Applications

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Mastercycler® pro with *vapo.protect*™ technology: Improved protection against evaporation of PCR solution

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Abstract

The new heated lid technology *vapo.protect*™, featured by the Mastercycler pro, achieves improved evaporation protection even at the corner and edge positions of the thermo block. The tighter sealing of the newly developed fluid filled cushion incorporated in the heated lid, leads to an improved evaporation protection. With its demonstrated minimization of technical variance on PCR, the Mastercycler pro makes a significant contribution to higher comparability and reproducibility.

Introduction

In order to achieve an efficient and robust PCR, all reaction parameters need to be optimized. Hence, it is usually necessary to determine optimal primer and magnesium ion concentrations experimentally. Other reaction parameters, such as concentrations of the PCR buffer components, are optimized by the manufacturer to achieve high polymerase activity and reaction specificity. Therefore, the stability of all optimized conditions throughout the course of the reaction is critical for the success of the PCR. One major criterion is minimization of evaporation of reaction fluids.
It can be assumed that the highest risk of evaporation stems from the seal of the single PCR reaction tubes, 8-strip tubes or PCR plates. Sealing options typically used are tube caps, adhesive tape or adhesive foil, or heat-sealing film or foil. Here, successful sealing of the reaction vessel depends heavily on the correct pressure applied by the lid of the thermocycler. Insufficient sealing pressure may lead to leaky sealing and subsequent loss of reaction liquid. These effects can also be observed in the case of excessive sealing pressure, as this may lead to deformation of the reaction vessels in the sealing area. In addition to correct intensity, uniformity of sealing pressure across all reaction tubes is of critical importance. In the case where cycler lids exert increased pressure on the samples in the center of the block, evaporation problems could arise in samples placed in the peripheral locations of the block. The data presented in this report will highlight the significance of improved evaporation protection provided by the Mastercycler pro with regards to obtaining optimal and reproducible PCR conditions.

Materials and Methods

The experiments were performed on the Mastercycler pro S and five thermocyclers made by other manufacturers. First, 20 µl of a solution containing 1 x concentrated PCR buffer and 1 µM fluorescein were pipetted into all 96 positions of a PCR plate using the ep*Motion* 5070 automated pipetting system (Eppendorf). For the Mastercycler pro, an Eppendorf twin.tec PCR plate 96 (semi-skirted) was used. While four other thermocyclers (A;B;C;D) were equipped with a PCR plate made by the corresponding manufacturer, the fifth thermocycler (E) was tested with an Eppendorf twin.tec PCR Plate 96 (skirted).

All plates were sealed with the Eppendorf PCR Foil (self adhesive). In order to obtain comparable seals on all plates, the foil was initially lightly attached to the plates. Following placing of a 4 mm rubber mat on top of the foil, each plate was sealed with an automatic sealer (Porvair MiniSeal). Thus, each foil could be adhered to the plates using equal force. Following sealing, each plate was placed in its corresponding thermocycler and subjected to the temperature profile of a typical PCR-run (Table 1). Additionally, one Eppendorf twin.tec PCR plate 96 (semi-skirted), pipetted as described above, was sealed using the Eppendorf Heat Sealing Film. Since sealing a PCR plate with heat sealing film is known to minimize evaporation of reaction solution this plate was used as a reference during the following analysis (deemed: reference plate). This plate was subjected to the same temperature profile on a Mastercycler ep (Table 1).

Table 1: Temperature profile.

Temperature	95 °C	95 °C	62 °C	72 °C	72 °C	10°C
Time	5 min	20 s	20 s	30 s	5 min	hold
		35 cycles				

Following the completed temperature profile, each PCR plate was centrifuged in the Eppendorf Centrifuge 5810R (1 min at ~660 rcf) and subsequently transferred to the ep*Motion* 5070. The ep*Motion* was programmed to transfer automatically 5 µl from each position of the PCR plate to the corresponding position of a black 96-well MTP. Previously, 95 µl of TE-buffer (pH 8) had been placed in each well of the 96 well MTP, thus achieving a 1:20 dilution of the fluorescein solution.
A different MTP was filled with 100 µl of TE-buffer in all 96 wells. This plate was used to determine the background signal (deemed: background plate).
Each MTP was then sealed with PCR Foil (self-adhesive) as described above, followed by a mixing step (Eppendorf MixMate) and centrifugation (1 min at ~660 rcf).
Finally, detection of fluorescein signal intensity was performed for all 96 positions of each MTP using the plate reader Safire^{2}™ (Tecan).

