Applications

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Cost savings in real-time PCR with Eppendorf twin.tec *real-time* PCR Plates

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Abstract

This application note demonstrates that the reaction volume in real-time PCR experiments can be significantly reduced by using Eppendorf twin.tec *real-time* PCR Plates. These plates enable multiplex analyses to be performed with a total volume of 5 µL without loss of sensitivity or reproducibility. Reducing the reaction volume can considerably cut costs in real-time PCR.

Introduction

Real-time PCR has become an established method in many areas because it has many advantages compared to conventional PCR. In addition to the possibility of quantifying nucleic acids, real-time PCR is characterized by high sensitivity, reproducibility and speed. The costs associated with this method are, however, significantly higher than in conventional PCR. The high acquisition costs associated with a real-time PCR device are negligible after many years of use since they are one-time costs only. In contrast, the consumables and reagents cause continuous costs in the laboratory, which are dependent on the amount of samples. Compared to the reagents the proportion of the consumables in these total costs is low and amount to approximately 5 %. This is shown in Table 2 by the total cost calculation with a conventional PCR plate and a reaction volume of 20 µL in each case.

To keep the costs in real-time PCR as low as possible, reaction volumes are being reduced. While reaction volumes of 50 µL were common at the beginning of real-time PCR, nowadays volumes of 20 µL and less are increasingly being used. However, reducing the volume is often at the expense of sensitivity because it also decreases the number of fluorescent molecules per reaction and consequently the intensity of the fluorescent signal. This may impair the signal-to-noise ratio of the measurement and make an early differentiation between the baseline and increasing fluorescence more difficult. Ultimately, this may result in the delayed detection of C_t values or, in the worst case, in the non-detection of these values in the case of samples with low nucleic acid concentrations.

Eppendorf twin.tec *real-time* PCR Plates can be used to compensate for the disadvantages resulting from the reduced volumes as the fluorescent signal is greatly increased by the reflection of the titanium dioxide added to the polypropylene. Even with a reaction volume of 5 µL the signal-to-noise ratio of the measurement is sufficiently good to provide comparable real-time PCR results with larger volumes. Furthermore, the reproducibility with regard to the replicates is also comparably good as the fluorescence is uniformly intensified without any interfering reflection from the thermoblock.

Materials and Methods

The genes SRY, GAPDH and beta-actin of the human genomic DNA were amplified and detected in parallel. For the detection the following probes were used: a Yakima Yellow®/BHQ1-labeled probe for the SRY gene, a BoTMR/BHQ1-labeled probe for beta-actin and a FAM/BHQ1-labeled probe for GAPDH. The primer and probe sequences as well as the concentrations used are summarized in Table 1.

Target	Primer and probe sequences	Primer and probe concentration
GAPDH gene	Forward primer: TGC CTT CTT GCC TCT TGT CT	200 nM
	Reverse primer: GGC TCA CCA TGT AGC ACT CA	200 nM
	Probe: FAM-TTT GGT CGT ATT GGG CGC CTG G-BHQ1	50 nM
Sex-related Y chromosome (SRY) gene	Forward primer: GCG ACC CAT GAA CGC ATT	200 nM
	Reverse primer: AGT TTC GCA TTC TGG GAT TCT CT	200 nM
	Probe: Yakima Yellow-TGG TCT CGC GAT CAG AGG CGC-BHQ1	50 nM
ß-actin gene	Forward primer: TGG GTC AGA AGG ATT CCT ATG TG	200 nM
	Reverse primer: TCG TCC CAG TTG GTG ACG AT	200 nM
	Probe: BoTMR-TCA CCC TGA AGT ACC CCA T -BHQ1	100 nM

Table 1: Primers and probes

Real-time PCR was performed in reaction volumes of 10 µL and 5 µL. The mastermix consisting of QIAGEN QuantiTect® Multiplex PCR NoROX Kit, TaqMan probe and primers was prepared manually. All further pipetting steps were performed using the ep*Motion*® 5070. First, a 1:3 dilution series consisting of human genomic DNA (Roche) was created in order to produce 111 – 9000 copies for each reaction volume for GAPDH and beta-actin. In the case of the SRY gene which only occurs once on the Y chromosome only half of the copies is available for each reaction. Subsequently, the mastermix was pipetted into the wells of an Eppendorf twin.tec *real-time* PCR Plate 96 skirted, after which the different DNA samples were also added. All reactions were performed in three replicates, consisting of the following:

