyclers settings

1.1. Temperature profile setup

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

1.2. Fluorescent channel setup

Set the fluorescent channels according to the manufacturer's IFU. The fluorophores FAM/SYBR, HEX, ROX and Cy5 mentioned in GENERI BIOTECH's kit manuals correspond to the same names in the Select Fluorophores window in the cycler's software.

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

See the results in the **Quantification** tab (1).

Use the default **Settings** (2), i.e., **Single Threshold** for the Cq Determination Mode, **Baseline Subtracted Curve Fit** for the Baseline Settings and **Fluorophore** for the Analysis Mode. Click the **Log Scale** check box (3) at the bottom of the Amplification chart to view the fluorescence traces on a semilog scale. Choose the fluorophores by checking the appropriate box (4). If needed, adjust the Baseline Cycles and the Single Threshold. Select **Baseline Threshold** (5) in the right-click menu to open the Baseline Threshold window. Baseline adjustment is important for the elimination of non-specific fluorescence signals which come from reactions with obviously poor/no amplification (as shown in **Fig. B**).

Determine the Ct as Cq values in the **Cq** column (6) of the spreadsheet.

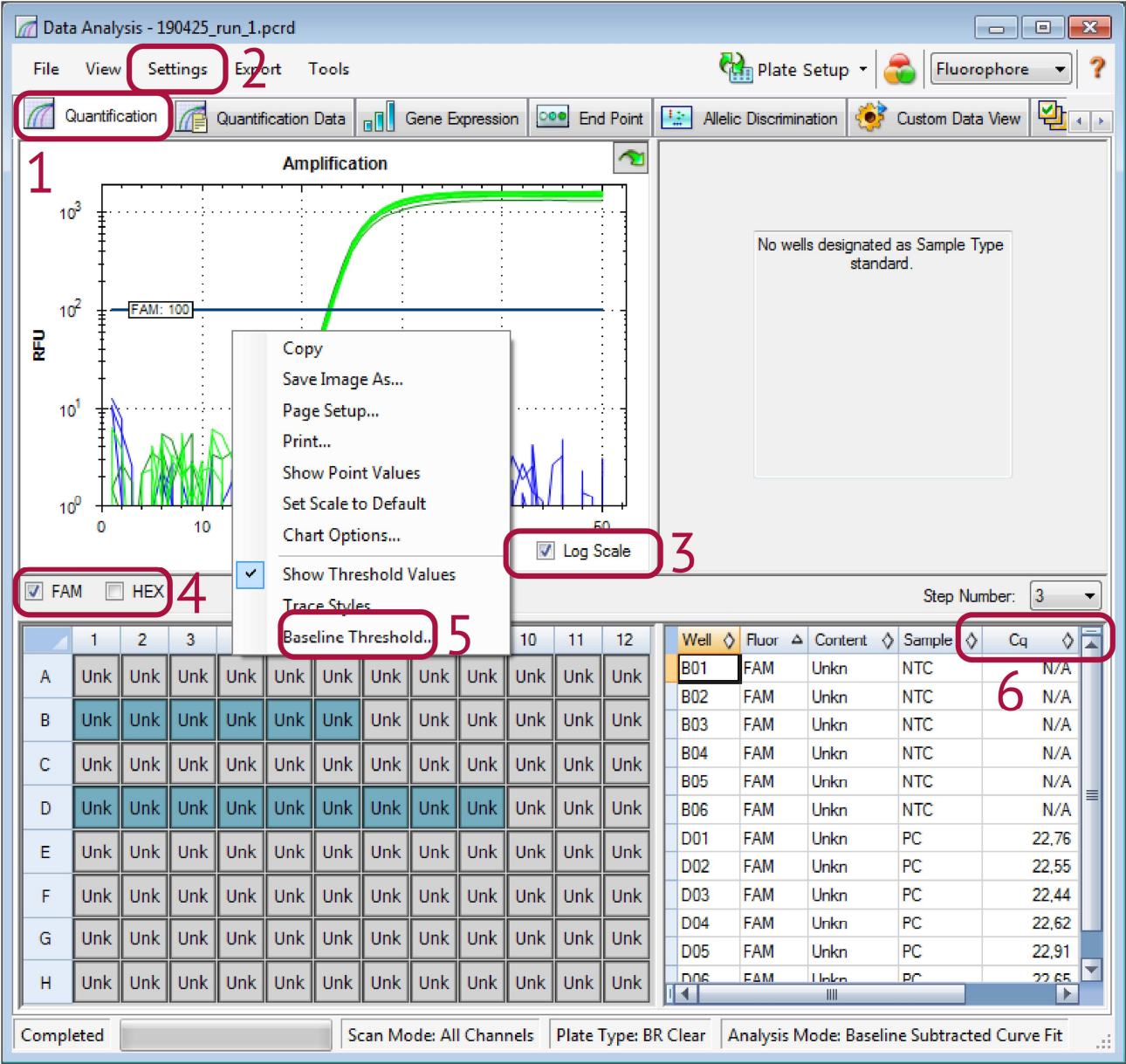


Figure A. Quantification tab window

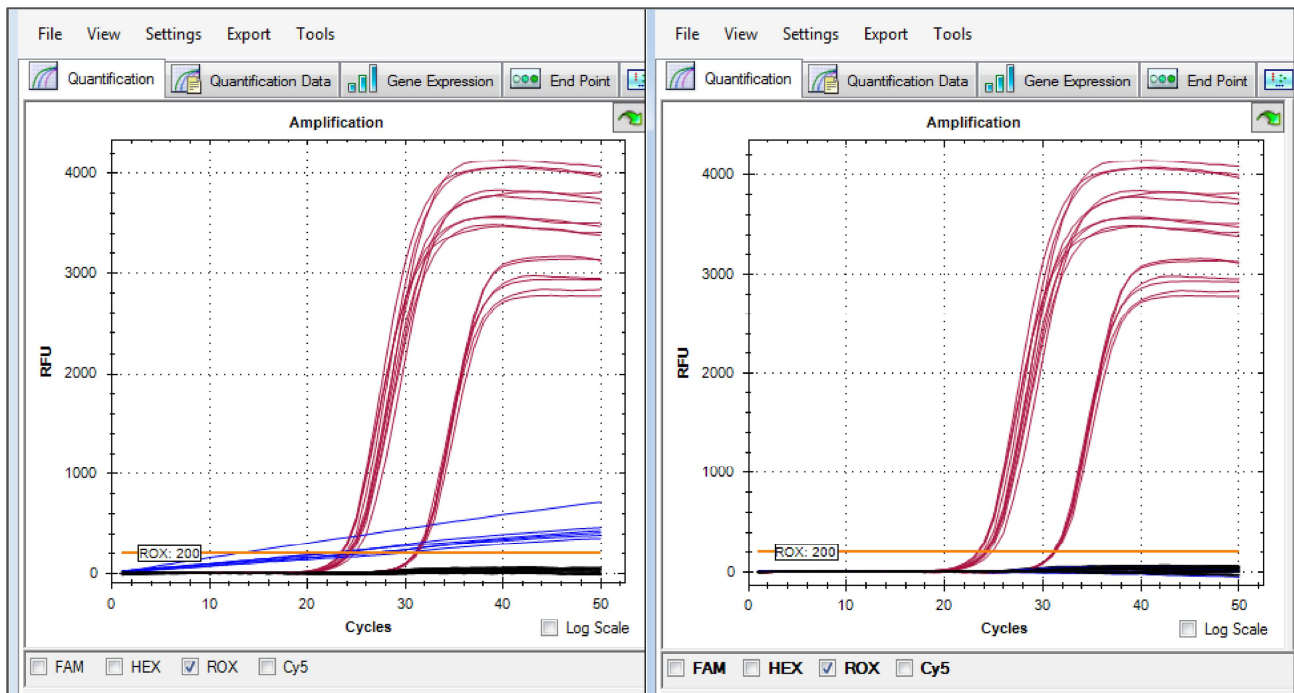


Figure B. Non-specific fluorescence signals (blue) with auto-calculated Baseline Cycles are eliminated by adjusting to user-defined Baseline Cycles (Begin/End 2–15). This can be done for the selected wells or all the wells in the analysis.

2.2. Genotype determination – Allelic discrimination method

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

Allelic discrimination enables assigning the genotype of the DNA samples. In CFX machines, it is based on the analysis of relative fluorescence (RFU) at the end of multiplex runs with two fluorophores.

See the results in the **Allelic Discrimination** tab (6).

Click the **Polar Coordinates** check box (7) at the bottom of the Allelic Discrimination chart to toggle the view between Cartesian and Polar coordinates. If needed, adjust allelic discrimination data by any of these methods: a) Select a fluorophore for each axis in the chart (X: and Y:) in the settings panel (8), b) Change a call manually by left-clicking and dragging to select the data point(s) in the chart and then selecting an option in the **Selected Wells** (9). You may select **Auto Call** to revert to the default call by the software.

