Challenge

Accurate and reproducible results for real-time PCR experiments.

Solution

Real-time PCR cyclers with best temperature properties and highest optical precision.

Highest precision with 1.5-fold discrimination for qPCR quantification applications

Introduction

A valid real-time PCR assay is characterized by four major properties: PCR efficiency, reproducibility, specificity and sensitivity. Several factors have an influence on these critical points; beside primer design, plastic ware and master mix, of course the performance of the real-time PCR cycler itself affects the results. For this reason, it is mandatory to guarantee best properties of the real-time PCR cycler to rely on the outcome of the applied real-time PCR.

One of the most challenging requirements is to discriminate between small dilution steps, even down to 1:1.5, which proves the dependability and accuracy of the performance of the real-time PCR cycler and therefore of all data created with the device.

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Materials and Methods

For this experiment primers specific for 16S rRNA gene of *E. coli* have been used for amplification of a 120 bp fragment in seven dilution steps with six replicates á 20 µl reaction volume for each concentration. The used master mix was innuMIX qPCR SyGreen Sensitive by Analytik Jena. The time-temperature-profile starts with 2 min at 95°C, followed by 45 cycles with 5 sec at 95°C, 5 sec at 58°C and 15 sec at 72°C and ends with a melting curve (60 – 95°C, 15 sec equilibration and 1°C increment).

Instrumentation

Data was obtained in color module 1 (470 nm/520 nm, gain = 5) of qTOWER³ G *touch* for SyGreen.

Results

The linear regression of the 1.5-fold dilution series results in a PCR efficiency of 97% (slope = -3.41) and R^2 = 0.998.

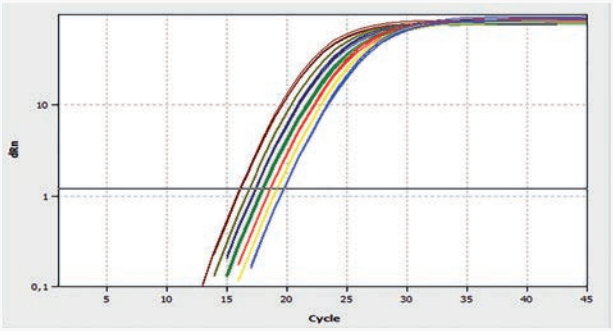

Figure 1: Amplification plot for 6-time 1.5-fold dilution of E. coli genomic DNA, amplified using primers specific for 16S rRNA gene. With automated threshold settings results are as follows for Ct (SD): 10^6 copies, dark red = 16.11 (0.04); $6{,}67 \cdot 10^5$ copies, light green = 16.83 (0.05); $4.44 \cdot 10^5$ copies, dark blue = 17.31 (0.04); $2.96 \cdot 10^5$ copies, green = 18.05 (0.03); $1.98 \cdot 10^5$ copies, red = 18.59 (0.03); $1.32 \cdot 10^5$ copies, yellow = 19.18 (0.03); $8.78 \cdot 10^4$, blue = 19.72 (0.03).

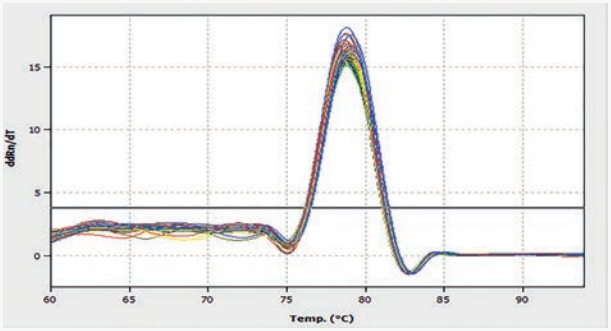

Figure 2: Melting peaks of the PCR amplicons result in a melting temperature of 78.80°C with a SD of 0.13°C.

Conclusion

The Analytik Jena qTOWER³ G *touch* is able to detect and clearly differentiate between minute concentration changes. In the performed 1.5-fold dilution series, high precision discrimination was demonstrated in a reaction with a PCR efficiency of 97% and a R^2 of 0.998.

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en · 07/2018