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APPLICATION NOTE No. 388

Highly reproducible low Volume PCR with Mastercycler® X50 and ep*Motion*®

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Abstract

Key requirements of a thermal cycler are naturally accuracy and reproducibility in terms of temperature performance. However, due to increasingly robust PCR reagent formulations in the market, temperature performance of a thermal cycler can be difficult to determine until one comes across a delicate PCR system. Optimization of such a system then becomes very tedious, laborious and can be extremely resource-consuming. Poor temperature performance of a thermal cycler and evaporation are two common issues that lead to poor PCR results. However, these two factors are difficult to simply assess based on numbers on a technical specification sheet. This paper demonstrates how Eppendorf ep*Motion* effortlessly handles low volume PCR set-up, hence cutting down preparation effort while simultaneously increasing accuracy. Furthermore, it presents the robustness of the new Eppendorf Mastercycler X50 even when running low volume PCR, demonstrating its excellent temperature performance and protection against evaporation.

Introduction

Modern scientific trends tend towards higher speed, greater convenience and lower volumes. Similarly, scientific equipment follows through this line of evolution to push such limits, giving rise to fancier equipment and techniques. Though easily overshadowed in the wake of such exciting advancement, performance breakthroughs in simple and established equipment can also be more useful as they are generally less resource intensive to operate.

Large PCR setups can be very time-consuming and tedious if done manually. Especially when handling very small volumes; imprecision and pipetting errors caused by the operator can lead to massive variations of the results. This operator-based error carries an even bigger implication when different individuals are involved in the same workflow. A way to overcome this issue is to use an automated liquid handling platform. This allows an increase of sample throughput while at the same time ensuring a reliable handling of small volumes with minimal variations between experimental runs.

Temperature performance of a thermal cycler is generally measured using an external temperature verification device. Such a measurement method can provide a good indication on the performance of the thermal block of a cycler. However, overall PCR performance relies on not just the thermal block but also the additional combination of consumable compatibility and ability to maintain reaction concentration by protecting against evaporation. Thus, the results of an actual PCR run using a temperature sensitive PCR system would present the best direct empirical proof of reliable performance of a thermal cycler. Additionally, a thermal cycler performance in terms of accuracy, homogeneity and robustness can be verified by performing the same PCR under stricter conditions such as lower reaction volumes, enzyme concentration and template quantity. The results from this Application Note will illustrate the convenience of the ep*Motion* and robustness of the Mastercycler X50 in handling low volume PCR setup and runs.

Materials and Methods

PCRBio Taq DNA polymerase (NIPPON Genetics) and Human Genomic DNA (Roche®) were used for the following amplification. PCR reaction master mix was prepared with 1X reaction buffer and 0.2 µM of each primer, for final volumes of 3 µL or 10 µL per reaction respectively. For 10µL reaction, 0.25U of enzyme and 20 ng DNA were used while when amplifying 3 µL reaction volume, the amount of enzyme and DNA was reduced to 0.15U and 10 ng, respectively. The master mix was dispensed into all 96 wells of Eppendorf twin.tec® skirted PCR plates.

The following primers were used for amplification of the human ß-actin gene:

Forward primer: 5'- ATCGCCGCGCTCGTCGTC-3'
Reverse primer: 5'- TGGGTCATCTTCTCGCGGTTGG-3'

Dispensing was carried out by Eppendorf ep*Motion* P5073 equipped with a 0.2–10 µL TS10 single channel dispensing tool using free jet dispensing in pipetting mode. The worktable setup is displayed in Figure 1. Furthermore, this dispensing protocol uses a device-default template and hence can be easily adapted for other Eppendorf ep*Motion* models equipped with a 0.2–10 uL dispensing tool.

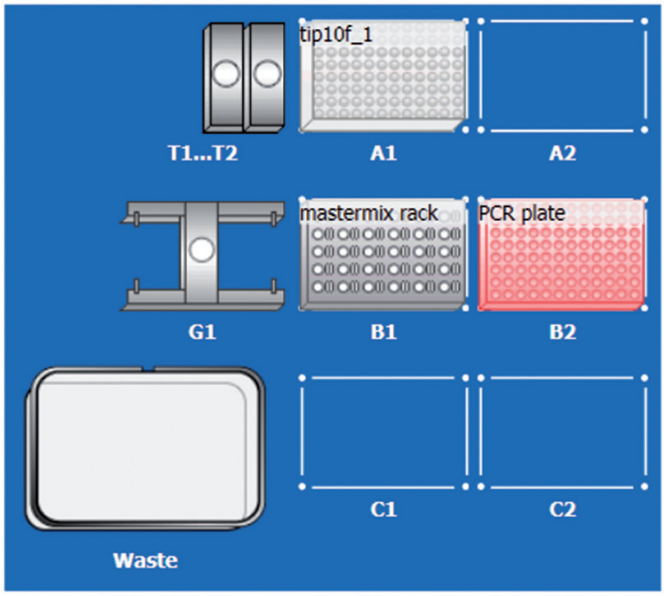

Figure 1: ep*Motion* P5073 worktable for PCR setup

Plates were sealed with Eppendorf Adhesive PCR Film and PCR were carried out on Mastercycler X50s. Cycling conditions are listed in Table 1. The PCR products were detected using GelRed™ (Biotium) following agarose gel electrophoresis and visualized using the Gel Doc XR+ (BioRad®).

