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Original Article

Evaluation of Palm PCR™ G1-12 System: a portable battery-operated PCR thermal cycler

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Abstract

Polymerase chain reaction (PCR) is the basis of recombinant and other molecular biological techniques. Availability of cheap and robust PCR platforms enables the tests to be performed easily, even in resource constrained settings. Herein we compared the efficacy of a portable thermal cycler (Palm PCR™ G1-12 System) for rapid DNA amplification against the standard Peltier-based thermal cycler using plasmid DNA and genomic DNA in single and multiplex PCR experiments. Our study revealed that the Palm PCR™ G1-12 System could be a portable DNA amplification system to conduct various molecular techniques, especially in places where resources are limited.

Keywords: hand-held PCR, convective PCR, portable thermal cycler

1. Introduction

Polymerase chain reaction (PCR) is a key procedure in molecular techniques used for amplifying specific DNA sequences (Saiki *et al.*, 1985). Since the first report in 1986, it has been extensively developed and widely applied in various fields, including molecular biology, medical diagnostics, food testing, and environmental monitoring (Hsieh *et al.*, 2006). Generally, the PCR thermal cycle consists of three steps: denaturation (90-99°C), annealing (40-72°C), and extension (72°C) (Mullis *et al.*, 1986). Theoretically, the number of amplicons is doubled during each cycle, and at the end of the process the amplicons are usually amplified to more than a billion copies and hence are readily detectable using conventional gel electrophoresis techniques (Hsieh *et al.*, 2006). The major breakthroughs in PCR technology were the development of recombinant DNA polymerases (Saiki *et al.*, 1988) and thermal cyclers (Schoder *et al.*, 2005). The first commercial thermal cycler, the Perkin-Elmer Cetus DNA thermal cycler, was introduced in 1987. Since then other companies have developed similar devices, but with different technical architectures, including heating-cooling systems (Peltier technology versus convection), internal temperature control (sample probe control versus calculated temperature control and block control), and evaporation control (heated lid versus oil overlay) (Saiki *et al.*, 1988; Saunders *et al.*, 2001).

In recent years, a growing number of studies have focused on developing a fast and portable PCR thermal cycler (Chou *et al.*, 2011). However, thermal cyclers that are based on thermal conductivity still require delicate controllers for their heating-cooling system. A convective PCR, on the other hand, is based on spontaneous and repeated liquid circulation to provide working temperatures for denatura-

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tion, annealing, and extension (Chou *et al.*, 2011). Accurate thermal cyclers are crucial for advanced molecular applications such as PCR and real-time qPCR in large sample formats, which rely on efficient heating-cooling, efficient heat transfer, and a reproducible temperature profile (Schoder *et al.*, 2005).

Thermal cyclers enable the automation of repetitive thermal processes and ensure temperature uniformity and rapid heat transfer to reagents. The early PCR thermal cyclers had limited application in laboratories and hospitals due to their size, cost, and long amplification time (Chou *et al.*, 2011). Later, improved thermal cyclers were developed that had simplified equipment, smaller size, and shorter ramping time via implementation of Peltier technology, which shortened the amplification time from 2-3 hours to 30-40 minutes (Feldkotter *et al.*, 2002; Emanuel *et al.*, 2003; Tafe *et al.*, 2007). Another alternative to the thermal block is a microfluidic chip that circulates the reagents through three zones at different fixed temperatures (Shoffner *et al.*, 1996; Kopp *et al.*, 1998; Lee *et al.*, 2004; Oh *et al.*, 2005; Lien *et al.*, 2007). This technology can greatly shrink the size of the device, but it requires external pumping equipment and a complex micro-channel design (Chou et al., 2011). The currently available PCR-based methods require an expensive thermal cycler, laboratory facilities, and training, making them unavailable and/or impractical for use in low resource settings. Thus, a simpler alternative thermal cycling technique that involves spontaneous and repeated liquid circulation to provide working temperatures for denaturation, annealing, and extension was proposed, which is applicable in places with scarce resources (Chou *et al.*, 2011).

