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Eppendorf Mastercyclers as robust PCR systems for GC-rich templates

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Abstract

The consideration for both thermal cyclers and reagents in performing PCR is commonly based on cost, availability and specific application needs. The interaction between thermal cyclers and reagents is often neglected as the prevailing expectation is that all enzymes are designed to work on any cyclers or vice versa. The aim of this paper is to evaluate the robustness of different cyclers in producing results with minimal effort when different enzymes are used. In this study, we investigated the amplification of a DNA template with high GC-content using different enzymes on five thermal cyclers. Some cyclers are highly robust and able to support the use of a wide range of enzymes without the need for extensive optimization.

Introduction

GC-rich regions contain regulatory sequences which make up approximately 28% of genes that are important for molecular biology studies [1] and often, Polymerase Chain Reaction (PCR) is utilized for the amplification of these regions. Despite the simple methodology of PCR, the process can often be complicated by the inherent formation of secondary structures like hairpins, where chemical additives [2, 3] are inevitably added to resolve these structures.

However, additional components in a PCR reaction tend to modify the melting behaviour of GC-rich regions which subsequently changes the primers' melting temperature and PCR cycling conditions as well. Therefore, an exact validation of various factors affecting PCR is still required to achieve optimal results. The first optimization step commonly involves finding the best annealing temperature which produces single and specific amplification.

Materials and Methods

Reaction master mix for the PCR was prepared per the protocols supplied by the manufacturers for a volume of 10 µL per reaction in 0.2 mL Eppendorf PCR tubes. The vessels are placed in wells that have the corresponding gradient temperatures in each cycler. BAIP3 forward (5'-AGTGCATGGAGGCGGACC-3') and reverse primers (5'-GCCAAGAAGCCCCTTGTGAG-3') were used at a final concentration according to manufacturer's recommendation for all three enzymes: i-Taq DNA polymerase (iNtRON Biotechnology), KAPA2G Robust DNA Polymerase (KAPA Biosystems) and AmpliTaq Gold® 360 (ATG360) DNA polymerase (Applied Biosystems). 100 ng of Human Genomic DNA, Female (Promega) was used per reaction in the first set. In addition, for the second set, dimethyl sulfoxide (DMSO) was added to the master mix at a final concentration of 4%. PCR was carried out on five 96-well thermal cyclers listed in Table 1.

Table 1: List of thermal cyclers in this study

Thermal cycler	Max block heating rate
Eppendorf Mastercycler® nexus GSX1	5°C/s
Eppendorf Mastercycler® nexus gradient	3°C/s
Applied Biosystems Veriti™ Fast	5°C/s
Life Technologies SimpliAmp™	4°C/s
Bio-Rad T100	4°C/s

Cycling conditions are listed in Table 2, with 2 sets of annealing temperatures; first set from 48.8°C to 61.2°C and second set ranging from 58°C to 68°C (Table 3). The first set of annealing temperatures was based on a range of ±5°C from predicted melting temperature for BAIP3, which is commonly employed during optimization of new primer sets. Meanwhile, the second set of temperature was modified accordingly to the nature of BAIP3, which contains high-GC content and requires higher annealing temperatures for higher specificity. For each of the thermal cycler models, the tests were performed on only one unit of each device.

Table 2: PCR program for each enzyme

	Step	Temperature	Duration		
			i-Taq	KAPA2G	ATG360
30 cycles	**Initial denaturation**	95°C	5 min	5 min	10 min
	Denaturation	95°C	15 s	15 s	30 s
	Annealing	[a]Gradient °C	30 s	30 s	30 s
	Extension	72°C	30 s	30 s	60 s
	Final extension	72°C	1 min	1 min	7 min

[a] Please refer to table 3.

Table 3: Annealing temperatures for first and second optimization

Thermal cycler	Maximum number of gradient temperatures	Annealing temperature used											
		1	2	3	4	5	6	7	8	9	10	11	12
		Set 1											
Mastercycler nexus GSX1	12 columns	48,8	49,2	49,9	51,1	52,7	54,2	55,8	57,3	58,9	60,1	60,8	61,2
Mastercycler nexus gradient	12 columns												
Veriti	6 zones*	49	50	51	52	53	54	55	56	57	58	59	60
SimpliAmp	3 zones*	49	50	51	52	53	54	55	56	57	58	59	60
T100	8 rows	48,8	49,6	51,1	53,5	56,3	58,6	60,1	61,1				
		Set 2											
Mastercycler nexus GSX1	12 columns	58,4	59,9	62	64	66	68						
Mastercycler nexus gradient	12 columns												
Veriti	6 zones*	58	60	62	64	66	68						
SimpliAmp	3 zones*	58	60	62	64	66	68						
T100	8 rows	58	60,1	62,2	64,7	66,8	68,2						

*Due to the limited number of permissible gradient temperatures per cycler, multiple PCR runs were performed to match the number of temperatures used in Mastercyclers.