Determine the genotype identity in the **Call** column (10) of the spreadsheet.

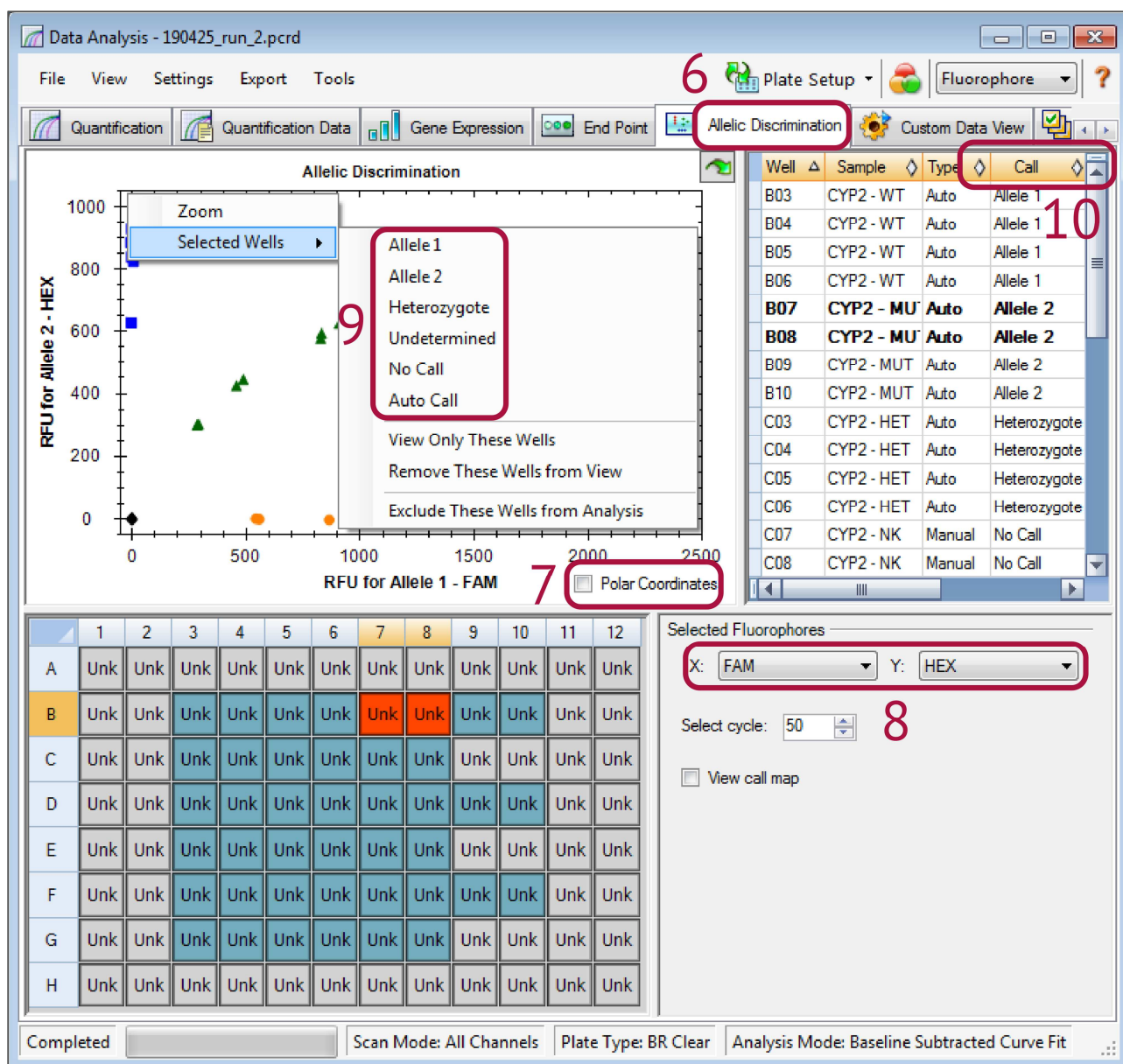


Figure C. Allelic Discrimination tab window

2.3. Melting temperature determination – Dissociation curve method

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

With 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 T_m 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.)

See the results in the **Melt Curve** tab (11).