Palm PCR™ G1-12 System (Ahram Biosystems Inc., Seoul, Korea) is a newly developed thermal cycler that operates based on temperature changes mediated by spontaneous and repeated liquid circulation. This stand-alone device is a modified version of a convective PCR thermal cycler in which the reagents are circulated across the temperature zones inside the PCR sample tube, thereby achieving the PCR amplification. The thermal cycler in this new device uses a convection system for heating-cooling, and three specially positioned heat blocks are maintained at designated temperatures for each of the three PCR steps (denaturation, annealing, and extension) to create a temperature gradient, causing the reagents to circulate through the thermal zones. In this system, the reaction liquid circulates between hot and cold regions of the tube without the need for the complicated channels and expensive pumps required in many microfluidic devices. The Palm PCR™ G1-12 System has the capacity to perform ultra-fast and highly efficient thermal cycling for 12 samples of 20 µL volumes. The manufacturer claims that the amplification sensitivity of this system can approach one copy, with a dynamic range of amplification up to 2 kb for simple templates such as plasmids and 1 kb for complex templates such as genomic DNA. Further information about the Palm PCR™ G1-12 System and related versions and products can be accessed from the official web page (http://www.ahrambio.com/products_palmpcr_G1-12.html). Thus, this portable, low power consuming, easy to operate thermal cycler may be well suited for point-of-care applications.

2. Materials and Methods

2.1 Instruments and programming

In this study, we sought to evaluate the amplification performance of the Palm PCR™ G1-12 System (Ahram Biosystems, Inc., Seoul, Korea) by using both the plasmid and genomic DNA as the template. To actualize this, we have compared the performance of the Palm PCR™ G1-12 System with that of a standard MyCycler™ Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). These two devices are based on completely different platforms. The PCR protocols were programmed into the devices following the guidelines provided by their respective manufacturers. Briefly, the S1 PCR speed level (standard slow mode) was selected for the Palm PCR™ G1-12 System and the amplification protocol was programmed as follows: annealing: 60°C; melting: 95°C; for 39 min for 30 cycles. The PCR amplification protocol for the MyCycler™ Thermal Cycler consisted of a preliminary denaturation step at 95°C for 2 min; 30 cycles of 95°C for 30 sec (denaturation), 60°C for 30 sec (annealing), 72°C for 30 sec (extension); and 72°C for 2 minutes (final extension).

2.2 PCR targets and reagents

Two types of DNA were used as the PCR template in this study, plasmid DNA and genomic DNA. Initially, two different plasmids of p100 and p600 were subjected to amplification using these two different systems to give 100 bp and 600 bp amplicons, respectively. Subsequently, in-house PCR for the detection of *Saccharomyces cerevisiae* and *Salmonella* typhi genomic DNAs were carried out. For the detection of *Saccharomyces cerevisiae,* the amplification target is the large subunit ribosomal (LSR) DNA, which produces an amplicon of 630 bp (Vilgalys and Hester, 1990). On the other hand, three different non-protein coding RNA (sRNA) genes were selected as the target for the detection of *S.* typhi; whereby amplicons of 204 bp, 304 bp, and 475 bp will be produced in this multiplex PCR system. Each 20 µL reaction mixture contained 1x PCR buffer, 1.5 mM $MgCl_2$, 0.35 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM of primers, 1U *Taq* DNA polymerase, and DNA templates. All reagents and *Taq* DNA polymerase were purchased from Biotools B&M Labs, S.A. (Madrid, Spain). A negative control reaction mix containing sterile deionized distilled water in place of DNA template was included in each PCR run.

2.3 Evaluation of thermal cyclers' performance

PCR amplification of the above targets and an in-house multiplex PCR assay of *S. typhi* were used to test the performance of the Palm™ G1-12 System as compared to the

Table 1. Differences between MyCycler™ Thermal Cycler and Palm PCR™ G1-12 System.