To visualize the evaporation effects a similar setup was performed as described above on selected thermocyclers. 20 µl of 1 x concentrated PCR buffer colored with Orange G was pipetted to the 96 well positions of a PCR plate. After the temperature profile (Table 1) was completed a picture was taken of the well positions A1-A12 (Figure 2).

Results and Discussion

The arithmetic mean of all 96 positions of the background plate was used as the background value for all analyses. To this end, the background value was subtracted from the reference value (arithmetic mean of all 96 positions of the reference plate) as well as from the 96 individual positions of every tested plate.

The determined reference value was considered the signal intensity where no evaporation occurs. Thus, the difference in measured signal intensity between an individual plate position and the reference value will allow determination whether evaporation has occurred during the course of the temperature profile. In the case of evaporation of reaction solution, an increased concentration of the fluorescein is observed, which is expressed in an increase in signal intensity in the corresponding positions (Table 2). If a measured signal intensity was lower than the reference value, the evaporation for this position was put to zero.

Table 2: Observed evaporation of reaction solution on the Mastercycler pro and 5 thermocyclers of other manufacturers.

Thermocycler	PCR plate: mean evaporation [%]		
	Corner (4 wells)	Edge (32 wells)	Center (60 wells)
Mastercycler pro S	3	2	0
A	45	32	10
B	30	5	1
C	4	3	6
D	49	5	0
E	>50*	30	0

*Thermocycler E: Since the measured signals of 2 corner positions correspond approximately to the background value it can be assumed that there was not enough reaction fluid left for transferring 5 µl to the MTP. This complies with the visual observation. Therefore, a mean evaporation of more than 50 % was estimated for the corner positions on this thermocycler.

The results demonstrate that the Mastercycler pro produces the lowest mean evaporation values (Table 2, Figure 1). Some of the devices of other manufacturers showed considerable evaporation values particularly with regard to the corner and edge positions. Evaporation values of more than 50% were observed in single positions.

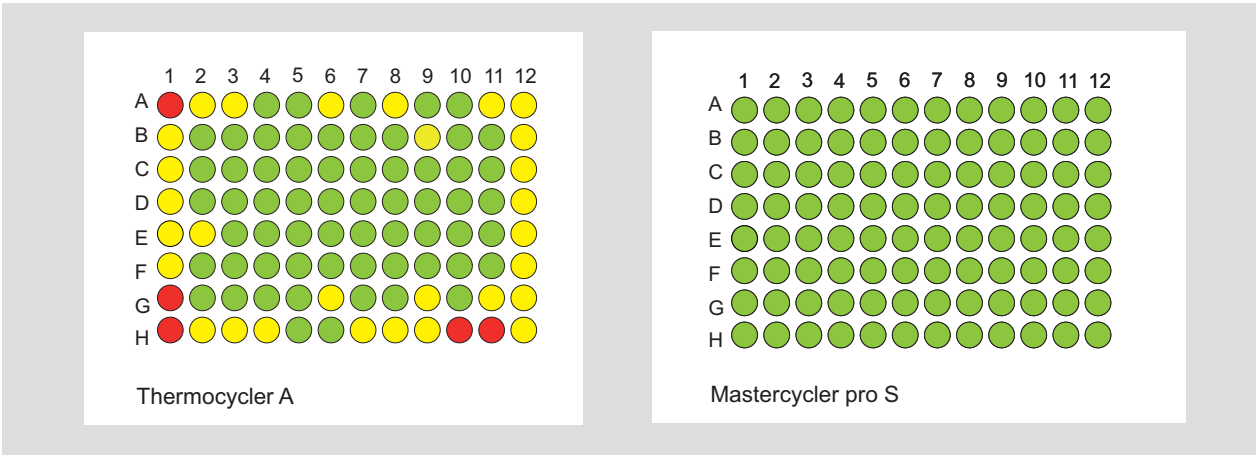

Figure 1: Flourescein experiment: Evaporation values of the single well positions on the Mastercycler pro and Themocycler A
Evaporation ● 0% to 20%
● more than 20% to 50%,
● more than 50%

