Standard Dilution Series with the xxpress® qPCR Thermal Cycler

The success of a qPCR reaction is evaluated using a standard dilution series, the data from which is used to calculate the reaction efficiency and R^2 values. Efficiency can be defined as the increase in amplicon per cycle, whilst R^2 is the coefficient of determination. Ideally, the efficiency of a qPCR reaction would be 100%, meaning that the PCR product is exactly doubling every cycle during the logarithmic phase. Criteria are put in place to determine how successful a qPCR protocol is, with an efficiency of 90-110% and an $R^2 > 0.985$ being deemed acceptable. The xxpress® qPCR thermal cycler performs successful, repeatable qPCRs even with low volume reactions, enabling optimal use of reagents.

1. Standard Dilution Series

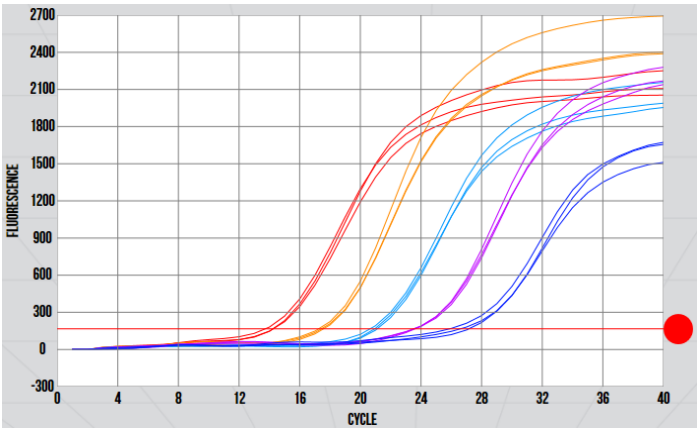
The following standard dilution series were performed in order to calculate the efficiency and R^2 values of qPCR reactions performed on each xxplate format using the xxpress® qPCR thermal cycler. Each dilution was run in triplicate with a final reaction volume of just 5µL per well.

Reaction mixture for each dilution: 20µL KAPA SYBR Fast Mastermix, 14µL nuclease free water, 2µL forward primer (10 µM), 2µL reverse primer (10µM), 2µL human genomic DNA template (100ng/µL, 10ng/µL, 1ng/µL, 0.1ng/µL or 0.01ng/µL).

Thermal protocol: initial denaturation at 95°C for 60 seconds, 40 cycles of denaturation at 95°C for 5 seconds, annealing & extension at 60°C for 30 seconds.

2. Results

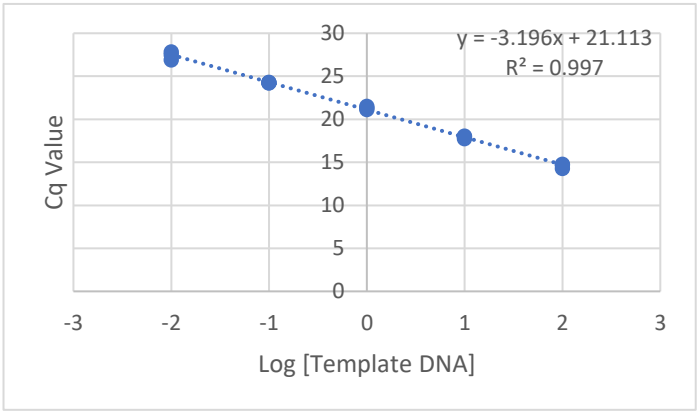
2.1 The xxpress® qPCR thermal cycler produced intuitive amplification graphs to display the successful qPCR standard dilution series data.



