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Easy Migration of PCR Protocols Using Eppendorf's Automated Runtime Adjustment

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Abstract

PCR optimization becomes a default practice when one implements a new PCR protocol as each experiment needs the right combination of samples, reagents, consumables, cycler settings, and amplification parameters. However, once optimized, it is desirable that the PCR protocol is reproducible across different thermal cyclers. Unfortunately, every thermal cycler in the market has its characteristics that are influenced for instance by hardware and software technology, construction design, and component sources.

As a result, the same optimized PCR protocol, when run on different thermal cyclers, may give different amplification results and hence needs to be validated on each thermal cycler model. This application note describes a solution for this problem: The Program Migration Feature (PMF) of the Eppendorf Mastercycler® X50 series allows easy transfer of PCR protocols simply by entering the total runtime of the protocol as the "desired runtime" in the Mastercycler X50 software. This increases the similarity of temperature profiles between the source device and the Mastercyler X50, enabling an easy, automated transfer of PCR protocols.

Introduction

PCR is a mature technique that is robust, simple, and sensitive enough for most molecular biology purposes. However, it can also be fastidious and may require a multitude of parameter tweakings, such as heating/cooling ramp rate, incubation time, denaturation and/or annealing temperature, number of cycles, and reagent formulation (Mg^{2+}, DNA concentration, primer matrix, etc.) [1]. Moreover, each thermal cycler manufacturer has its own design, technology, and default device settings, all of which could impact the effectiveness of DNA amplification. As a result, the same system protocol that has been optimized on one thermal cycler might not give comparable results when performed on another thermal cycler of a different brand.
Fortunately, most protocols can be transferred between different thermal cyclers without adjustment. However, when it does happen, especially when using multiple cyclers with big differences in speed, re-optimization becomes a tedious, time- and material-consuming task.

To prevent such waste of resources and time, some thermal cyclers in the market implement "simulation" functions to mimic the thermal profiles of other cyclers in order to facilitate protocol adaptation. Unfortunately, the list of simulated cyclers is usually neither comprehensive nor up to date. In contrast, the Mastercycler® X50's desired runtime option circumvents this shortcoming by allowing users to control the thermal simulation according to the run time of a PCR protocol. As the Mastercycler X50 is the thermal cycler with the fastest speed and ramping rates in the market [2], this feature enables it to simulate the ramping profile of any other cycler.

Material and Methods

Human Genomic DNA (Promega®) was used as a template for the fast PCR protocol with SpeedSTAR™ HS DNA Polymerase (Takara Bio®). The tests were performed in Eppendorf 0.2 mL PCR tube strips on Mastercycler X50s and Cycler S.

The following PCR reaction master mix (Table 1) and cycling conditions (Table 2) were used for the amplification of a 536 bp sequence from the human ß-globin gene. The PCR products were detected via agarose gel electrophoresis using GelRed™ (Biotium) and visualized using the Gel Doc™ XR+ (Bio-Rad®).

Table 1: PCR reaction components and primers

Component	Final concentration	Details
Fast buffer I	1X	
dNTP-Mix	0.2 mM	
SpeedStar HS	0.25 U	
Forward primer	0.5 µM	5'-GCT CAC TCA GTG TGG CAA AG-3'
Reverse primer	0.5 µM	5'-GGT TGG CCA ATC TAC TCC CAG G-3'
DNA	20 ng	
Water	To make up to 10 µL	

Table 2: Cycling parameters

Eppendorf Header Settings	Lid	105 °C
	Energy-saving mode	ON
	Block settings	Silver 96
	Temperature mode	Fast
Initial denaturation		94 °C/60 s
35x cycles	Denaturation	96 °C/2 s
	Annealing	65 °C/6 s
Final elongation		72 °C/60 s
Hold		10 °C

Result and Discussion

The current PCR protocol has been previously optimized on a slower thermal cycler (Cycler S) to give a good amplicon yield. The time taken for the run to complete was about 28 min. When running this protocol on the Mastercycler X50s, the cycler only took 15 min to complete this run. However, the amplification results as shown by the visualized bands are faint (Figure 1).

Initial denaturation		94 °C/60 s
35x cycles	Denaturation	96 °C/2 s
	Annealing	65 °C/6 s
Final elongation		72 °C/60 s
Hold		10 °C

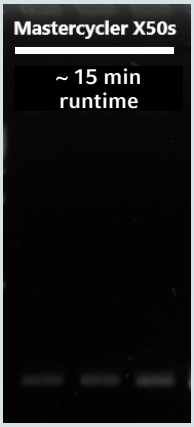

Figure 1: Certain PCR protocol may not transfer well from slower to faster thermal cyclers and require re-optimization (Note: Amplification of human ß-globin gene in triplicates).