Results and Discussion

In this study, polymerases from three amplification kits are tested under various annealing temperatures in five different thermal cyclers to evaluate the ease of optimization under different conditions.

BAIP3 was amplified to produce amplicons of 788 base pairs with 64.6% GC content. All cycling conditions were based on the parameters recommended by the respective amplification kit manufacturer. Minimal optimization was carried out in this study. Addition of DMSO, a common additive used for GC-rich DNA amplification, was tested at concentration 2, 4, 8 and 10% when the supplied enhancers were found to yield no/ sub-optimal results for some cycler-enzyme combinations. For every polymerase used, it was substantiated that the addition of 4% DMSO, a common additive used for GC-rich DNA amplification, is required to achieve result where a specific product was produced in an observable amount (Figure 1; only representative result from i-Taq enzyme is shown).
In general, amplification of high GC template is more difficult than standard DNA template and requires some amount of optimization [4, 5]. Three commercial *Taq* DNA polymerases chosen in this study represent different types of formulations available in the market: KAPA2G Robust, which is formulated for GC-rich templates; ATG360, which is formulated for a broad range of targets including GC-rich sequences with addition of an enhancer; and i-Taq DNA polymerase reagent, which is designed for general PCR. The concoction of PCR reagents would respond in different ways during the amplification process. Hence, the thermal cycler plays a crucial role in this situation to simplify the process of protocol optimization or primers validation by reducing interfering factors contributed by the instrument itself. Overall, Eppendorf Mastercyclers nexus GSX1, Eppendorf Mastercycler nexus gradient and Life Technologies Veriti produced positive amplification comparable across all 3 enzymes. In comparison, amplification with Life Technologies SimpliAmp and Bio-Rad T100 were only successful with KAPA2G enzyme. Optimization can be dramatically affected by the characteristic (such as heating/cooling efficiency) and functions (such as gradient availability) of a thermal cycler. The data presented herein this study was a result of minimal optimization effort, whereby the maximum (or comparable) number of permissible gradient temperatures was used in the first optimization and only six gradient temperatures were used in the second targeted optimization (Table 3).

Thus, it is possible that better results can be obtained with further modification in various parameters. However, the aim of this study is to evaluate the robustness of various cyclers in running difficult PCR applications, with the goal of utilizing minimal optimization effort. In fact, if more detailed optimization is desired, thermal cyclers with more advanced gradient function (such as 2D-gradient in Eppendorf Mastercycler X50 [6, 7]) would also greatly simplify optimization effort. At this point in discussion, a robust thermal cycler that can produce results when used with various enzyme formulations can save users much effort in optimizing each PCR system.

With a better thermal cycler, users will not be limited to use only advanced enzyme formulations, which can be costlier to obtain satisfactory results in difficult templates. In summary, the results showed that both enzymes and thermal cyclers together play important roles in successful PCR amplification.

