Application Note A09-001A

Thermal uniformity across the block

PrimePro48

Prime Pro 48 Thermal System

For the highest accuracy in qPCR, temperature must remain uniform across the entire plate during all cycling steps, ensuring that all samples are processed equally. The unique thermal block design of the Techne Prime Pro 48 achieves this with an innovative heating and cooling system that provides accurate ±0.1°C temperature control and quickly cycles from one temperature to the next. This unique design delivers industry leading thermal stability in a convenient block format, resulting in higher qPCR performance, tighter Cq values and the ability to perform HRM applications.

In this application note we demonstrate the uniformity in both Cq and melting temperature (T_m) for samples run in each of the 48 wells of the block.

Method

To demonstrate uniformity we first ensured that every well contained the same amount of starting material. A master mix was prepared (sufficient for all 48 wells) containing the equivalent of $1x10^8$ copies of template per well. 10µl of the mix was pipetted into each well, the plate sealed with a Pro adhesive seal and the plate centrifuged for 1 minute at 1200rpm. The plate was then cycled for 40 cycles in the Prime Pro 48 followed by a melt stage to determine the T_m. The entire run took approximately 43 minutes to complete. The results were analysed using the ProStudy software to determine the Cq and T_m values for each sample.

Results

Figure 1 shows the baseline corrected amplification plot for all 48 wells. The graph clearly demonstrates the precision of amplification across the entire plate. Analysis of the data showed an average Cq of 13.31 with a standard deviation of ± 0.061. This equates to a %CV across the plate of just 0.46%.

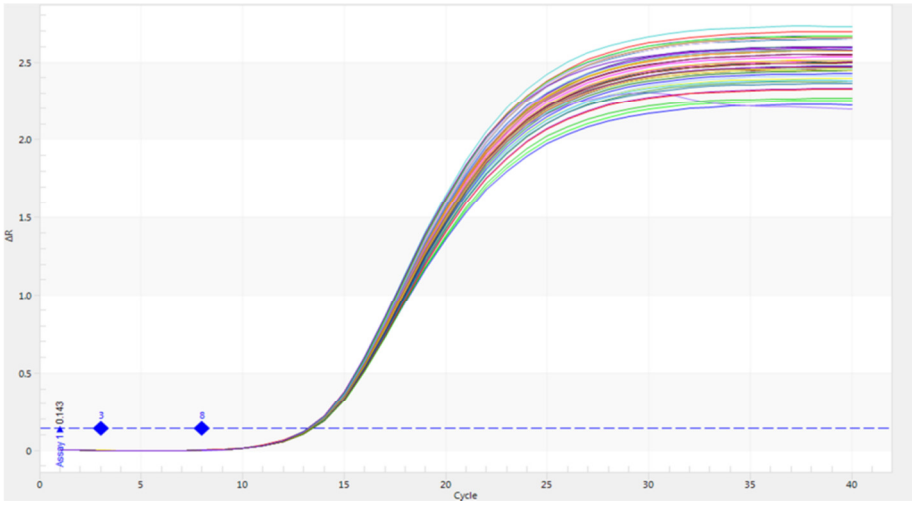

***Figure 1**: Baseline corrected amplification plot showing the data from all 48 wells of the plate. The template (100bp template based on Lambda phage DNA) was amplified for 40 cycles (95°C, 10s; 60°C, 30s) using the GoTaq® QPCR Master Mix (2x) from Promega (part code A6001). Fluorescence data was collected at the end of the 60°C step using the Green channel.*

One of the best measures of block uniformity is to determine the T_m of the PCR product. This can be readily done by running a melting stage following the amplification steps. The determined temperatures depend purely on the chemical composition of the product and are not reliant on the accuracy of external temperature probes.

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The amplified products were melted over the range 75°C to 95°C, collecting fluorescence data throughout. The graph is shown in Figure 2.