Parameter	MyCycler™	Palm PCR™ G1-12 System
Heating cooling system	Peltier Technology	Convection system
Cycling condition	User defined	User can only set annealing temperature. Melting and extension is controlled by system
PCR speed	Depends on the program set by user	7 pre-defined speed levels can be chosen
Size (w x d x h)	23.6 x 44.3 x 20.5 cm	71 x 121 x 47 mm
Sample capacity	96 wells	12 wells
Input power	220-240 V, 4 A	11-15 V, 4 ABattery operated

MyCycler™ Thermal Cycler. Each reaction mix was prepared as described previously in a total volume of 40 µL and aliquoted into two tubes (20 µL/tube), which were run separately in the two thermal cyclers for comparison without bias. The PCR products amplified using each device were run on a 2% agarose gel prior to visualization under UV light. To identify the detection levels for plasmid DNA and genomic DNA, the DNA templates were serially diluted tenfold before being added into the reaction mix, yielding a range of DNA concentrations per reaction. The detection level was defined as the lowest dilution producing visible amplicon bands on an agarose gel after PCR. The multiplex PCR consisted of three pairs of primers that yielded three amplicons of 204 bp, 304 bp, and 475 bp.

3. Results and Discussion

Table 1 shows the difference between the two PCR machines used in this study. MyCycler™ incorporates a Peltier driven heating and cooling system for thermal cycling process. Palm PCR™ G1-12 System however, uses a convection system for the heating and cooling process. MyCycler™ allows user to define their own cycling condition by setting up the melting, annealing and extension steps. The cycling condition will in turn define the PCR speed or in other words, how long is the PCR run. This is not the case for the Palm PCR™ G1-12 System, where users can only set the annealing temperature. The melting and extension is controlled by the system. Therefore, there are seven pre-defined speed levels available, categorized into three modes: 'Standard Fast', 'Standard Slow', and 'Turbo Fast' mode. Getting the name from its size, the Palm PCR™ G1-12 System is only 71 x 121 x 47 mm. Because of the small size, the Palm PCR™ G1-12 System can only accommodate 12 samples for each run, and therefore can be operated using batteries. The MyCycler™ instead is bigger, with the size of 23.6 x 44.3 x 20.5 cm and can accommodate 96 samples in each PCR run.

3.1 Evaluation based on detection limits

Comparisons between the PCR efficiencies of the Palm PCR™ G1-12 System and the MyCycler™ Thermal Cycler based on their detection levels using both plasmid DNA and genomic DNA are shown by agarose gel electrophoresis after completion of the PCR amplification. The agarose gel image in Figure 1a shows that the detection levels for plasmid DNA of 100 bp amplicon for both the MyCycler™ and Palm PCR™ G1-12 were 10 pg per reaction. Thus, the level of detection was equal in both cases, but, the band intensity was higher in MyCycler™ Thermal Cycler (Figure 1b) compared to Palm PCR™ G1-12 System (Figure 1c) as was evident with the surface plot profile of the band intensity measurement. However, the detection limit of the MyCycler™ Thermal Cycler was better than that of the Palm PCR™ G1-12 System for plasmid DNA of 600 bp amplicon (Figure 2a). Palm PCR™ G1-12 System also resulted in smearing at a