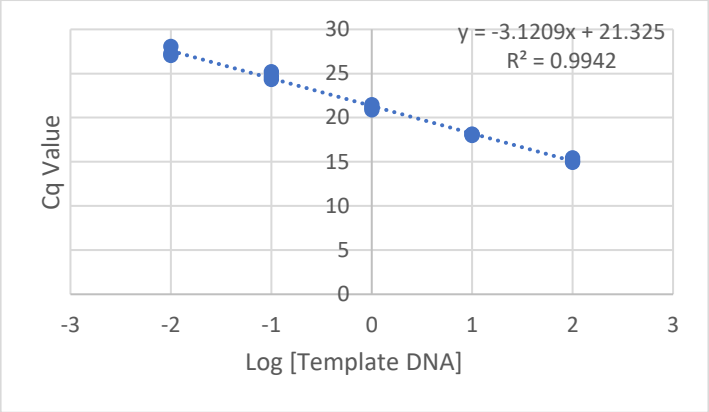
Graph 1. Amplification graph as displayed on the xxpress® thermal cycler, presenting qPCR standard dilution series data produced using a 96-well xxplate.

2.2 The xxpress® qPCR thermal cycler produced successful, linear standard curves with all xxplate formats.

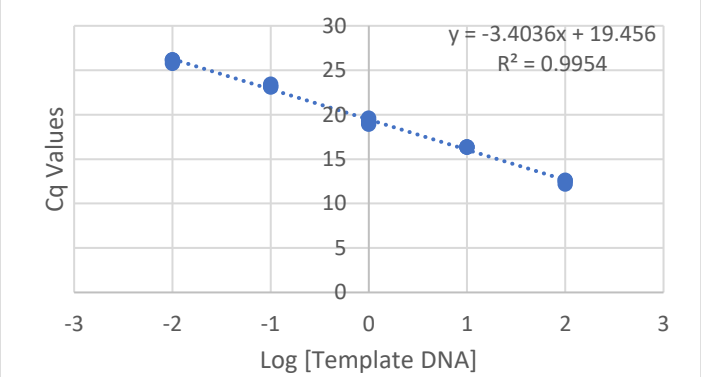
For each xxplate, the data collected was used to produce a standard curve by plotting the Cq value of all replicates against the log[template DNA] (see graphs 2, 3, 4).



Graph 2: The standard curve produced from the detailed standard dilution series on a 96-well xxplate using the xxpress®.



Graph 3. The standard curve produced from the detailed qPCR standard dilution series on a 54-well xxplate using the xxpress®.



Graph 4. The standard curve produced from the detailed qPCR standard dilution series on a 24-well xxplate using the xxpress®.

2.3 The xxpress® qPCR thermal cycler performed efficient qPCR reactions with all xxplate formats.

For each standard curve a linear trendline was added and the equation of the line displayed (as seen in graphs 1, 2, 3). From this, the gradient could be determined and used to calculate the efficiency of the qPCR reaction. Efficiency is defined using the formula displayed in figure 1.

$$\text{Efficiency} = 10^{(-1/\text{gradient})} - 1$$

Figure 1: The formula used to calculate the efficiency of a qPCR reaction.

All three xxplate formats produced good qPCR efficiency values within the accepted criteria. The reaction efficiencies were 96.7%, 109% and 105.5% for the 24-, 54- and 96-well xxplate qPCR standard dilution series respectively.

2.4 The xxpress® qPCR thermal cycler performed linear standard dilution series with all xxplate formats.

The R^2 value of a standard dilution series indicates how linear the data is, in other words how variable the assay replicates are. All three xxplate formats produced linear qPCR data with little replicate variation when run on the xxpress® thermal cycler. Each xxplate format produced good R^2 values (see table 2).

xxplate Format	Reaction Efficiency	R^2 Value
24-well	96.7 %	0.9954
54-well	109 %	0.9942
96-well	105.5 %	0.997

Table 1. The standard dilution series R^2 values and reaction efficiencies for each xxplate format on the xxpress® qPCR thermal cycler.

3. Conclusions

The data demonstrates the ability of the xxpress® qPCR thermal cycler to perform highly efficient, linear qPCR reactions with low replicate variation and good dynamic sensitivity. This applies to each xxplate format, all of which are interchangeable on the same thermal cycler.

The 24- and 96-well xxplates have the same well spacing as 96- and 384-well microtiter plates respectively. This makes the xxpress® system compatible with automatic pipettes, saving you time and increasing throughput!

4. References

- Bustin, S., *et al.* (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry.* **55** (4), 611–622.
- Saunders, N and Lee, M. (2013). Real-time PCR Advanced Technologies and Applications. Norfolk: Caister Academic Press.

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