Technical note

For research purposes only

PCRmax Eco 48: The world's most uniform block based qPCR system and what that means for your data.

PCRmax Eco 48 Thermal System

Temperature control is at the heart of qPCR. Without accurate and exact temperature control confidence in data can drift and more replicates need to be performed to improve confidence.

The unique thermal block design of the Eco 48 achieves the highest uniformity of any block based system by applying unique and proprietary technologies to solve the problem of poor thermal uniformity and by extension poor data quality: the Eco 48 is able to achieve ±0.1°C uniformity at 95°C

Unlike most other cyclers we challenged the system to achieve this now gold standard in uniformity at 95°C, many other qPCR systems record uniformity at much lower, easier to achieve, temperatures. This unmatched uniformity is also recorded with no settle time, which means that with the Eco 48 you can shorten the hold times massively as you are sure all your samples are immediately at the correct temperature and completely uniform. This gives end users confidence in their data, the ability to reduce the number of replicates and to save time. The Eco 48 can easily achieve 40 cycle protocols in 40 mins and often much faster, using completely standard plastics and reagents.

In this application note we demonstrate the uniformity in both Cq and melting temperature (Tm) for samples run in each of the 48 wells of the block. Future application notes will highlight the Eco 48 ramp speeds, cycle times and dwell times at set temperatures to highlight how the Eco 48 can be used to get qPCR run times, for 40 cycles, down to comfortably below 20mins and still achieving gold standard thermal uniformity which is unmatched in any other block based qPCR system.

Method

To define the thermal uniformity across the Eco 48 block 48 replicate samples were put through one of the most thermally demanding, in terms of accuracy, protocols, High Resolution Melt (HRM). Each of the 48 wells had 1x10^8 copies of template in a 10µl final volume. The plate was sealed with and Eco Seal plate centrifuged for 1 minute at 1200rpm. The entire 40 cycle protocol and associated HRM was performed in 43 minutes total. The results were analysed using the Eco Study software to determine the Cq and Tm values for each of the 48 replicates and note the degree of uniformity.

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%.

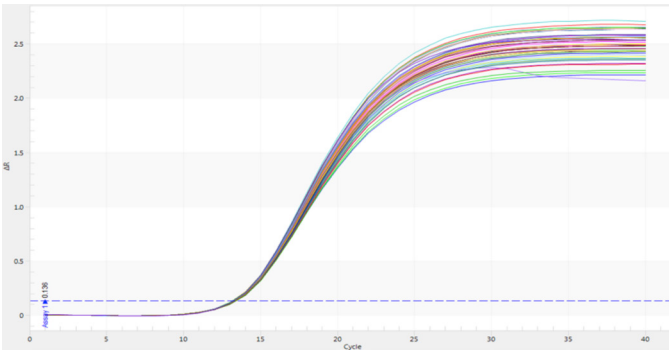


Figure 1: Baseline corrected amplification plot showing the data from all 48 wells of the plate. The template (100bp template based on Lambda phage DNA) was amplified for 40 cycles (95°C, 10s; 60°C, 30s) using the GoTaq® QPCR Master Mix (2x) from Promega (part code A6001). Fluorescence data was collected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

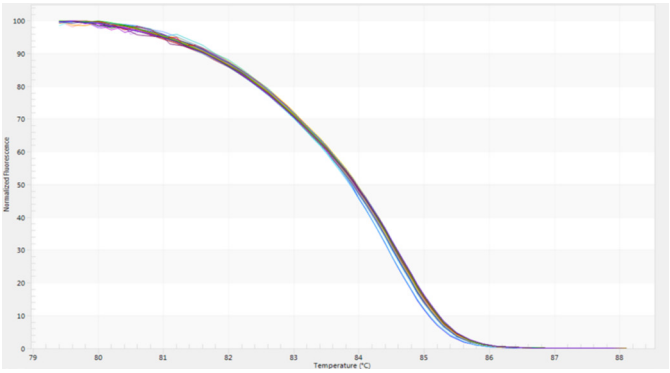


Figure 2: Normalised melt plot showing the data from all 48 wells of the plate. The 100bp PCR product template was melted over the range 75°C to 95°C. Fluorescence data was collected.

The Tm average across all 48 wells was recorded as 84.45°C with a standard deviation of ± 0.058, which equates to a %CV across the plate of just 0.07%. Figure 3 shows a summary of the results obtained.

One of the best measures of block uniformity is to determine the Tm 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.

Amplified product melted in the 75°C to 95°C range, the Eco 48 measures the fluorescence with every 0.1°C of temperature change which confers the accuracy required to detect class IV SNPs with greater than 99% accuracy. Figure 2 shows the normalised melt curve.

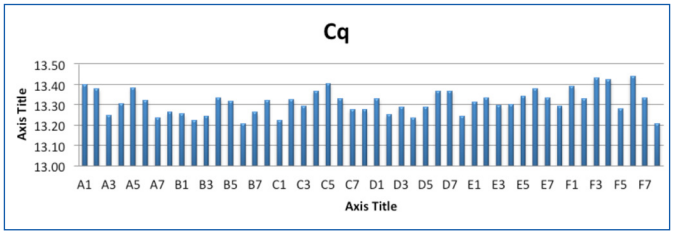
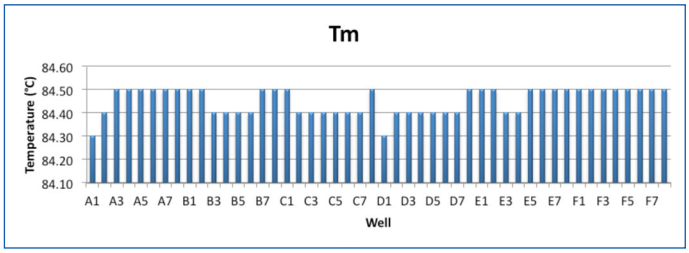


Figure 3(A and B): Summary of Cq and Tm data from each of the 48 wells of the plate. 0.24 cycles was the largest recorded variation across all 48 samples with a maximum range in Tm of 0.2 °C (84.3°C to 84.5°C) across the entire plate



Conclusions

Fast, uniform temperature control is crucial to qPCR because accurate dwell temperatures ensure that the primers bind efficiently and the polymerase enzymes work optimally, generating the maximum yield of DNA and limiting machine derived artefacts. This uniformity across the plate is essential for accurate quantification of any sample and any application.

Extreme block uniformity, like you get in the Eco 48, 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 as well as improving the confidence in the data and the conclusions that can be drawn from those data. The unique design of the Eco 48 block ensures that it is the most thermally accurate and uniform block based system currently on the market.

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