Tech Note
qTOWERiris

Exceptional precision with 1.3- and 1.5-fold discrimination in qPCR

Introduction

A valid real-time PCR assay is characterized by four major properties: PCR efficiency, reproducibility, specificity and sensitivity. Several factors influence these critical aspects; beside primer design, plasticware and master mix, the performance of the real-time PCR cycler itself also affects the results. For this reason, it is essential to ensure the best performance of the real-time PCR cycler to rely on the outcome of the applied real-time PCR.
One of the most challenging requirements is the ability to discriminate between small dilution steps, even down to 1:1.3 or 1:1.5, which demonstrates the dependability and accuracy of the real-time PCR cycler's performance, and consequently, the reliability of all data generated with the device.

Your Benefits

- Robust and calibration-free qPCR cycler
- Highest precision due to silver sample block technology
- Best reproducibility realized by unmatched temperature homogeneity

Application

In this experiment, specific primers for the 16S rRNA gene of *E. coli* K12 were used to amplify a 120 bp fragment. The amplification was performed across seven dilution steps, with six replicates per concentration, each in a 20 µL reaction volume using the master mix 2x SensiFAST SYBR no-ROX Mix (Meridian Bioscience). The time-temperature profile and device settings are provided in table 1.

Table 1: Time and temperature protocol of qTOWERiris

Step	Cycle	Profile	Temperature	Holding time	Ramp rates
1	1	Initial denaturation	95°C	3 min	8 °C/sec
2	40x	Denaturation	95°C	5 sec	8 °C/sec
		Annealing	58 °C	5 sec	5.5 °C/sec
		Elongation*	72 °C	20 sec	6 °C/sec
3		Melting curve*	60 °C - 95 °C	30 sec	

** Data acquisition: Color module 1 using gain 5*

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Results

The evaluation of the standard curve demonstrates a clear distinction between the individual dilution levels for both dilution series (see table 2). Figures 1 and 2 prese the amplification plots in the logarithmic view.

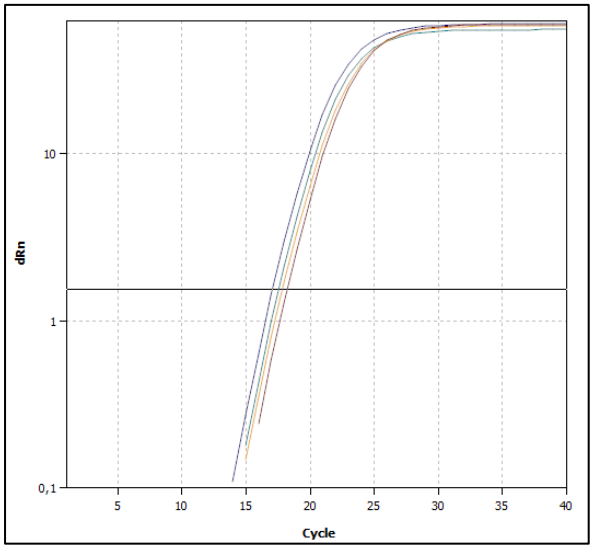

Figure 1: Amplification plot for 4-time 1.3-fold dilution of *E. coli* genomic DNA, amplified using primers specific for 16S rRNA gene. With automated threshold settings results are as follows for Ct: blue = 17.10, 7.69 × 10^6 copies; green = 17.53, 5.91 × 10^6 copies; orange = 17.86, 4.55 × 10^6 copies; violet = 18.21, 3.50 × 10^6 copies

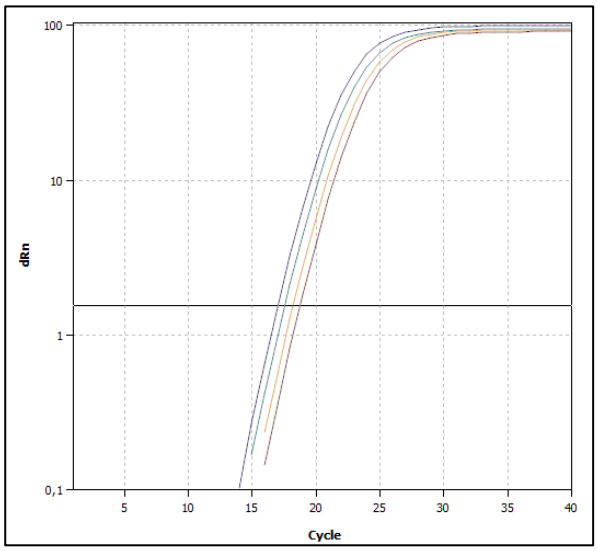

Figure 2: Amplification plot for 4-time 1.5-fold dilution of *E. coli* genomic DNA, amplified using primers specific for 16S rRNA gene. With automated threshold settings the results are as follows for Ct: blue = 17.03, 6.66 × 10^6 copies; green = 17.51, 4.44 × 10^6 copies; orange = 18.19, 2.96 × 10^6 copies; violet = 18.74, 1.97 × 10^6 copies

Table 2: Values of the standard curve calculation

Sample	Dilution	
	1:1.3	1:1.5
R^2	0.997	0.996
Slope	-3.23	-3.29
Offset	39.35	39.47
PCR efficiency	1.04	1.01

This TechNote demonstrates that the qTOWERiris from Analytik Jena enables highly precise and reproducible quantification, reliably distinguishing even the smallest dilution steps (1:1.3 or 1:1.5). This underscores the cycler's accuracy and ensures dependable data quality.

Reference: TechNote_qTOWERiris_0021_Exceptional Precision 1.3-fold discrimination_en.docx

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