

Applications

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Excellent temperature homogeneity contributes to high PCR performance of the Mastercycler[®] nexus

Nils Gerke¹, Henner Tasch², Kirsten Schicke², Andrea Hellberg²

¹ Eppendorf AG, Hamburg, Germany, ² Eppendorf Instrumente GmbH, Hamburg, Germany

Abstract

Temperature measurements for the purpose of evaluating block temperature homogeneity were performed on the Mastercycler nexus and on two other thermal cyclers by competing manufacturers. The results illustrate the characteristics of the selectable temperature control modes and demonstrate the excellent homogeneous temperature control of the Mastercycler nexus. Furthermore, reliable PCR performance is shown by using a commercially available PCR test kit.

Introduction

Many factors influence PCR – apart from PCR components such as primers and quality of template DNA and reagents, the technical parameters influencing PCR play a major role. For example:

- Thermal cycler (performance of the thermoblock)
- PCR setup (e.g. liquid handling)
- Quality of the reaction vessels

The cyclical approach of the different temperature levels is a core function of the thermal cycler during the course of a PCR reaction [1]. All block positions are to be controlled as homogeneously as possible to the set temperature. If this condition is fulfilled, a major contribution to the minimization of technical variability has been achieved. As a consequence, the certainty that the PCR products obtained may be interpreted as actual biological results, rather than the result of technical artifacts, increases. Such artifacts may manifest as non-specific products, false positive results or, in the worst case scenario, as false negative results.

A high quality thermal cycler with a high level of temperature accuracy and homogeneity in all positions fulfills the basic technical requirements for minimization of technical variability, thus playing a major part in obtaining accurate and reproducible results.

A further aspect is highlighted by the fact that thermal cyclers made by different manufacturers may differ considerably when it comes to temperature control. Consequently, a PCR system which has been optimized and established on one thermal cycler may not deliver a comparable PCR product, despite employing the same PCR program, when a thermal cycler by a different manufacturer is involved.

In this Application Note, the homogeneous and accurate temperature control of the Mastercycler nexus is demonstrated by temperature measurements and by the performance of a commercially available PCR test kit for the purpose of quality control.

Materials and Methods

1) Temperature measurements

The data for temperature homogeneity were collected at temperatures 95 °C, 72 °C and 60 °C. For each temperature level a holding time of 45 s was programmed. Prior to approaching the next temperature level, the thermoblock was cooled to 35 °C for 15 s (Figure 2).

Block temperatures were continuously recorded at a frequency of 2 Hz across the entire time of the run. The data collected after 30 s at the respective temperature level were used for the determination of temperature homogeneity. Data collection was performed using the Eppendorf Temperature Verification System – Multi channel, which records the temperatures of 14 block positions in parallel (Figure 1).

Measurements were performed with two of the three selectable temperature control modes of the Mastercycler nexus (“fast”, “standard” and “safe”) [2].

For comparative observations of the fast mode, measurements were performed on a thermal cycler made by a competitor A. This thermal cycler offers a setting pertaining to temperature control which corresponds to the fast mode. Comparative measurements to the standard mode of the Mastercycler nexus (MC nexus) were carried out at a volume setting of 50 µL using a thermal cycler from competitor B.

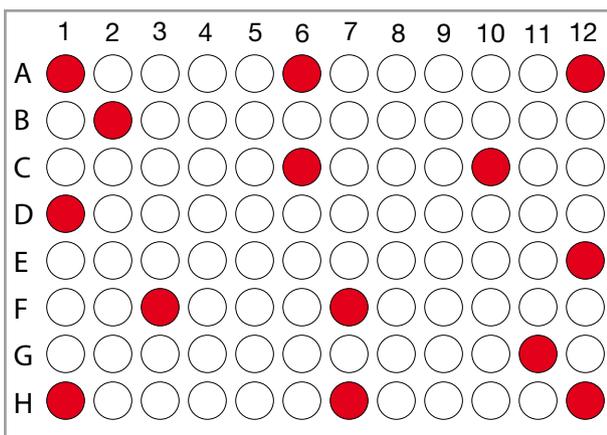


Figure 1: The sensor plate of the Eppendorf Temperature Verification System – Multi channel measures 14 block positions in parallel (marked red).