As mentioned previously [2], PCR is influenced by multiple variables such as lid heating, lid cooling, heat dissipation, heat sink retention, overshoot/undershoot, and ramping patterns. Thus, some challenging PCR systems, especially those that are sensitive to ramping speed or heat transfer, may not produce a desirable result when running on cyclers with very different thermal profiles.
Usually, when facing such problem, the user can manually program the heating and cooling rate on the target cycler (e.g. Mastercycler X50s) according to the desired result on the original cycler (e.g. Cycler S). However, due to the multitude of hidden parameters that the user cannot directly access, a few runs might be needed to find the right corresponding ramp rates. By activating "Desired runtime" in the software of the Mastercycler X50, one can easily mimic the thermal profile of the cycler of interest, thereby saving time and reagents.
The desired runtime enables the transfer of a PCR program from thermal cyclers with a lower ramping speed to a Mastercycler X50. The run time of the slower cycler was measured from after lid heating (at the beginning of block heating to 94 °C in the first denaturation step) to reaching 10 °C final block temperature in the hold step.
This way of run time measurement would negate the influence of lid heating time and minimizes contributing factors in measuring cycler speed. The time needed for the Cycler S to complete the PCR run measured this way was 28 min.
In the header settings of the Mastercycler X50, the function <<Desired runtime>> is displayed under <<Block Settings>>. The Mastercycler X50 automatically calculates the appropriate ramping rates according to the desired runtime without the need to further interference by the user. This also solves the need for multiple trial-and-error to find the right ramp rates when transferring protocol to other thermal cyclers. Figure 2 shows improved amplification yield of Mastercycler X50s after adaptation of protocol speed using the desired runtime for this challenging PCR protocol.

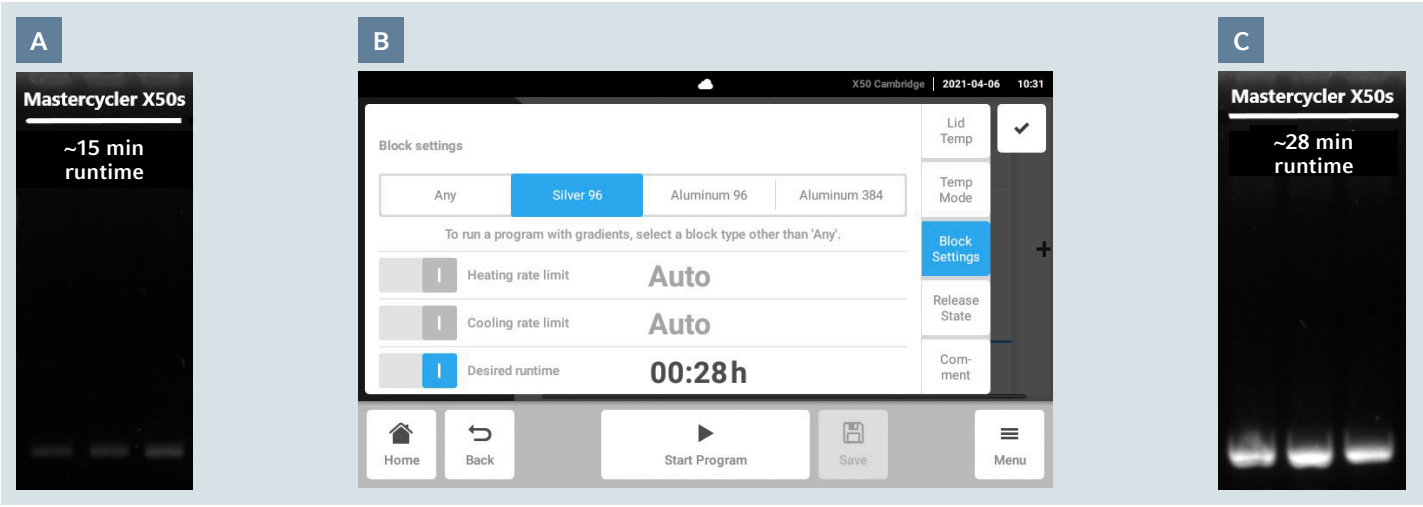

Figure 2: PCR results before (A) and after (C), the Program Migration Feature (PMF) was activated using the <<Desired runtime>> option (B).

In conclusion, PCR optimization can be fastidious and time-consuming, with each change requiring revalidation of the protocol. Hence, thermal cyclers which offer additional features that can simplify such processes can vastly boost user convenience and make life in the lab easier.

Conclusion

The Mastercycler X50 brings the advantages of fast ramping and shorter PCR run time. Additionally, if speed adjustments are necessary when transferring protocol from slower cyclers, the desired runtime option offers a simple and fast solution.

Literature

[1] Eppendorf Mastercycler®: Meeting all your PCR needs with reliability and flexibility. Eppendorf SE, White Paper No. 32 I April 2016.
[2] Gerke N & Phang A. Comparative run time evaluations of PCR thermal cyclers. Eppendorf SE, Application Note 274.

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