

Brilliant III Ultra-Fast SYBR Green qPCR Master Mix Outperforms the Competition

Agilent scientists evaluated qPCR kits according to each manufacturer's protocol. Amplification of the 305 bp genomic DNA target was performed on the AriaMx Real-Time qPCR system.

In the results below, Brilliant III exhibits superior specificity, sensitivity and reproducibility within a shorter thermal cycling time vs competing kits. The same was true for the Agilent Brilliant III SYBR qRT-PCR kits.

Findings were compared in the following data.

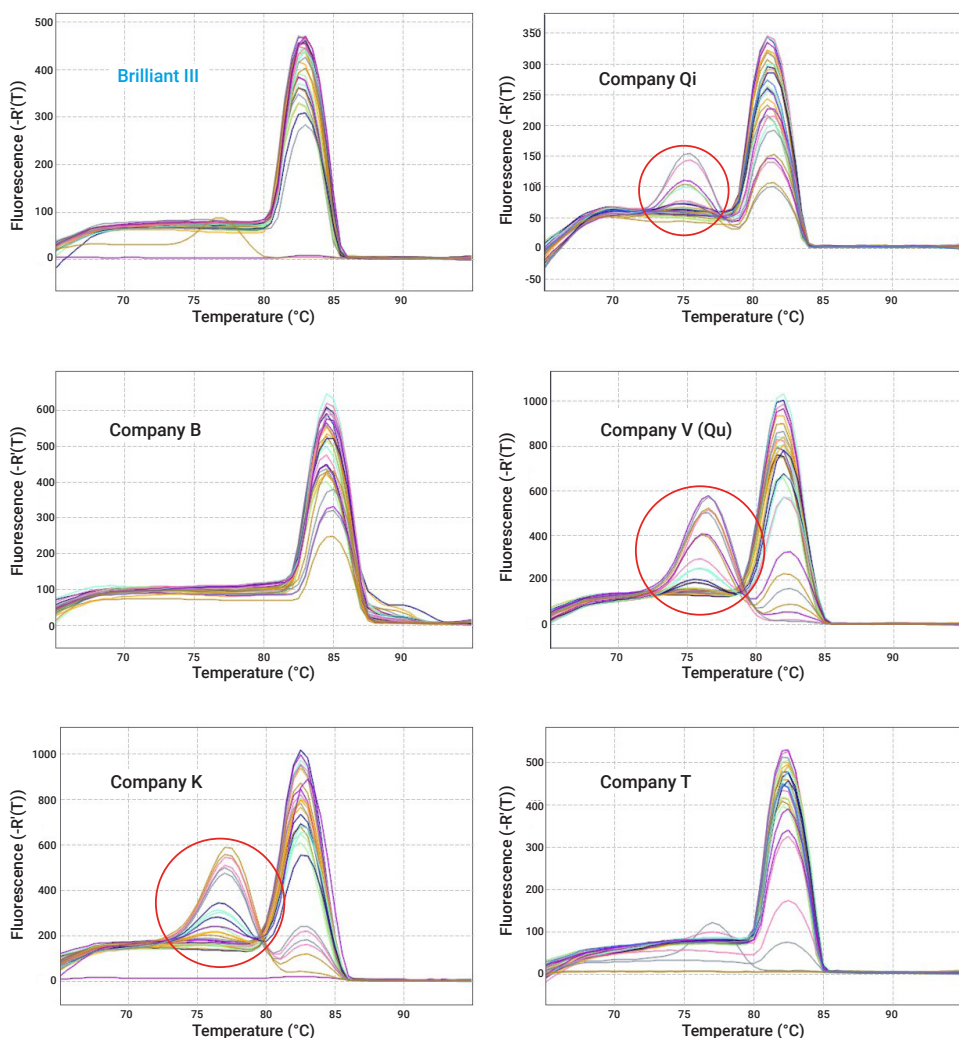


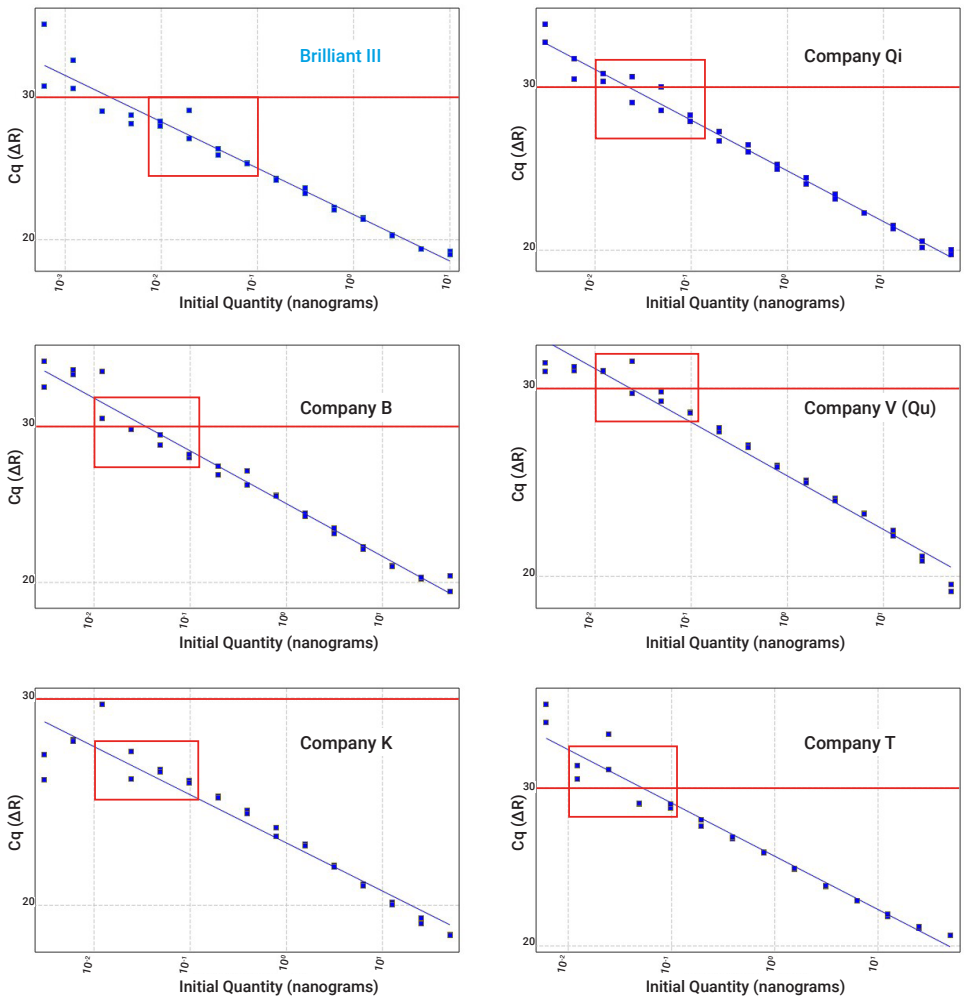
Figure 1. shows the melting curve analyses for Brilliant III versus its competitors. Brilliant III outperforms company K, company Q, and company V. The absence of primer-dimer formation, as shown in red circles on the dissociation curve data—shows the improved specificity of Brilliant III compared to other kits. Reactions were run in duplicate for a 15 x gDNA dilution series, 50 ng - 3 pg per reaction. The NUMB1 fragment was PCR amplified on the AriaMx Real-Time qPCR instrument. Melting curve thermal profiles are shown (one cycle of 95 °C, 30 s > 65 °C, 30 s > 95 °C, 30 s).

Figure 1. The Agilent Brilliant III Ultrafast SYBR Green qPCR Master Mix demonstrates higher amplification specificity than competitors.

Table 1. Comparison of Brilliant III's amplification efficiency and R² values against competitors.