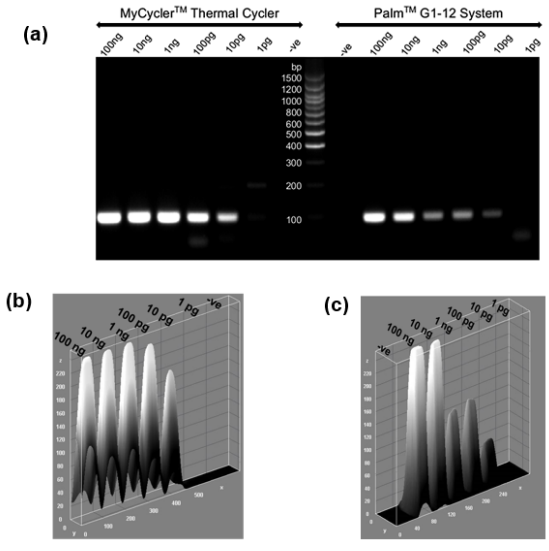

Figure 1. Detection levels of the PCR amplification using plasmid DNA p100 as the template using MyCycler™ Thermal Cycler and Palm PCR™ G1-12 System. (a) PCR products obtained from PCR run using 10 fold serially diluted template. –ve: negative control without template. (b) ImageJ surface plot profile analysis of the band intensity for MyCycler™ Thermal Cycler (c) ImageJ surface plot profile analysis of the band intensity for Palm PCR™ G1-12 System.

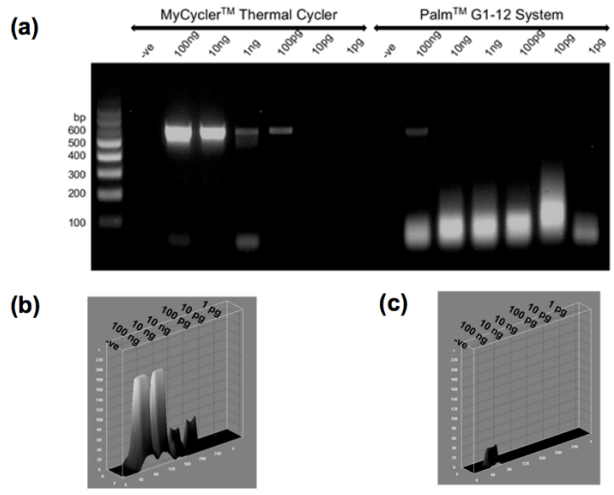

Figure 2. Detection levels of the PCR amplification using plasmid DNA p600 as the template using MyCycler™ Thermal Cycler and Palm PCR™ G1-12 System. (a) PCR products obtained from PCR run using 10 fold serially diluted template. –ve: negative control without template. (b) ImageJ surface plot profile analysis of the band intensity for MyCycler™ Thermal Cycler (c) ImageJ surface plot profile analysis of the band intensity for Palm PCR™ G1-12 System.

lower size as shown in Figure 2a. As corroborated by ImageJ surface plot profile, the detection limit of the MyCycler™ Thermal Cycler (Figure 2b) is 100 pg, which is better than Palm PCR™ G1-12 System, that achieved a detection limit of 100 ng (Figure 2c).

The detection level for amplification of the target gene from genomic DNA was slightly better for the Palm PCR™ G1-12 System (100 fg per reaction) than for the MyCycler™ Thermal Cycler (1 pg per reaction) (Figure 3a). ImageJ surface plot profile showed that the band intensity for MyCycler™ Thermal Cycler at 100 fg has an arbitrary value of 0 (Figure 3b) while Palm PCR™ G1-12 System has a value of of 20 (Figure 3c). Palm PCR™ G1-12 System has a detection sensitivity that is 10-fold higher than the MyCycler™ Thermal Cycler. In addition, the PCR products generated by the Palm PCR™ G1-12 System did not show a gradual decreasing pattern of amplicon band intensity, whereas this decline was apparent for the PCR products generated by the MyCycler™ Thermal Cycler in parallel with the serial tenfold dilution of the DNA template in the reactions (Figure 3a). This result was further validated with ImageJ surface plot profile, whereby amplicons produced by MyCycler™ Thermal Cycler resulted in decreased band intensity at 10 pg and dropped further at 1 pg (Figure 3b). As a comparison, the amplicons generated by the Palm PCR™ G1-12 System from 100 ng to 1 pg have similar band intensity (Figure 3c). This showed that Palm PCR™ G1-12 System has a higher sensitivity of detection for longer template (for example genomic DNA). In addition, unexpected bands of 100-200 bp were observed in the Palm PCR™ G1-12 System results.

