Challenge

Improving the reproducibility of the real-time results in 384 well format

Solution

Application of a 384 well qPCR using a pipetting robot and standardized sample setup

Real-time PCR to Measure Minute Volumes in High Throughput

Introduction

Steadily-increasing sample volumes and new options for molecular biology experiments are changing the demands on quantitative real-time PCR. For these, the transition to the multi-well format with 384 samples opens up new application areas for gene expression, mutation analysis, and diagnostics.

Real-time PCR in 384-well high-throughput format requires a steady homogeneous readout of minute sample volumes, which, in addition to such factors as speed and experimental accuracy in the high-throughput format, will play an increasingly important role. The qTOWER³ 84 provides precise performance in 384-well high-throughput format by means of an aluminum block with guaranteed temperature uniformity. In addition, the patented high-performance optics enables a readout time of only six seconds for a complete 384-well plate – regardless of the number of fluorescent dyes that are used.

The preparation effort required for performing a high-throughput qPCR is increasing. In addition, the setup for the reaction batch for qPCR requires certain skills and expertise. Along with a quadrupling of the number of samples of the 96-well standard format, the reduced sample volume also plays an important role. These micro-volume ranges must be pipetted with extreme precision, because even the minutest variations in raw material can lead to major differences in the amplification.

analytikjena
An Endress+Hauser Company

Material and methods

Using DNA extracted from Escherichia coli, a sequence-specific primer pair and the double-concentrated innuMIX qPCR MasterMix SyGreen (Analytik Jena), an E.coli-specific target sequence of 120 bp is amplified in real-time in the 384-well format.

A) Manual			B) Automated		
Reaction volume	**Ct**	**SD(Ct)**	**Reaction volume**	**Ct**	**SD(Ct)**
2 µL	14.926	0.05	2 µl	14.896	0.03
5 µL	14.464	0.04	5 µl	14.430	0.02
10 µL	14.128	0.05	10 µl	14.168	0.02
20 µL	14.12	0.08	20 µl	14.096	0.04

Figure 3: Comparison of the automatically generated Ct values of the qPCRsoft in the qTOWER^3 84.

In a variation of the reaction volume, five technical replicas 5 µl to 20 µl of the master mix were used. Subsequently, the specificity of the amplification product was verified in a melting curve. The amplification is carried out in a FrameStar® qPCR plate (4titude) with an initial denaturing of 120 s followed by 35 cycles, with the denaturation at 95 degrees Celsius for 15 s, annealing at 58 degrees Celsius for 15 s, and elongation at 72 C for 30 s. The fluorescence signal was recorded at 72 degrees Celsius in each cycle. A manual reaction setup using a manual pipette was compared with an automated reaction batch that was pipetted using the pipetting robot GeneTheatre (Analytik Jena).

Results and Discussion

The results of the volume variation show that it is possible to reduce the reaction volume up to 2 µl for this assay without compromising precision and reproducibility. Even this small sample volume shows a standard deviation of the Ct values of only ± 0.05 over five replications. The amplification line plot from the automatically generated reaction setup using the pipetting robot also shows a homogeneous distribution of the volume-varied real-time PCR curves. This shows that the standard deviation of the Ct values calculated by the software are again lower compared to the Ct values in the experiment with the manual pipette. This means that substantiated and reproducible results are achieved using a pipetting robot, especially in the 384-well high-throughput format, through standardization of the sample setup and the pipetting routine – even in the smallest volume range.

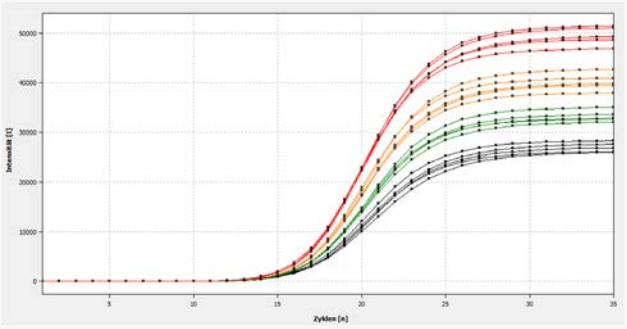

Figure 2a: Representation of the amplification of a 120 bp E.coli-specific target sequence in the qTOWER^3 84. In the image above, a manual reaction setup has been used, using the manual pipette for the carrying out the volume variation.

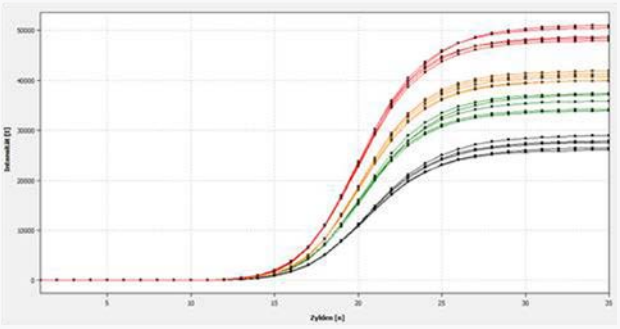

Figure 2b: Representation of the amplification of a 120 bp E.coli-specific target sequence in the qTOWER^3 84. At this point, the qPCR was carried out with an automated reaction batch, produced using the GeneTheatre pipetting robot.

Conclusion

It is recommended to use a pipetting robot in order to standardize and improve the precision and reproducibility of real-time PCR reactions. This makes it infinitely easier to perform all pending pipetting tasks in the laboratory and enables full automation of PCR and real-time PCR reaction batches. The standardization of processes significantly reduces errors regarding reactions involving 10 or 5 µl batch volumes – regardless of the experience and skill of the user. An automated reaction setup offers many advantages: the utmost precision, for example, as well as the possibility to routinely process smaller reaction volumes than those which would be possible with manual batches.

References

Labo 5/2017: Kleinstvolumina im Hochdurchsatz messen

Headquarters
Analytik Jena AG
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

en · 03/2018