Tech Note
qTOWERiris

Homogeneity - a Key Factor in Real-time PCR Performance

Introduction

Real-time PCR or qPCR has become an essential tool in molecular biology for detecting and quantifying nucleic acids. This method is indispensable across a wide range of fields, including R&D, pharmaceutical sector, food and animal feed quality assurance, and forensics, to name a few. As qPCR becomes increasingly important, devices on the market must meet ever-higher standards of quality and performance. Key factors include the ability to employ various analysis methods, the capability to display multiple targets simultaneously in a multiplex assay, and the high sensitivity and reproducibility of measurement results. These are primarily achieved through uniform excitation and detection across the entire sample block. This Tech Note demonstrates that the real-time qPCR thermal cycler qTOWERiris meets these high standards of optimal measurement homogeneity by using the Luna® Universal qPCR Master Mix from New England BioLabs® GmbH.

Your Benefits

- Patented high performance optical system of qTOWERiris series
- Optimal homogeneous excitation and detection in each of the 96 wells
- High reproducibility and Sensitivity
- Maximum flexibility and constant results in different sample volumes

Application

A standard qPCR experiment with 96 samples was performed in the real-time qPCR thermal cycler qTOWERiris. In the analysis, Ct values were considered as well as the homogeneity of the measurements based on the standard deviation of the Ct and melting point values, and the deviation of the final fluorescence of all samples.

Results

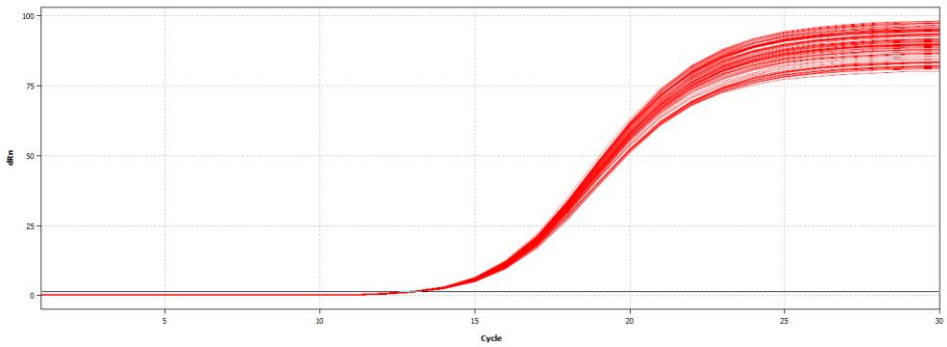

Figure 1: qPCR amplification curves of the standard qPCR experiment across 96 samples.

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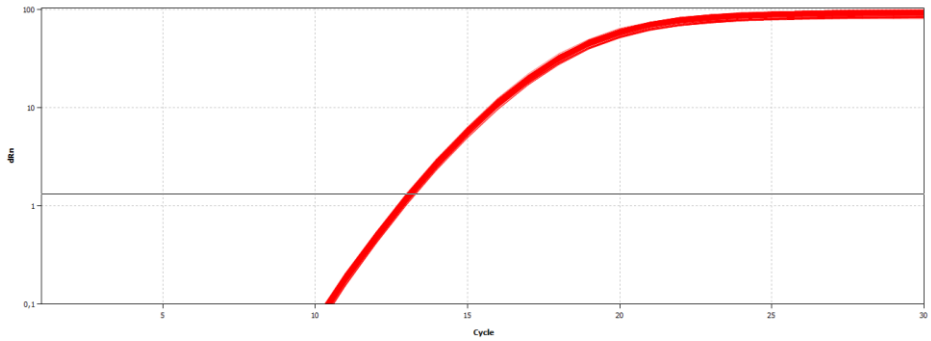

Figure 2: Semi-logarithmic scaling of qPCR melting curves of standard qPCR experiment across 96 samples.

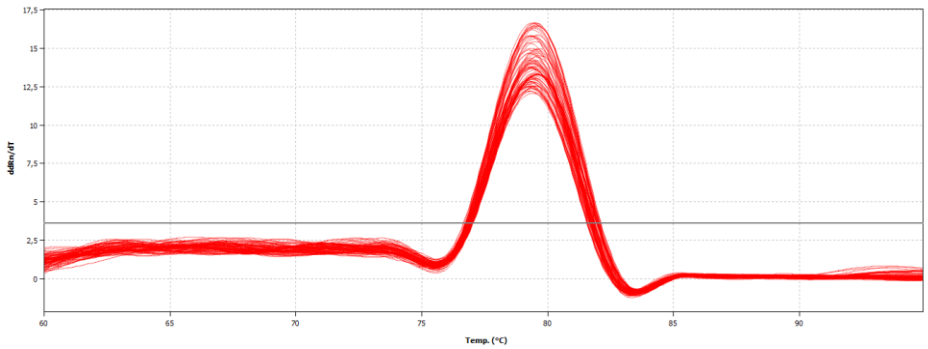

Figure 3: Melting points of qPCR melting curves of standard qPCR experiment across 96 samples.

Table 1: Analysis data of qPCR amplification and melting curve.
Ct: Cycle threshold. Std dev. Ct: Standard deviation of Ct, Tm: Melting point, Std dev. Melt: Standard deviation of melting point.

Ct value	Std dev. Ct	Min. Tm	Max. Tm	Diff. Tm	Std dev. Tm
13.08	0.05	79.2 °C	79.6 °C	0.4 °C	0.11

Min. final fluorescence	Max. final fluorescence	Diff. final fluorescence	Deviation final fluorescence
52,688	63,801	11,113	17%

Conclusion

Figures 1, 2, and 3 clearly demonstrate the highly homogeneous distribution of curves in both the amplification plot and the melting curve plot of the standard qPCR experiment across 96 samples. The standard deviation of the Ct value, at 0.05, is within a very precise range. The standard deviation of the melting points of the different curves is similarly low at 0.11, with an overall difference of 0.7°C. Furthermore, the variation in final fluorescence across the 96 samples was 17%, which is well within the acceptable range of 30%. These results show a very precise and homogeneous performance of the qTOWERiris using the Luna® Universal qPCR Master Mix from New England BioLabs® GmbH.

Reference: TechNote_qTOWERiris_0015_Homogeneity_en.docx

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