2) Quality control of PCR performance

To demonstrate the comparable performance of all 96 thermoblock positions by means of a PCR application the Cyclercheck kit (Protrans, Hockenheim, Germany) was run on the Mastercycler nexus. Cyclercheck is a commercially available PCR test kit. According to the description of the manufacturer, this kit is a tool to monitor temperature profiles of thermal cyclers used for routine PCR. The Cyclercheck kit was set up and run in accordance with the

manufacturer’s instructions. The Cyclercheck PCR protocol was carried out in standard temperature control mode on the Mastercycler nexus [2]. After completion of the PCR, the PCR products were separated by electrophoresis on a 2 % agarose gel and subsequently stained with ethidium bromide.

Results and Discussion

1) Temperature measurements

For the Mastercycler nexus, the measurements were carried out in temperature control modes fast and standard (Figure 2). In fast mode, regulation of the programmed protocol is immediately linked to the temperature control of the thermoblock.

The standard mode is recommended for typical PCR applications with a volume range between 20-50 µL [2]. In this mode, a slight overshoot, or undershoot, respectively, in temperature at the beginning of each temperature step helps that the PCR solution will reach the programmed temperature more quickly. The holding time of the temperature step starts approximately when the reaction solution has reached the desired temperature.

The third selectable temperature control on the Mastercycler is the safe mode. The principle of the control characteristics of this mode is comparable to standard mode, but it is recommended for reaction volumes >50 µL and/or GC-rich PCR targets [2]. Safe mode was not tested in this investigation.

The overview of the temperature profile illustrates the uniform performance of the 14 measured block positions on the Mastercycler nexus (Figure 2). In contrast, the same temperature profile revealed a poor parallelism and tightness of the temperature trajectories on thermal cycler A; certain curves reach the respective area of the programmed temperature only towards the end of the 45 s holding time.

The standard-analog mode on thermal cycler B revealed a remarkably high temperature overshoot when approaching the programmed temperature step. For example, the block heated up to temperatures around 107 °C at the beginning of the 95 °C temperature step. In contrast, the Mastercycler nexus performed an overshoot of approximately 2-3 °C in standard mode. This clearly demonstrates the possible differences in the temperature performances on cyclers of different manufacturers – even when the same protocol with analog control mode is programmed on both cyclers. Hence, a transparent description of the temperature performance by the thermal cycler manufacturer helps the user to understand the process of PCR applications and to optimize protocols. This is especially important when transferring a PCR application from one thermal cycler to another.