Kit Manufacturer	Amplification Efficiency (%)	R ²
Agilent Technologies	111.0	0.98
Company Qi	110.5	0.99
Company K	133.1	0.98
Company Qu	124.2	0.97
Company T	106.7	0.97
Company B	101.3	0.98

The table shows that Brilliant III amplification efficiency and R² values are very competitive. The higher amplification efficiencies in kits from companies K and Qu are attributed to significant primer dimerization evident in the melt curve analysis.



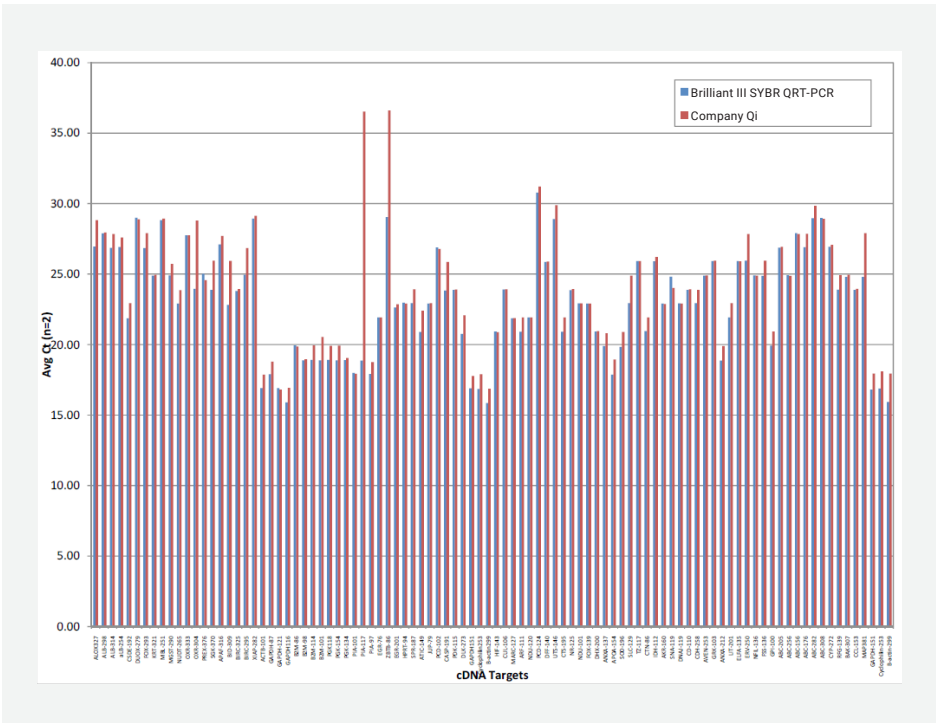
Brilliant III generates equivalent Ct values up to two cycles earlier than competitors B, Qi, V(Qu) and T for the NUMB1 target at all concentrations; 0.1 - 0.01 ng (bounded by red rectangles) were amplified in fewer than 30 cycles (red horizontal lines) for Brilliant III compared to the other master mixes. At the lowest DNA input, the Ct value discrepancy between Brilliant III and company T represents nearly a threefold difference in detection sensitivity. Brilliant III duplicates exhibit a narrower range of Cts at low target amounts and more closely fit the standard curve versus competitors (region highlighted in red rectangles). Reactions were run in duplicate for a 15 x gDNA dilution series, 50 ng – 3 pg per reaction. The NUMB1 fragment was PCR amplified on the AriaMx Real-Time qPCR instrument; cycling conditions were used according to the manufacturer's protocol.