Use the fluorophore selector (12) to choose the fluorophore you want to analyse. If needed, adjust the melt curve data by any of these methods: a) Click and drag the threshold bar (13) in the Melt Peak chart to include or exclude peaks in the data analysis, b) Select **Negative** (or Positive, e.g., for SYBR Green I) in the **Peak Type** drop-down menu (14) to show the spreadsheet data correctly.

Determine the T_m values in the **Melt Temp** column (15) of the spreadsheet.

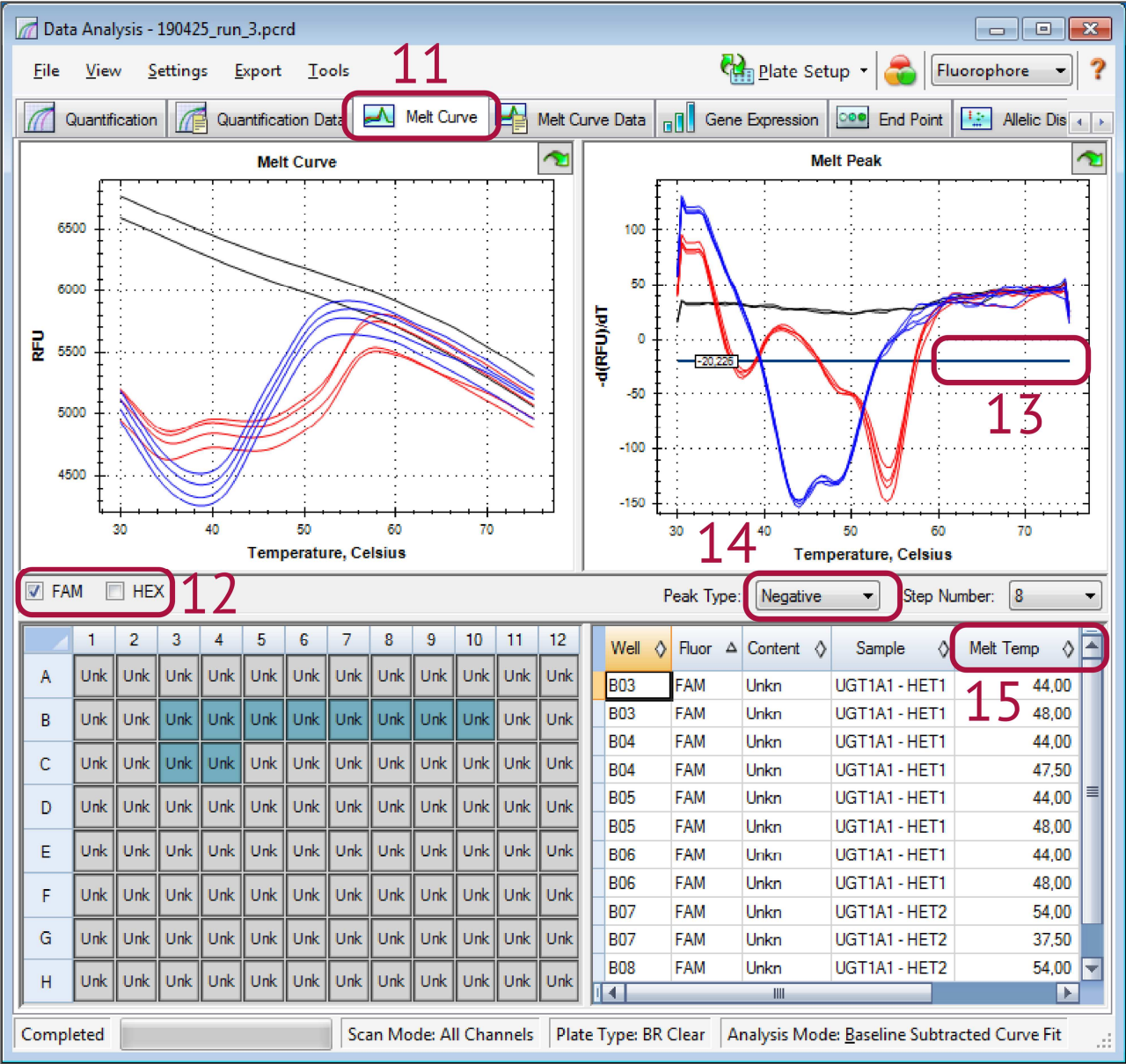


Figure D. Melt Curve tab window

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