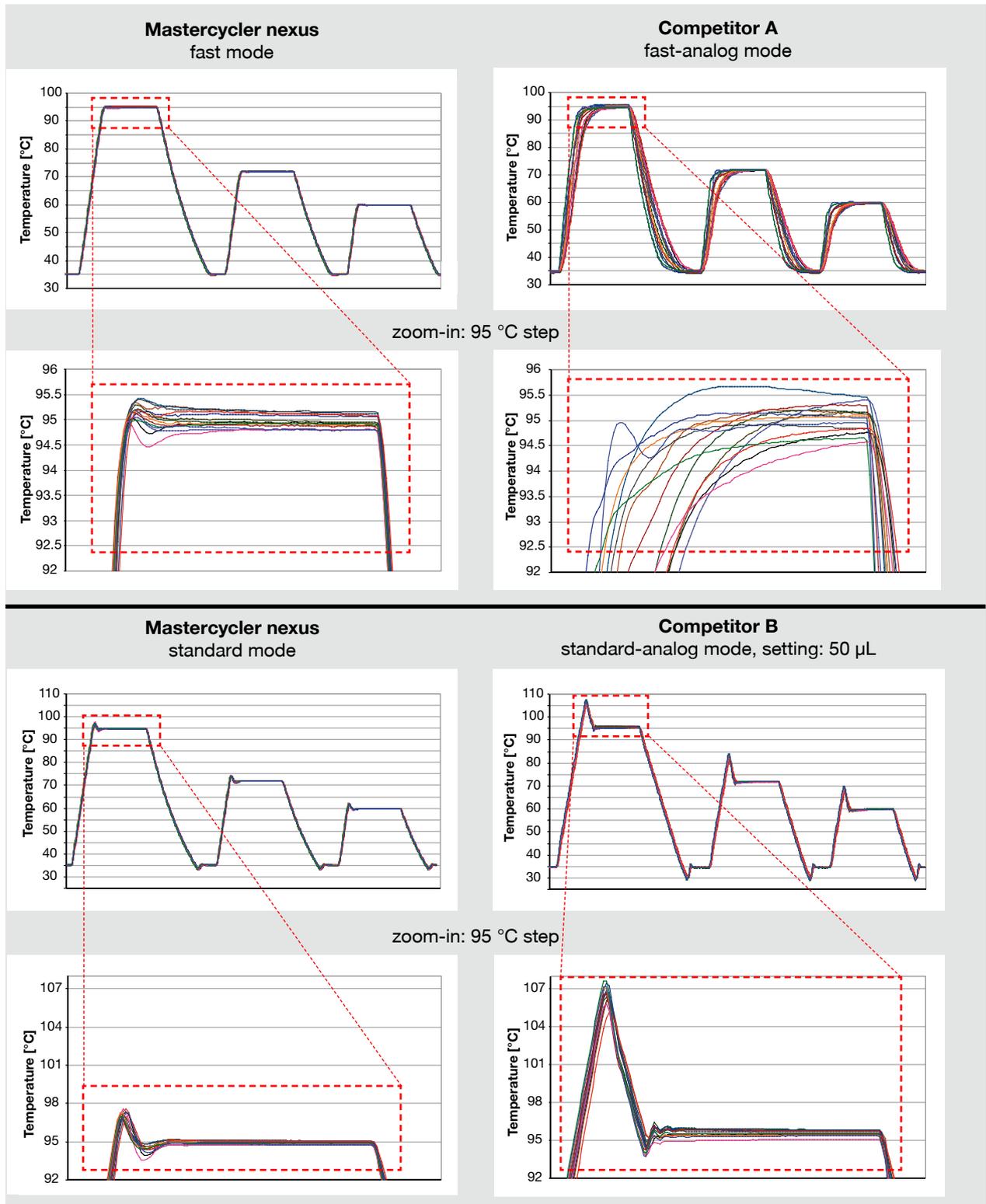


Figure 2: Recorded temperature profiles of the Mastercycler nexus and two other thermal cyclers. In both modes, the 95 °C step was selected for a zoom-in view.

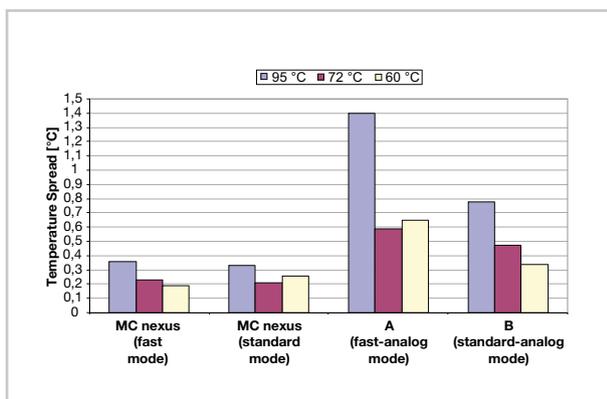


Figure 3: Thermoblock homogeneity: Temperature spread between the lowest and the highest recorded temperature value at the three different temperature steps.

For each of the three tested temperature steps the Mastercycler nexus showed the best block homogeneity (Figure 2 and 3). In particular, the 95 °C step revealed a significantly smaller temperature spread than the two other thermal cyclers.