Figure 2. Brilliant III Ultrafast SYBR Green QRT-PCR Master Mix offers higher sensitivity and reproducibility at low target concentrations.

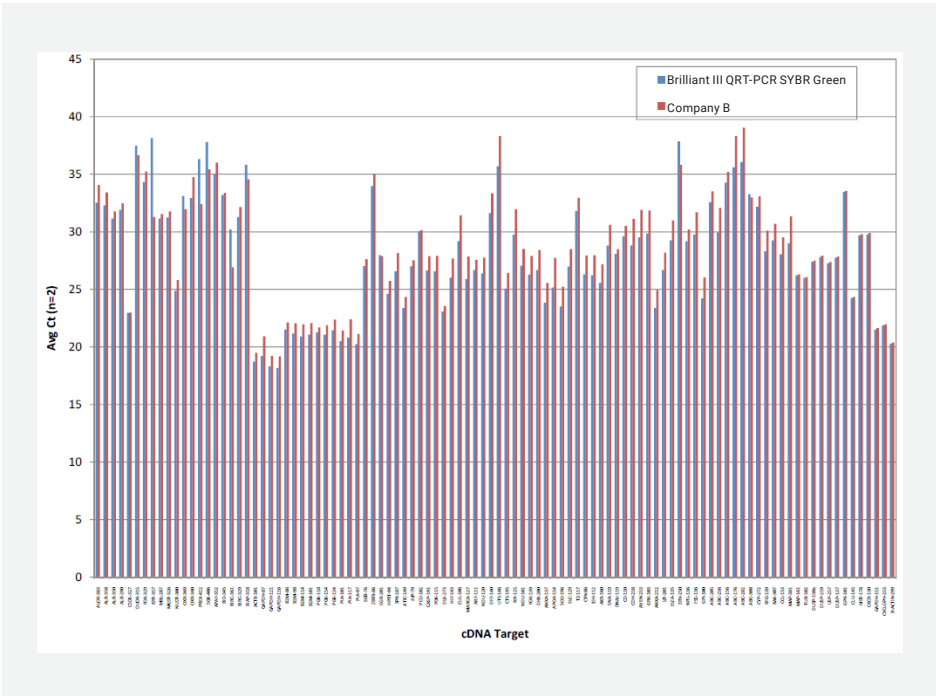
Table 2. Agilent Brilliant III Ultrafast SYBR Green qPCR Master Mix is one of the fastest and most reasonably priced master mixes.

Kit Manufacturer	Total Reaction Time (min)	List Price/Reaction (\$)
Agilent Technologies	45	0.9
Company Qi	45	1.2
Company K	89	0.7
Company Qu	89	0.9
Company T	90	0.9
Company B	125	0.9

The thermal cycling reaction run time for each individual kit was the optimal reaction time recommended by the manufacturer. All reactions were run on the AriaMx Real-Time qPCR instrument. Prices were calculated from the manufacturer website.



Comparison of robustness of Brilliant III Ultrafast qRT-PCR SYBR Green (blue bar) to company Qi qRT-PCR Master Mix (red bar) across 99 cDNA targets (10 ng each) on the ABI StepOnePlus Real-Time qPCR system. Brill exhibited lower Ct values for equivalent starting DNA amounts vs the competition.



A similar result as above was observed for company B qRT-PCR SYBR Green master mix on the BioRad CFX96 system. The labels of each cDNA target are denoted on the graph as well as the length of the expected product. Thermal cycling conditions followed the manufacturer protocol.

Figure 3. Agilent Brilliant III Ultrafast SYBR Green qRT-PCR Master Mix kits are more robust across multiple targets and can be used on non-fast cyclers.

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