10 µL total reaction volume: 6 µL mastermix + 4 µL template DNA
5 µL total reaction volume: 3 µL mastermix + 2 µL template DNA

Afterwards, the twin.tec *real-time* PCR Plate 96 skirted was sealed with Eppendorf Heat Sealing Film and centrifuged at 1,500 x g. Amplification was performed on the Eppendorf Mastercycler® ep *realplex*4 S with the following PCR program:

Initial Denaturation	Denaturation	Annealing / Elongation
95 °C	94 °C	60 °C
15 min	10 s	30 s

Results and Discussion

It could be shown that the use of Eppendorf twin.tec *real-time* PCR Plates makes it easy to perform multiplex analyses even in small reaction volumes of 10 µL and 5 µL (Figure 1). Although the reduction of the total volume is accompanied by a decrease of the fluorescent signal, due to the reflective properties of titanium dioxide the signal is still sufficiently strong enough to ensure optimum analysis of multiplex assays. Thus all three genes examined could be amplified in a reaction volume of just 5 µL with PCR efficiencies of 90 to 95 %. This way the amplification was comparable to or even more efficient than in a reaction volume of 10 µL. Furthermore, the volume reduction had no effect on the reproducibility of the replicates, as shown by the comparison of the correlation coefficient R^2. This is a measure of how close the individual points are to the linear regression lines. In the analysis performed here, values were achieved for all standard curves from 0.995 to 0.998, which were extremely close to the best possible value of 1.

Table 2 shows a comparison of the average costs of a SYBR Green assay using different reaction volumes. The average list price of standard real-time PCR reagents with SYBR Green for 200 reactions was used as the basis. When performing an experiment with 20 µL per reaction volume, a fully loaded 96-well PCR plate incurs costs of approx. 90 €. The proportion of consumables is low and amounts to less than 5 % of the total costs. If the reaction volume is reduced to 10 µL using Eppendorf twin.tec *real-time* PCR Plates, the relative costs for consumables increases, but the total costs are cut by more than 44 %. The reduction to only 5 µL for each reaction volume cuts the price for a completely filled PCR plate to just 29 €, which amounts to a cost reduction of 69 % compared to a plate with a filling volume of 20 µL.

Component	Clear or frosted wells	White wells	
	20 µL	10 µL	5 µL
twin.tec PCR Plate	4.20 €	-	-
twin.tec *real-time* PCR Plate	-	6.70 €	6.70 €
SYBR MasterMix	89.00 €	44.50 €	22.30 €
300 nM primer	1.00 €	0.50 €	0.25 €
costs / 96 reactions	94.20 €	51.70 €	29.25 €

Table 2: Total costs of components in a real-time PCR experiment with 96 reactions based on average list prices in 2009

The difference in cost becomes more marked with the use of more sophisticated real-time PCR systems such as reagents for one-step RT-real-time PCR, which can be up to three times more expensive than the more simple real-time PCR reagents.

With real-time PCR experiments based on probes the cost savings are even greater than with SYBR Green applications as the reduction in volume decreases the mastermix and also the quantity of probe applied. Furthermore, using Eppendorf twin.tec *real-time* PCR Plates, the concentration of the probes can also be reduced as the fluorescence intensity is still sufficiently good even in 5 µL to ensure optimum detection of the remaining fluorescence molecules. The multiplex assay shown in Figure 1 used comparatively low probe concentrations (see Table 1). While probe concentrations of approx. 200 nM are often used in clear consumables, only 50 nM or 100 nM per probe were used with twin.tec *real-time* PCR Plates. The advantage for the user is that he can analyze a considerably higher amount of samples with a probe synthesis depending on reaction volume and the probe concentration applied. Furthermore with half the amount of probe used, user can possibly maximize their sample run with just one batch of probe. This results in the reduction of the experimental variation that may be introduced by batch-to-batch probe quality. Besides the cost savings, this also has the advantage that the renewed verification of the real-time PCR system which is necessary for a new synthesis does not need to be performed so often.