2) Quality control of PCR performance

Each of the 96 well positions of a Cyclocheck plate contains two primer pairs that amplify two fragments of 540 and 90 bp, respectively. According to the manufacturer both primer pairs are designed to work optimally under the same conditions in all individual positions of the plate (further information is provided by the Cyclocheck manufacturer Protans). The separation of the PCR products on an agarose gel revealed a reliable amplification of the expected two PCR products at all 96 well positions of the Mastercycler nexus (Figure 4). Thus, the high technical performance of the Mastercycler nexus shown by the temperature measurements (see above) could be confirmed using this quality control PCR application.

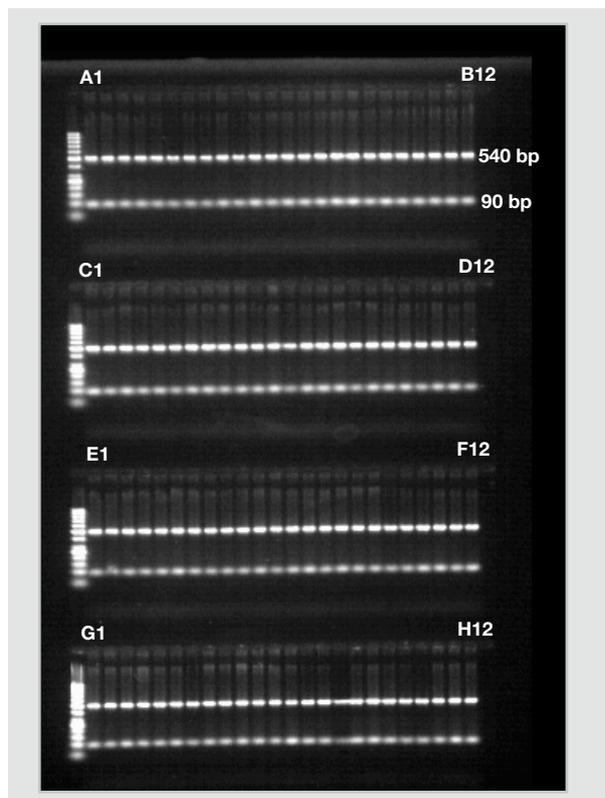


Figure 4: Performance of the Cyclocheck kit (Protans) in all 96 well positions of the Mastercycler nexus (Length standard in the first position of every row on the gel: GeneRuler™ 50bp DNA Ladder, Thermo Fisher Scientific).

Conclusion

The Mastercycler nexus is characterized by high technical performance which contributes to minimizing technical variability in PCR sample processing. The results presented here demonstrate the outstandingly homogeneous and accurate temperature control of the Mastercycler nexus.

References

- [1] Kim YH, Yang I, Bae Y-S, Park S-R. Performance evaluation of thermal cyclers for PCR in a rapid cycling condition. *BioTechniques* 2008; 44:495-505.
- [2] Manual of Mastercycler nexus. Available at: www.eppendorf.com

Ordering information

Product	Description	Order no. International	Order no. North America
Mastercycler® nexus gradient	230 V / 50 - 60 Hz,	6331 000.017	-
	120 V / 50 - 60 Hz, with US-plug	-	6331000025
Mastercycler® nexus	230 V / 50 - 60 Hz,	6333 000.014	-
	120 V / 50 - 60 Hz, with US-plug	-	6333000022
Mastercycler® nexus eco*	230 V / 50 - 60 Hz,	6332 000.010	-
	120 V / 50 - 60 Hz, with US-plug	-	6332000029
CAN_BUS connection cable	50 cm	5341 612.006	950014008
CAN_BUS connection cable	150 cm	5341 611.000	950014016
Selftest USB key		6320 071.001	950030040
Temperature Verification System – Single-channel		0055 000.298	950008059
Temperature Verification System – Multi-channel		6328 000.006	on request

* A Mastercycler nexus gradient or Mastercycler nexus is needed to run a Mastercycler nexus eco. Up to 2 Mastercycler nexus eco can be controlled by one Mastercycler nexus gradient or Mastercycler nexus.

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