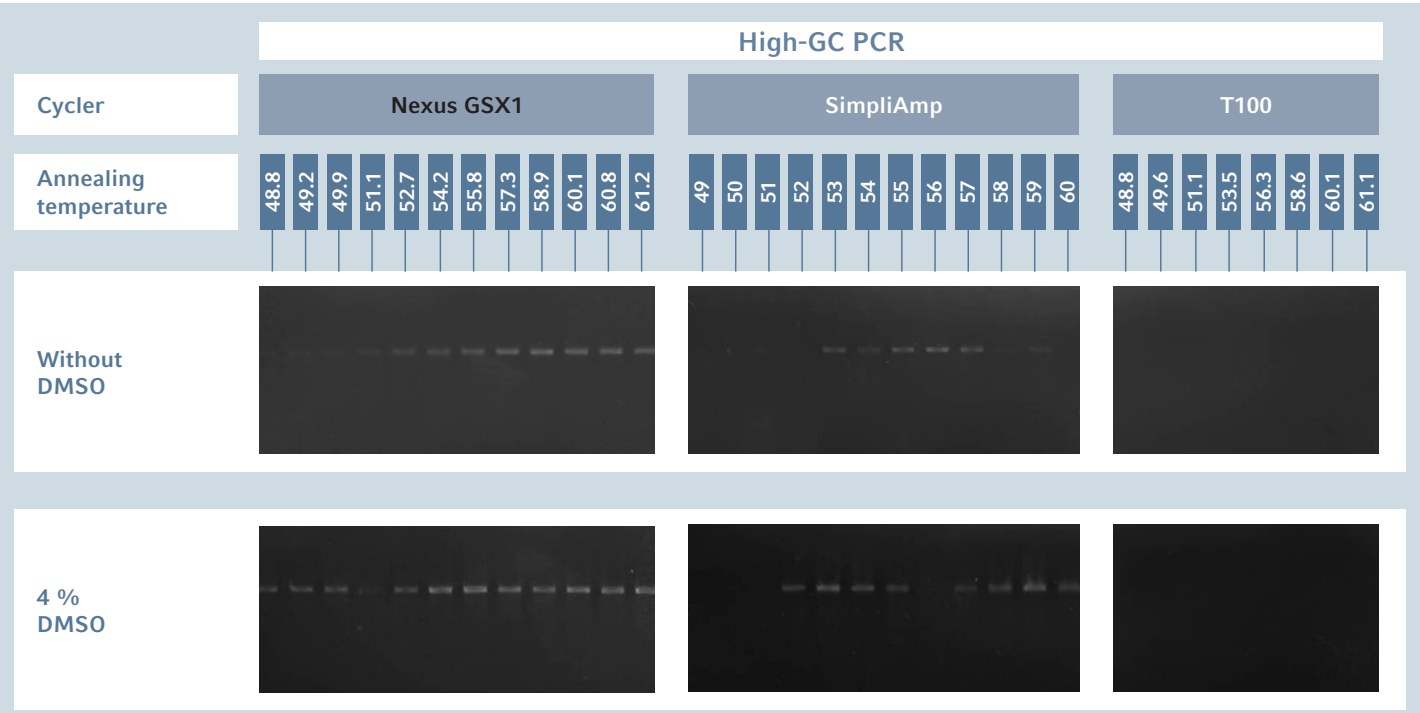

Figure 1: 3-step amplification of BAIP3 with and without addition of DMSO using i-Taq DNA polymerase. Addition of 4% DMSO increased amplification performance in Mastercycler Nexus GSX1 and SimpliAmp. However, even with addition of 4% DMSO, no amplification was observed with T100.