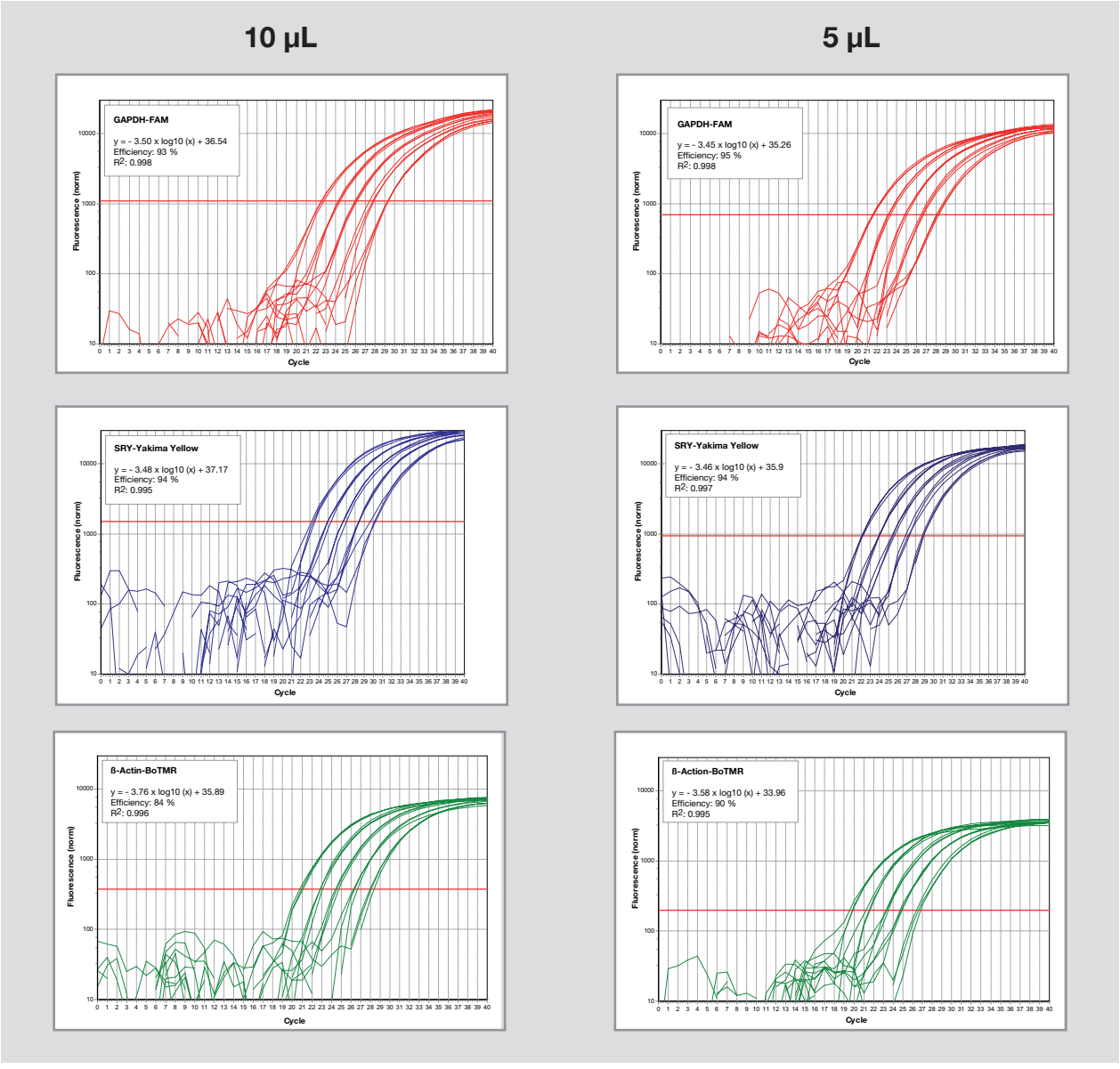

Fig. 1: Multiplex assay with reaction volumes of 5 and 10 µL.

Conclusion

Using Eppendorf twin.tec *real-time* PCR Plates it is possible to reduce the reaction volume for real-time PCR experiments down to 5 µL. The associated reduction of the reagents used results in considerable cost savings for the user. Even multiplex assays can be performed in a reaction volume of just 5 µL without loss of sensitivity and reproducibility

Ordering Information

Product	Description	Order no. international	Order no. North America
twin.tec *real-time* PCR Plate 96, skirted	white frame	0030 132.513	951022015
	blue frame	0030 132.505	951022003
	black frame	0030 132.521	951022027
twin.tec *real-time* PCR Plate 96, semi-skirted	white frame	0030 132.548	951022055
	blue frame	0030 132.530	951022043
	black frame	0030 132.556	951022067
Mastercycler® ep *realplex*^{4} S	with silver block and 4 emission filters	6302 000.601	950020318
ep*Motion*® 5070	Automated pipetting system	5070 000.000	960000005
Heat Sealing Film	10 x 10 units	5390 000.024	951023078
Heat Sealer		0030 127.650	951023060

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