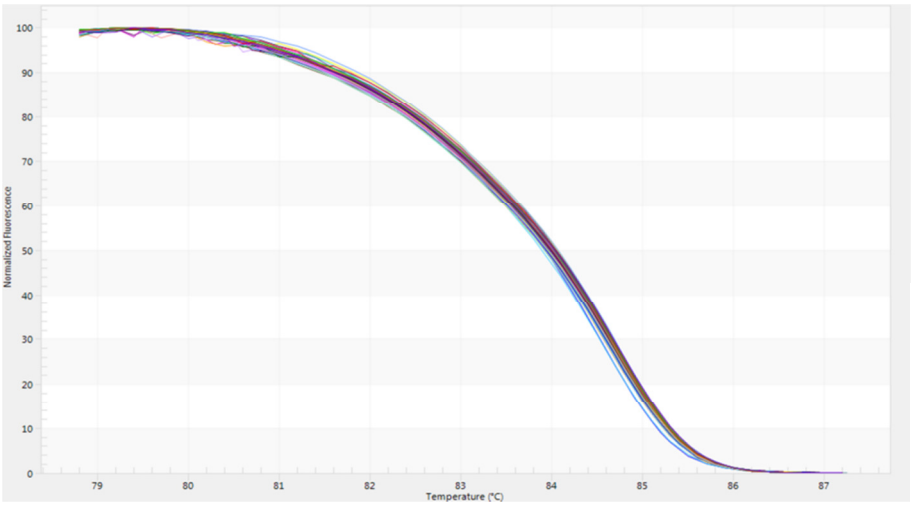

***Figure 2**: Normalised melt plot showing the data from all 48 wells of the plate. The 100bp PCR product template was melted over the range 75°C to 95°C. Fluorescence data was collected throughout using the Green channel.*

Analysis of the data showed an average T_m of 84.45°C with a standard deviation of ± 0.058. This equates to a %CV across the plate of just 0.07%. Results from each of the individual wells are summarised in Figure 3.

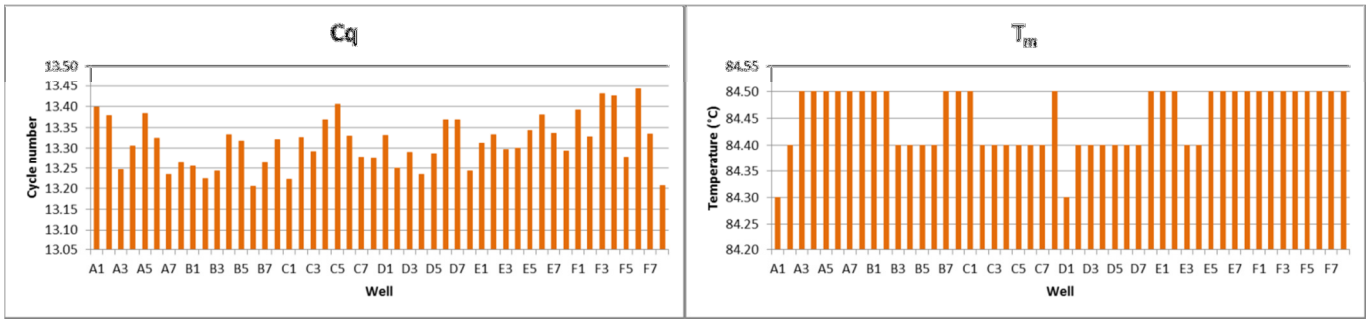

***Figure 3**: Summary of Cq and T_m data from each of the 48 wells of the plate. The total range across the Cq values (maximum – minimum) was 0.24 cycles and the temperature range of the T_m was a maximum of 0.2°C between samples.*

Conclusions

Fast, uniform temperature control is important in qPCR because accurate dwell temperatures ensure that the primers bind efficiently and the polymerase enzymes work optimally, generating the maximum yield of DNA. In addition to this, uniformity across the plate is essential for accurate quantification, whether performing absolute or relative quantitative assays. Block uniformity removes one more variable from the assay ensuring that any variation measured is from the samples themselves and not due to the instrument. This increases accuracy and reduces the requirement for numerous replicates. The unique design of the Prime Pro 48 block ensures that it is the most thermally accurate and uniform block based system currently on the market.