3.2 Evaluation based on multiplex PCR assay

We have also carried out multiplex PCR using genomic DNA as the template to evaluate the ability of the devices to simultaneously amplify more than one target per reaction, as multiplex PCR is used frequently in various molecular applications today. Although the PalmTaq™ High-Speed PCR Kit (Ahram Biosystems, Inc.) is recommended for use with the Palm PCR™ G1-12 System, it was not used in this experiment. In order to compare the performance of the thermal cyclers, all reagents and the DNA polymerase used for this experiment were those used for standard PCR amplifications, and they were synchronized for both devices. The standard slow mode (S1) was selected for the Palm PCR™ G1-12 System and the reactions were run in triplicates. The agarose gel image in Figure 4a shows that all of the targets in the *S. typhi* strains were successfully and evenly amplified by the MyCycler™ Thermal Cycler, whereas only the 202 bp and 304 bp amplicon sizes were successfully amplified by the Palm PCR™ G1-12 System. The band intensity of 304 bp amplicon differs from each other while the amplicon of 202 bp exhibited similar band intensity in Palm PCR™ G1-12 System (Figure 4b). However, the band intensities constituted by amplicons of 202, 304 and 475 bps are equal in MyCycler™ Thermal Cycler (Figure 4c). Furthermore, additional smeared bands were present in the Palm PCR™ G1-12 System results

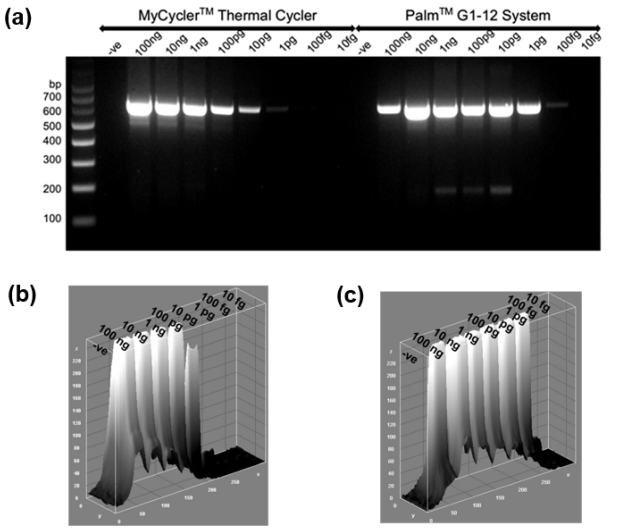

Figure 3. Detection levels of the PCR amplification using genomic DNA extracted from *S. cerevisiae* as the template. (a) PCR products obtained from PCR run using 10 fold serially diluted template. –ve: negative control without template. The lower panel shows the band intensity analyzed using ImageJ software. (b) ImageJ surface plot profile analysis of the band intensity for MyCycler™ Thermal Cycler (c) ImageJ surface plot profile analysis of the band intensity for Palm PCR™ G1-12 System.

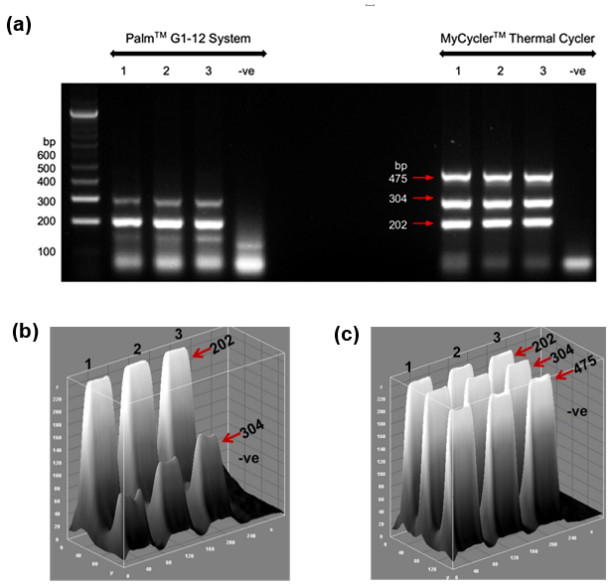

Figure 4. Evaluation of the two thermal cyclers' performance using multiplex PCR assay. The genomic DNA used was extracted from *S. typhi* at 100 ng per reaction (in triplicate). (a) Lanes 1-3 represent the PCR products obtained from the run. (b) ImageJ surface plot analysis of the band intensity for Palm PCR™ G1-12 System (c) ImageJ surface plot analysis of the band intensity for MyCycler™ Thermal Cycler.