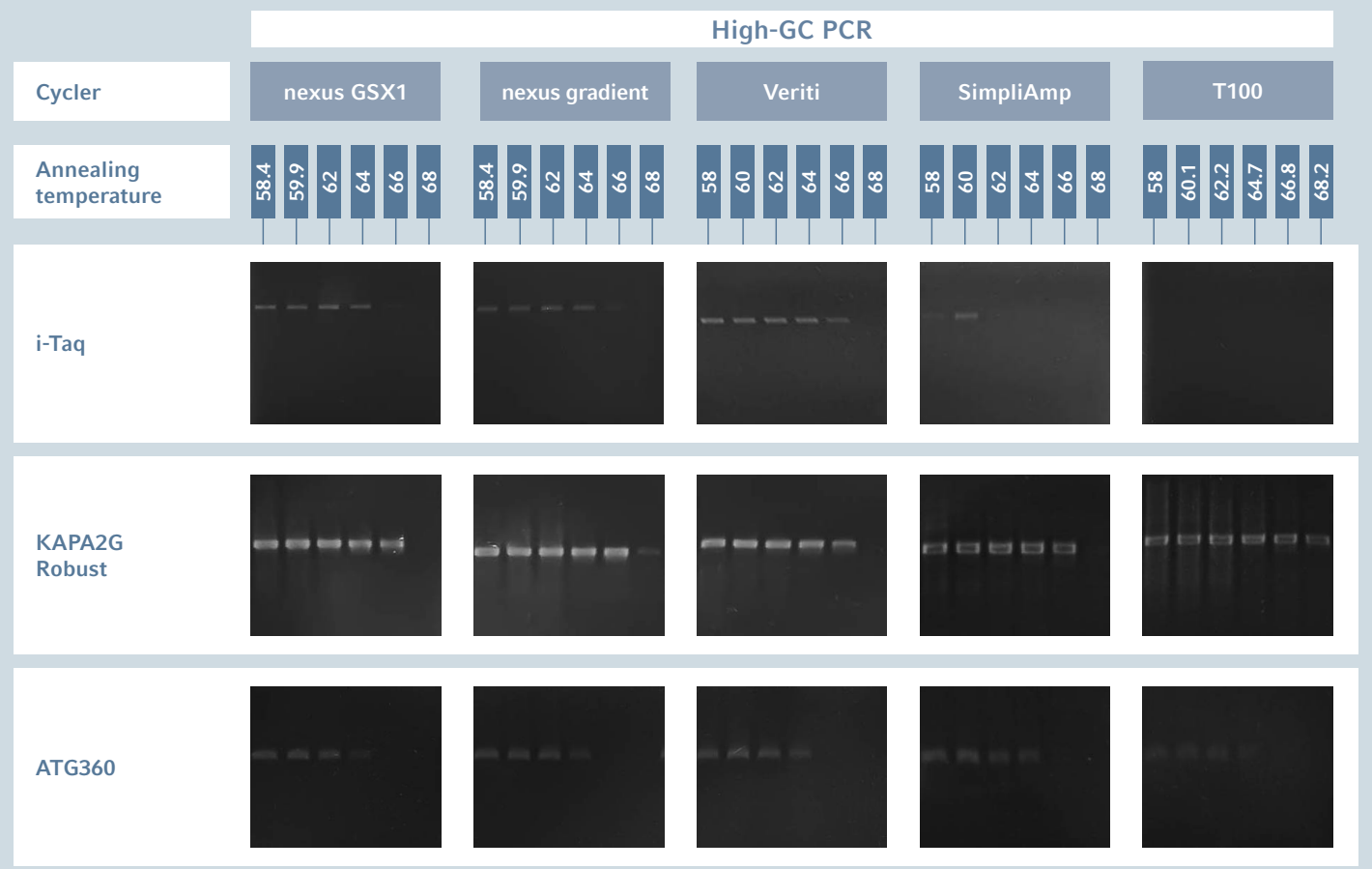

Figure 2: 3-step amplification of BAIP3 with 3 commercial Taq polymerases; i-Taq DNA polymerase, KAPA2G Robust and AmpliTaq Gold 360 (ATG360) DNA polymerase. BAIP3 was successfully amplified with in Mastercyclers GSX1, nexus gradient and Veriti using all three enzymes with the addition of DMSO.

Conclusion

The right choice of thermal cycler and enzymes are critical in determining the success of amplifying DNA template containing high GC content. Thermal cyclers with gradient function enable researchers to save time and effort by running several annealing temperatures instead of one in a single run.

Thermal cyclers with robust design and high temperature performance support a wide range of enzymes, saving researchers much effort in optimization work. A robust thermal cycler will not only save time and ultimately cost, but also yield reliable reproducible results that are of key importance in scientific research.

Literature

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[3] Henke, W., Herdel, K., Jung, K., Schnorr, D., & Loening, S. A. (1997). Betaine improves the PCR amplification of GC-rich DNA sequences. Nucleic acids research, 25(19), 3957-3958.
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[6] Eppendorf Application Note 387. Phang, A., Schommartz, T. (2017). Ultimate PCR Optimization with Eppendorf Mastercycler ® X50 2D-gradient.
[7] Eppendorf Application Note 388. Phang, A., Schommartz, T. (2017). Highly reproducible low volume PCR with Mastercycler® X50 and epMotion.

Ordering information

Description	International Order no.	North America Order no.
Mastercycler® nexus GSX1, 230 V/50 – 60 Hz	6345 000.010	6345000028
Mastercycler® nexus GSX1e*, 230 V/50 – 60 Hz	6347 000.017	6347000025
Mastercycler® nexus gradient, 230 V/50 – 60 Hz	6331 000.017	6331000025
Mastercycler® nexus gradient eco*, 230 V/50 – 60 Hz	6334 000.018	6334000026
PCR tubes, 0.2 mL, PCR clean, colorless	0030 124.332	951010006

* To run a Mastercycler® nexus with the suffix »eco« or »e«, a Mastercycler® nexus model without such a suffix is needed. Up to 2 units with the suffix »eco« or »e« can be connected to a Mastercycler® nexus without such a suffix.

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