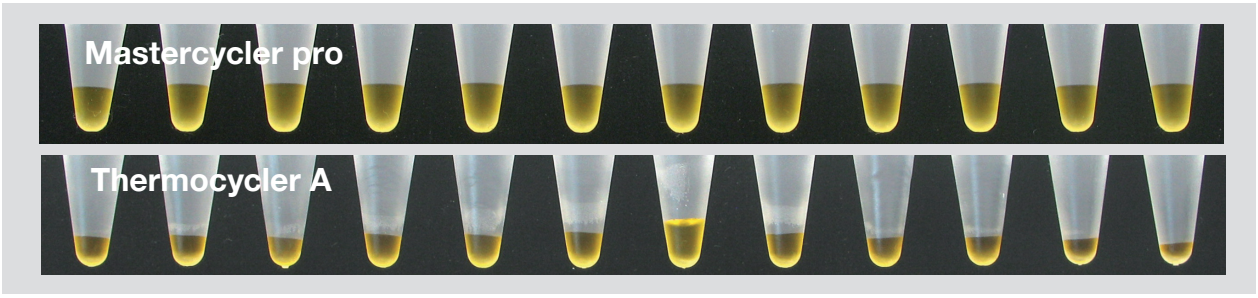

Figure 2: Orange G experiment: Liquid levels of the well positions A1-A12 of the PCR plate after a run on the Mastercycler pro and Themocycler A.

The different liquid levels of highly affected positions are obviously recognizable when taking a closer look at the wells (Figure 2).
Evaporation values, which were observed in the fluorescein experiment, may lead to a considerable increase in the concentration of reaction components. Higher primer and magnesium ion concentrations may result in an increased risk of formation of non-specific PCR products, for example through the formation of primer dimers or non-specific primer annealing. These undesirable side products are considered as parallel competitive reactions which partly use up available resources (such as primers, NTPs, Mg^{2+}). Hence, these are no longer available for the specific PCR, which becomes evident in a reduced PCR efficiency of the specific product. This effect can be of high significance during the amplification of difficult templates, such as small DNA template amounts or low quality DNA. Specific amplification of DNA fragments from such templates is often dependent on optimized and stable PCR conditions.

Conclusion

The decrease of evaporation risk provided by the ***vapo.****protect*™ technology featured by the Mastercycler pro can be considered as an important prerequisite for work with small reaction volumes. This can be advantageous when only small amounts of DNA template are available, such as in forensic samples or single cell PCR. A smaller reaction volume requires less total template in order to achieve optimal template concentration for a successful PCR.

Furthermore, faster run-times can be achieved, as smaller reaction volumes reach the desired temperature faster; hence, by utilizing of the very fast heating and cooling rates of the Mastercycler pro S, very short total run times can be achieved.

In addition, depending on sample throughput, considerable savings in reagents are possible when working with smaller reaction volumes.

Ordering Information

Product	Description	Order no. international	Order no. North America
Mastercycler pro (96 well aluminum block)	230 V / 50 - 60 Hz 120 V / 50 Hz, with US-plug	6321 000.019 6321 000.027	- 950030010
Mastercycler pro S (96 well silver block)	230 V / 50 - 60 Hz 120 V / 50 Hz, with US-plug	6325 000.013 6325 000.021	- 950030020
Mastercycler pro 384 (384 well aluminum block)	230 V / 50 - 60 Hz 120 V / 50 Hz, with US-plug	6324 000.010 6324 000.028	- 950030030
Control Panel	incl. connecting cable	6320 000.007	950030050
twin.tec PCR Plate 96, skirted	wells colorless, 25 pcs.	0030 128.648	951020401
twin.tec PCR Plate 96, semi-skirted	wells colorless, 25 pcs.	0030 128.575	951020303
PCR Foil	self-adhesive, 100 pcs.	0030 127.471	951023001

Disclaimer

This product is licensed under U.S. patent Nos. 5,525,300, 5,779,981 and 6,054,263. The heated cover device is licensed under US 5,552,580 and foreign equivalents.

The user of the Eppendorf Mastercycler pro might require additional rights for kits, reagents and other components required for his/her application. Such accompanying rights for these kits,reagents and other may be obtained by the respective holder of such rights. No rights are conveyed expressly, by implication or estoppel to any patents on real-time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit. Mastercycler pro upgraded to a Mastercycler ep realplex requires a Real-Time Thermal Cycler License under Applera's United States Patent No. 6,814,934 and corresponding claims in non-U.S. counterparts.

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