and absent in the MyCycler™ Thermal Cycler results. Thus, the difference in performance between the thermal cyclers became more significant and pronounced with multiplex PCR. The differences in performance might have also been caused by the interplay of two factors in the Palm PCR™ G1-12 System, liquid convection and *Taq* DNA polymerase.

The Palm PCR™ G1-12 System uses a convection system for heating-cooling, whereby the reagents circulate through three thermal zones (denaturation, extension, and annealing) for DNA amplification. The challenge facing convective PCR is that the circulation pattern and flow rate of the liquid might be inconsistent and therefore limit the PCR applicability for larger amplicons and more complex DNA like genomic DNA or chromosomal DNA. This is shown in the inability to amplify the larger amplicons for the multiplex PCR (Figure 4a). Chou *et al.* (2011) previously studied single isothermal convective PCR and suggested that smaller volume reactions would have a shorter effective time for annealing and extension by DNA polymerase, as it takes time for the denaturation temperature to decrease. Furthermore, a longer capillary tube might cause turbulence in the liquid circulation and split into more than one circulation pathway (Chou *et al.*, 2011). The above challenges of convective PCR could explain the observed lower performance of the Palm PCR™ G1-12 System compared to the MyCycler™ Thermal Cycler. In the Palm PCR™ G1-12 System, the annealing and extension steps performed by the DNA polymerase may not have had time to catch up with the liquid circulation in the capillary tube, causing failure or incomplete amplification. This can be seen in the failure to amplify the 600 bp plasmid, but instead producing smearing with much smaller size around 100 to 200 bp (Figure 2a). Another phenomenon observed was the presence of additional amplicon bands of smaller sizes (100-200 bp) in the Palm PCR™ G1-12 System results; these might be due to incomplete extension by the DNA polymerase. A possible solution to this limitation is to replace the DNA polymerase used in normal PCR with a high-speed DNA polymerase, like the one provided in the PalmTaq™ High-Speed PCR Kit.

Our results demonstrated that the PCR of small amplicons (100 and 600 bp) from plasmid DNA performed by the Palm PCR™ G1-12 System has lower sensitivity compared to that of standard thermal cycler used in this study. Palm PCR™ G1-12 System have also shown better performance for longer templates (i.e., genomic DNA). For multiplex PCR, there is a variation in the performance between Palm PCR™ G1-12 System and MyCycler™ Thermal Cycler, this can be further optimized for more optimal performance. However, we would like to highlight the limitiations of the data from this study. First, the PCR condition used were those for standard PCR amplifications and not optimized for the Palm PCR™ G1-12 System. Second, the PalmTaq High speed PCR kit was not used for the Palm PCR™ G1-12 System, which might have affected the outcome of the results.

4. Conclusions

Based on these analyses, we conclude that the Palm PCR™ G1-12 System still need optimization for amplification of templates with varied lengths. Although the performance of the Palm PCR™ G1-12 System is shown to give variable results, there is no doubt that it is suitable for performing wide range of PCR diagnostic assays especially in low resource areas. Use of this portable, low power consuming, battery-powered, and easy to operate system could improve surveillance systems for infection outbreaks beyond the laboratory. This portable amplification system is in tandem with the World Health Organization (WHO)'s criteria of ASSURED (A-Affordable, S-Sensitive, S-Specific, U-User-friendly, R-Robust and rapid, E-Equipment-free and D-Deliverable to those who need the test) (Rozand, 2014).

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