

Increasing the call rate in TaqMan® Genotyper Software



Passive reference dyes

Passive reference dyes are frequently used in real-time PCR to normalize the fluorescent signal of reporter dyes and correct for fluctuations in fluorescence that are not PCR-based. Normalization is necessary to correct for fluctuations from well-to-well caused by changes in reaction concentration or volume and to correct for variations in instrument scanning. Most real-time PCR instruments use ROX™ dyes as the passive reference dye, because ROX™ dye does not affect the real-time PCR reaction and has a fluorescent signal that can be distinguished from that of any reporter or quencher dye used.

The quality and quantity of ROX™ dye is specific to each master mix and can impact the autocalling in TaqMan® Genotyping software.

This document explains two simple modifications of the default settings in the TaqMan® Genotyper software to increase the autocall rate:

- Modifying the ROX™ dye threshold
- Modifying the Quality Value

TaqMan® Genotyper Software is a SNP genotyping data analysis tool for use with TaqMan® SNP Genotyping Assays (Pre-Designed, Custom, and DME assays) in combination with 48-, 96-, and 384-well microtiter plates, and OpenArray® Genotyping Plates. The software, powered by a state-of-the-art genotype calling algorithm, showcases an intuitive user interface, and introduces an enhanced multi-plate analysis feature.

The software allows the user to choose between two call methods to call genotypes:

- Autocalling
- Classification scheme

The autocalling method generates occasional low call rates for an experiment based on default values in the Analysis Settings. These default values are the thresholds for the ROX™ passive reference dye and for the Quality Value. Modifying the ROX™ dye threshold and the Quality Value threshold tends to increase the overall call rate when using the autocalling method but doesn't affect the call rate when using the classification scheme method.

Modifying the ROX™ dye threshold

If invalid data points are present in the study, the call rate may be increased by adjusting the default ROX™ dye threshold. The invalid call may be caused by a ROX™ dye value below the default threshold set in the Analysis Settings. When the threshold is lowered, the invalid call may be converted to an actual genotype call.

To change the default ROX™ dye threshold:

1. Click on **Analysis Settings** at the top of the screen. A new window will open showing details of the Analysis Settings (Figure 1).
2. Click the **QC Settings** tab and lower the ROX™ threshold value under the **Well-Level QC Flags** section.

- Click **Apply** at the bottom of the window.
- Click **OK** to close the window. For some instrument types, the ROX™ dye threshold may be set to zero.

Modifying the Quality Value threshold

If undetermined data points are present in the study, the call rate may be increased by lowering the default Quality Value threshold.

To change the default threshold:

- click **Analysis Settings** at the top of the screen. A new window will open showing details of the Analysis Settings (Figure 2).
- Click the **QC Settings** tab and lower the **Quality Value** under the **Well-Level QC Flags** section.
- Click **Apply** at the bottom of the window.
- Click **OK** to close the window.

In the example shown in Figure 3, the default value of 0.95 was lowered to 0.85.

Conclusion

This document outlines two ways to increase the call rate in a TaqMan® Genotyper Software study by adjusting default values in the Analysis Settings. When the ROX™ dye value associated with a data point is below the default ROX™ dye threshold, lowering the ROX™ dye threshold may convert invalid calls to actual genotype calls. Similarly, lowering the Quality Value threshold may convert undetermined calls into actual genotype calls.

