Thermal Uniformity with the xxpress® qPCR Thermal Cycler

qPCR is a molecular biology technique that utilises temperature cycles to amplify and quantify DNA. Thermal uniformity is essential when performing qPCR to ensure repeatable, reliable results. Undershoots can prevent reaction steps from reaching completion, whilst overshoots can cause the sample to degrade. At BJS Biotechnologies we have developed the xxpress® qPCR thermal cycler which runs our patented heating algorithm to control the system thermals. This combined with its resistive heating technology and low thermal mass consumable is extremely effective in producing unbeatable thermal uniformity of ±0.3°C across the whole plate.

1. Quantifying Uniformity

This qPCR experiment amplifies the 18s rRNA housekeeping gene using Human Genomic DNA (HgDNA) as the template and SYBR-based chemistry. An automated pipetting robot aliquots 20 µL of the reaction mixture into each well of a 24 well xxplate™ (see Figure 1). The results are used to calculate the Cq variation.

Reaction Mixture: 250 µL KAPA SYBR FAST qPCR mastermix, 175 µL Nuclease Free Water (NFW), 25 µL forward primer (10 µM), 25 µL reverse primer (10 µM), 25 µL HgDNA (10ng/µL).

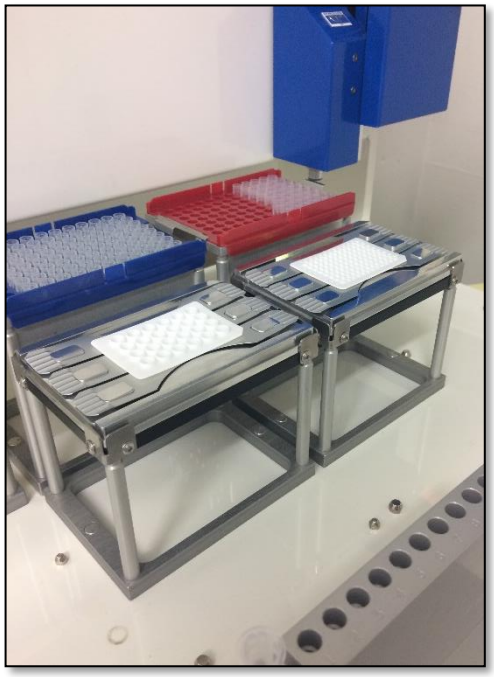


Figure 1: The set up for a 24 well xxplate™ being filled with qPCR reaction mixture by an automated pipetting robot.

The xxpress® is an ultra-fast thermal cycler and operates at ramp rates of 10°C per second during heating and 8°C per second during cooling. The thermal protocol used for this experiment can be seen in Table 1. All steps were performed using maximal ramp rates.

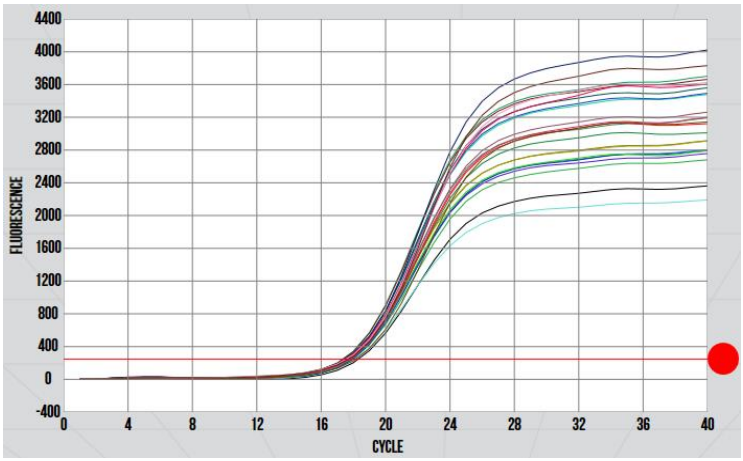
Stage	Temperature	Dwell	Repeats
Initial Denaturation	95°C	120 secs	x1
Denaturation	95°C	5 sec	x40
Annealing & extension	60°C	30 secs	
Melt	50°C	5 secs	x1
	50°C - 95°C	-	x1

Table 1: The thermal protocol used by the xxpress® in an experiment to quantify the uniformity of a qPCR reaction.

2. Results

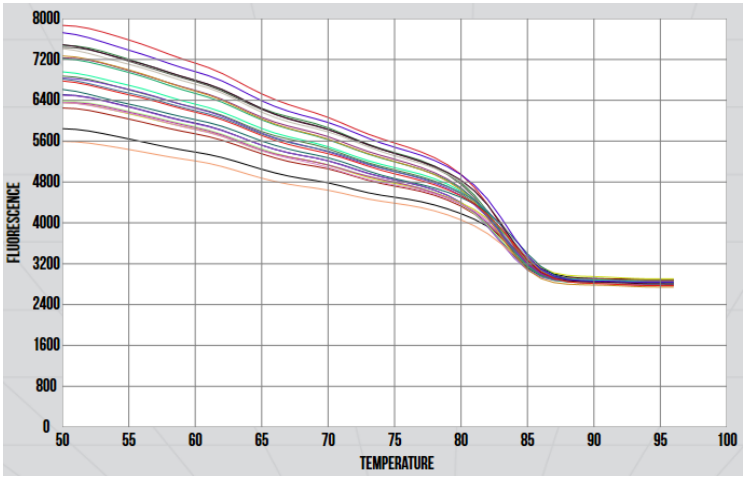
The entire qPCR and melt were completed in just over half an hour. In this time the xxpress® also generated an amplification graph, a melt graph, the Cq values and Tm values for each well. This data was easily exported as a .csv or .RDML file for further analysis.

2.1 The xxpress® produces intuitive amplification graphs.



Graph 1: The amplification graph produced by the xxpress® from the SYBR-based qPCR detailed.

2.2 The xxpress® produces fast, informative melt curves.



Graph 2: The melt curve produced by the xxpress® from the SYBR-based qPCR detailed. The xxpress® is also able to plot the temperature against the first derivative of the fluorescence to display the melt peaks.

2.3 The xxpress' excellent uniformity of ±0.3°C across the whole plate produces repeatable results.

Mean Cq	Standard Deviation	Coefficient of Variation
17.7	0.26	1.5%

Table 2: The statistics resulting from the analysis of the detailed SYBR-based qPCR experiment on the xxpress®.

3. Conclusions

The excellent thermal uniformity of the xxpress® thermal cycler enables repeatable results across the whole plate with low amounts of variation in Cq value. This was reflected in the results of the SYBR-based qPCR experiment performed here. The mean Cq value was 17.7 (±0.26).

The xxpress® not only reaches each target temperature much faster than traditional peltier based systems, but is also much more stable in maintaining the desired temperature. The xxpress® does not experience temperature undershoots and overshoots which ensures that each reaction step reaches completion and prevents sample damage.

It's the unique resistive heating technology combined with its patented heating algorithm and the low thermal mass of the consumable that makes the thermal uniformity of the xxpress® unbeatable.

Figure 2: The xxpress® qPCR thermal cycler.

4. References

- Broadway, K. and Karteris, E. (2015) Amplification efficiency and thermal stability of qPCR instrumentation: Current landscape and future perspectives. *Exp Ther Med.* **10** (4), 1261–1264.
- Nolan, T. and Bustin, S. (2013), PCR Technology: Current Innovations, Third Ed. CRC Press.
- Bustin, S., *et al.* (2009), The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry.* **55** (4), 611–622.

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