Table 1: PCR condition with two concurrent gradient setting at denaturation and annealing steps.

Eppendorf Header Settings	Lid	105 °C
	TSP/ESP	ON
	Lid auto-off	ON
	Temperature mode	Fast
Initial Denaturation		96 °C/5 min
Cycles: 35x	Denaturation	Gradient at 90–99 °C/20 s
	Annealing	Gradient at 52–72 °C/20 s
	Elongation	72 °C/30 s
Post-Cycle Elongation		72 °C/2 min
Storage	Hold	4 °C

Results and Discussion

The new Eppendorf epMotion TS10 pipetting tool and Mastercycler X50 are a powerful combination in running low volume applications. Not only does an automated pipetting system provide superior accuracy, it is also comparatively more pleasant than setting up PCR reactions manually, especially at high throughput. The error tolerance range is naturally narrower when working with lower liquid volumes. Therefore, automated systems with defined and controlled force provide more reproducible dispensing of PCR reagents, which in turn result in higher confidence in PCR results. The use of an automated liquid handler also allows the operator to reduce hands-on setting-up time to a bare minimum. Additionally, evaporation issues in a thermal cycler are intensified at lower reaction volumes due to the lower amount of water present in the mastermix. Hence, a change in even a small amount of liquid will lead to a big shift in reagent concentration that will affect PCR amplification. As evaporation in each well across the block is uncontrollable, irreproducibility is the most common consequence of evaporation. Therefore, a good thermal cycler is one that has good thermal performance as well as a design that protects against evaporation.
These evaluation criteria can be easily tested using a temperature sensitive PCR system. The human ß-actin gene used in this study is one such system. Specific amplification will yield 484 bp fragments while sub-optimal condition will give rise to non-specific amplification visible as a 350 bp artefact in the gel. This characteristic can be used to clearly portray how finely temperature control a cycler has. Figure 1A showed the PCR amplification of the ß-actin gene across different temperatures using the gradient function while Figure 1B shows the comparative result of the same system under reduced volume.

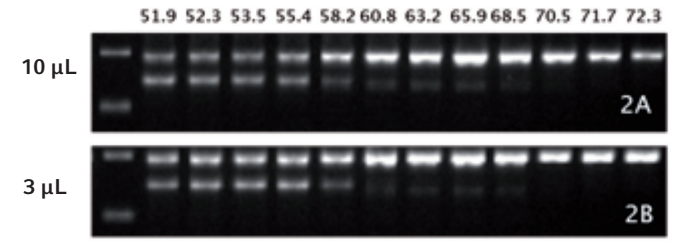

Figure 2A and 1B: PCR amplification of human ß-actin gene.

The results showed that each different annealing temperature gives a different specific and non-specific fragment yield, with highest yield at 65.9 °C while highest specificity at 70.5 °C. Even though the difference in each tested temperature is small, the effect was nonetheless obvious due to the sensitive nature of this PCR system. These differences hence illustrate the fine control and accuracy the Mastercycler X50 has over the temperatures across the block.
Additionally, these results were highly reproducible at vastly reduced reaction volume (3 µL), showing not only that automated pipetting was very precise, the wells also did not suffer from evaporation problems. At 3 µL, the DNA bands seem to have higher intensity. A possible explanation is that while the amount of enzyme and DNA were reduced accordingly in the 3 µL setup, the overall concentration of enzyme and DNA per total volume is slightly higher, hence resulting in slightly higher yield. Furthermore, it is expected that the effect caused by condensation/evaporation in 3 µL is larger than in 10 µL at the same rate due to the lower amount of water present in the 3 µL setup. This will thus further drive up the concentration of reagents in 3 µL setup and may result in slightly higher final yield at the same temperatures.
The respective reductions in enzyme consumption and DNA amount required for successful amplification at low reaction volumes have two important implications. While many inexpensive PCR reagents exist in the market, DNA polymerases remained one of the most cost-consuming elements in PCR. This is especially true for users with high-throughput or when more expensive reagents are needed to increase efficiencies when amplifying difficult targets. Similarly, for users working with precious samples or low concentration targets, the ability of a thermal cycler to reproducibly amplify low amounts of samples can be the determining factor between PCR success or failure.

Conclusion

The results from this study showed that the Eppendorf Mastercycler X50 is a thermal cycler with excellent temperature performance and evaporation protection that gives highly reproducible result even for low volume PCR applications or delicate PCR system.

Combined with the Eppendorf ep*Motion* to handle low volume liquid handling set-up, a PCR can be set up effortlessly and completed with minimal time and effort for maximal accuracy and reproducibility.

Ordering information

Description	Order no. International	Order no. North America
Mastercycler® X50a	6313 000.018	6313000018
Mastercycler® X50p	6315 000.015	6315000015
Mastercycler® X50h	6316 000.019	6316000019
Mastercycler® X50l*	6303 000.010	6303000010
Mastercycler® X50r*	6305 000.017	6305000017
Mastercycler® X50t*	6306 000.010	6306000010
Mastercycler® X50i*	6301 000.012	6301000012
Accessories		
Ethernet cable, 5 m	6313 070.008	6313070008

* To operate this unit, it needs to be connected to a Mastercycler X50 s,a,p, or h. Up to 9 units can be connected to a Mastercycler X50 s,a,p, or h.

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