Tech Note
qTOWERiris

Wide Linear Range with accurate Quantification across 10 Orders of Magnitude

Introduction

Reliable and reproducible results in real-time PCR are prerequisite for trustworthy analysis in various application fields. For instance, when performing gene expression analysis of various genes at once, some genes can be less expressed while others can be highly expressed. In addition to a high-quality real-time PCR device, optimized assay chemistry is needed. Typically, real-time PCR assays should have a linear dynamic range of 10^1 copies of up to 10^9 copies. Detection of less than ten copies is challenging because the concentration is so low that aliquots of the same volume may or may not even contain one copy. In contrast, detection of more than 10^9 copies is difficult because either assay components are limiting, or the initial amount of template introduces strong background in the instrument's detection.

Your Benefits

- Patented high performance optical system of qTOWERiris
- Optimal homogeneous excitation and detection across all 96 wells within 6 seconds
- High reproducibility and sensitivity
- Excellent temperature uniformity of ± 0.15 °C across the entire sample block

Application

The template of the experiment was a synthetic DNA of 118 bp length with a concentration of 3* 10^{10} copies. Specific primers and a FAM probe for the target region were used to amplify and detect the DNA sequence precisely. Serial dilutions of the stock template solution were prepared, spanning ten orders of magnitude. These were measured in technical replicates in 20 µL qPCR reaction volume using the New England Biolabs Luna® Universal Probe qPCR Master Mix and the real-time PCR system qTOWER iris.

Results

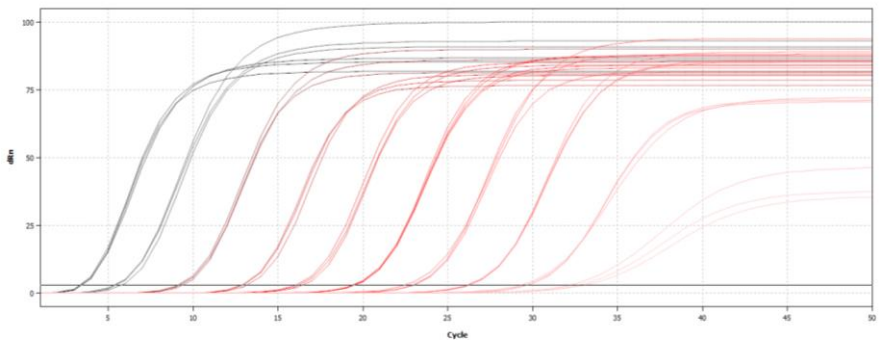

Figure 1: Amplification plot of the 10-fold serial dilutions.

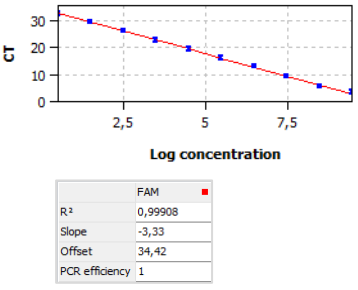

Figure 2: Standard curve and PCR efficiency of the 10-fold template dilutions.

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Table 1: Mean Ct values, standard deviation and difference of Ct values between adjacent dilution steps of the serial dilutions.

Sample	Mean Ct	SD (Ct)	Δ Ct
1	4.13	0.04	
2	6.37	0.17	2.24
3	10.14	0.09	3.77
4	13.9	0.18	3.76
5	17.2	0.15	3.30
6	20.52	0.05	3.32
7	23,89	0,21	3.37
8	27,35	0.02	3.37
9	30,93	0.16	3.58
10	34.54	0.54	3.61

Serial dilutions of synthetic human DNA were amplified across a wide linear range with accurate quantification across 10 orders of magnitude using the New England Biolabs Luna® Universal Probe qPCR Master Mix on the real-time PCR cycler qTOWERiris. The standard deviations of the Ct values of the technical replicates were less than 0.6 within an acceptable range. A Ct value difference of 3.3 per dilution step is optimal to ensure high PCR efficiencies. Here, an average Ct value deviation value of 3.31 fulfils that criterion. This real-time PCR trail illustrates that a wide range of high and low concentrated samples can be detect and simultaneously analyzed the qTOWERiris device.

Reference: TechNote_qTOWERiris_10 orders of magnitude_0012_en.docx

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