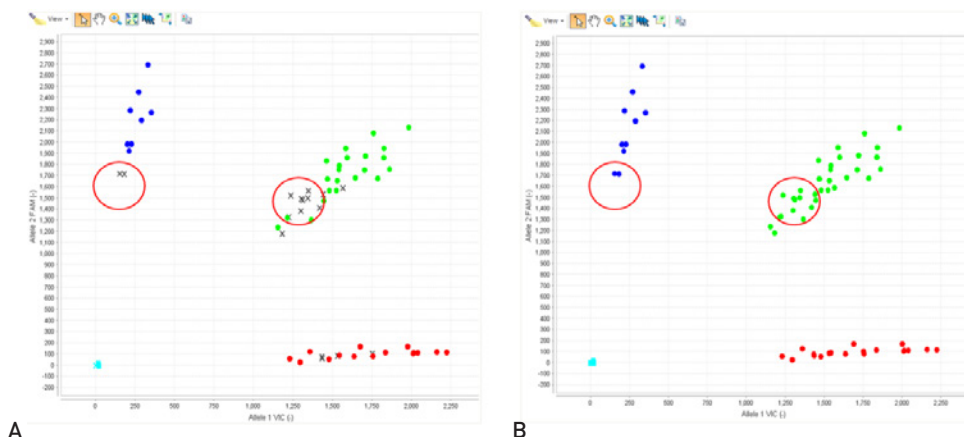


Figure 1. Example of ROX™ dye threshold impact on autocalling. Graph (A) shows the result of autocalling using a ROX™ dye threshold of 500. Graph (B) shows the same samples analyzed with a ROX™ dye threshold of 0. Invalid samples that were not assigned a genotype call (red circles in graph A) with the default value are assigned a genotype call with a ROX™ dye threshold of 0 (red circles in graph B).

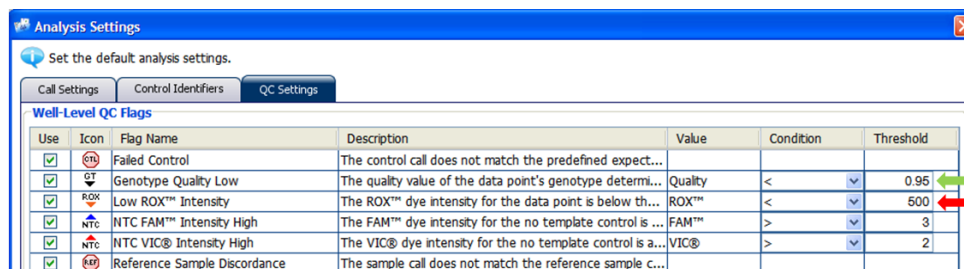


Figure 2. Analysis Settings window. The green arrow shows the Quality Value threshold. The red arrow shows the ROX™ dye threshold.

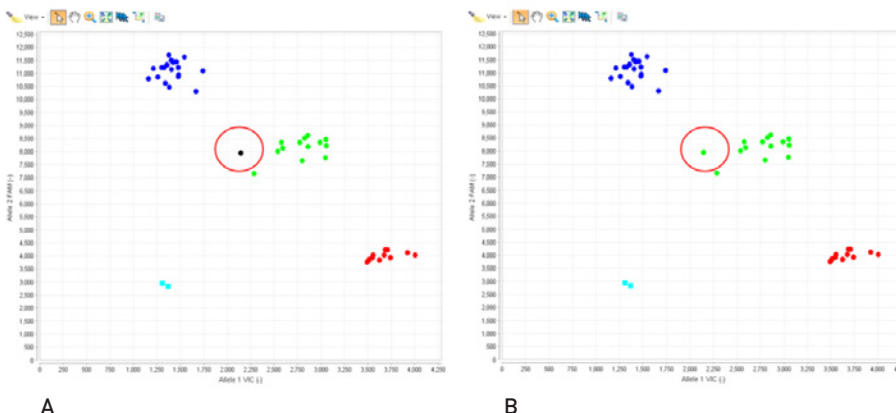


Figure 3. Example of Quality Value impact on autocalling. Graph (A) shows the result of autocalling using a default Quality Value of 0.95. Graph (B) shows the same samples analyzed with a lowered Quality Value threshold of 0.85. The highlighted sample that is assigned an undetermined call with the default Quality Value threshold (red circles in graph A) is assigned a heterozygous call with a lowered Quality Value threshold of 0.85 (red circles in graph B).

Download TaqMan® Genotyper Software at lifetechnologies.com/taqmangenotyper

