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Development and Manufacturing of World Grade Programmable Thermal Cyclers for Polymerase Chain Reaction in Pakistan: A Case of Biomedical Engineering

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Abstract

Manufacturing is the backbone of a modern state and has a direct impact on life style of a common person of a society. We present here the development and manufacturing of Polymerase Chain Reaction (PCR) machines by the Pakistan Atomic Energy Commission (PAEC), Pakistan. Steps of the development and manufacturing of PCR machines are explained. Important design features of MERADD PCR machines and their implementation are documented here. Results of the MERADD PCR machine are compared with those of competitive imported machines. MERADD is the sole manufacturer of PCR machines in Pakistan. Quality assurance and cost analysis are also given. MERADD PCR machines produce as good results as by the imported machines, but cost is significantly lower. This paper is useful for a broad community of developers/manufacturers of medical devices and biomedical engineering in developing countries around the world.

Keywords: Biotechnology; Medical devices; Biomedical engineering; Polymerase chain reaction; Peltier effect; DNA

Introduction

Biotechnology is the application of science and engineering to the world of organisms [1]. Polymerase Chain Reaction (PCR) is a method used in biotechnology to multiply a single copy or a few copies of a specific segment of DNA into thousands to millions of copies of a specific DNA sequence. It mimics the basic mechanism of DNA replication [2-4]. Quantitative measurements are possible using advanced version of PCR, called real time PCR [5]. PCR was invented by Dr. Kary Mullis in the 1980s. Mullis was awarded Nobel Prize in Chemistry in 1993 for this invention. PCR has various applications, including molecular diagnostics of human and animal diseases [6-8], forensics [9], food technology [10] and environmental studies [11,12]. Figure 1A shows three stages of a PCR cycle. These stages are called denaturation, annealing and extension. In the denaturation stage, the template DNA is heated beyond the melting point of its two complementary strands. It separates the two strands of the DNA. In the second stage annealing, the temperature is reduced to allow the primers to bind to their complementary sequences on the single strands. In the third stage of extension, the DNA polymerase extends the primers by integrating nucleotides to the developing DNA strand [2]. Amplification of the target DNA takes place in the repetition of a PCR cycle. A variety of PCR machines are in use around the world [2,13-15] (Figure 1B). Advantages of PCR include its simplicity, fast speed and high sensitivity. Applications of PCR range from agriculture to biomedical research. Experimental steps of the PCR procedure are sample preparation, DNA amplification and product detection. During amplification, the sample is cycled between denaturation, annealing and extension temperature. The typical ranges of denaturation and annealing are, respectively, 90-95°C and 50-65°C. The extension happens around 72°C [16]. The next section describes PCR machines.

Programmable Thermal Cyclers or PCR Machines

A typical PCR machines

Figure 2 shows the general function of a typical/conventional PCR. Temperature cyler in the figure is to conduct three steps of PCR, denaturation, annealing and extension. DNA micture contains the target DNA, primers, nucleotides and DNA polymerase. Heater at the top of the mixture is to supress vapor formation in the tube. Temperature of the heater remains the same and is kept above the denaturation temperature.

Types of PCR machines

Many variants of PCR machines have been developed. Some of them are commercially available. We do not review here all the available or reported PCR machines but are documenting only leading or general types.

Conventional PCR machine: It is also called qualitative PCR. Its components are of macroscopic size. Normally, it uses Peltier cell [17] thermal cycling. Other parts include power system and control system. DNA mixture of macroscopic size (fraction of a milliliter) is needed. Several samples can be processed or analyzed simultaneously. DNA amplification up to millions is possible in hours' time scale. Please see details later in the paper.

Multiplex PCR machine: In a multiplex PCR machine, more than one DNA targets are amplified simultaneously in the same reaction. Since its first description in 1988 [18], multiplex PCR has been used in several areas of DNA testing and diagnostics. Important considerations in the Multiplex PCR are DNA primers, recipe of the primer mixture, optimization of Multiplex PCR cycling conditions and optimization of Multiplex reaction components [19,20].

Real-time PCR machine: The same principal of conventional PCR is implemented in real-time PCR with the additional capability

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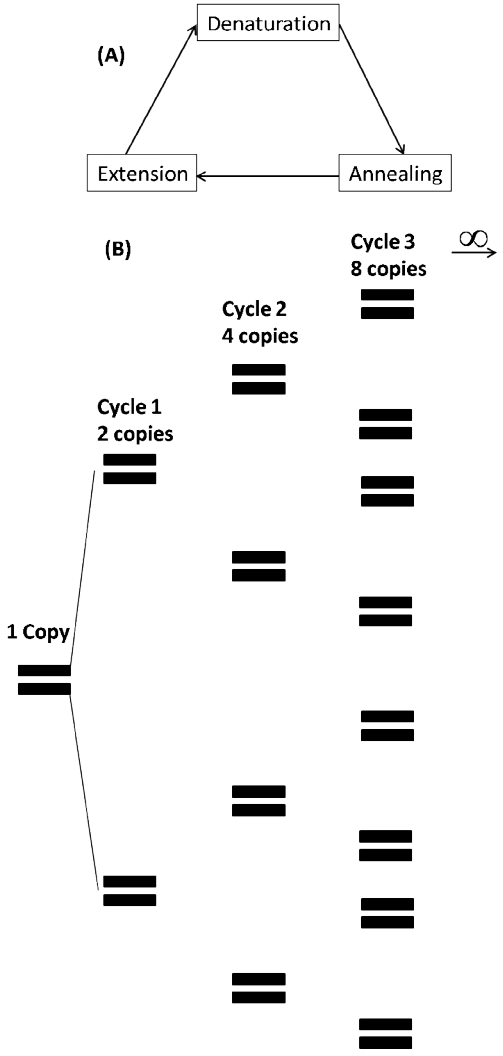


Figure 1: (A) Three stages of a PCR cycle, (B) Amplification of DNA fragments in PCR cycles.

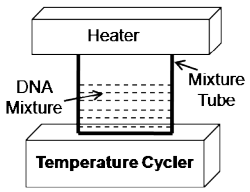


Figure 2: A schematic showing the function of a typical PCR.

of monitoring the DNA amplification in real time using a camera or a detector. The amplification of DNA is coupled the fluorescence production during the process. The fluorescence is produced by the added dye like SYBR green or a detection solution like [21].

Micromachined/Nano machined chip PCR: PCR amplification is necessary for applications of integrated microchips in genetic analysis. Micromachined/nanomachined [22,23] DNA PCR chips with different groove depths can be fabricated in glass, silicon and plastic [24,25]. Microchip PCR machines have advantages of minute sample consumption, simultaneous analysis of large number of samples and enhanced integration ability with the supporting equipment.

MERADD PCR Machines

MERADD has developed several models of conventional PCR machines. The latest model is Amplicon PTC-15. We describe below the R&D work and manufacturing of PCR machines at MERADD.

MERADD infrastructure

MERADD has two Research & Development (R&D) labs, one Assembly Hall in which machines are assembled from components, one technical store where electronic and mechanical components are catalogued with easily usable records of the components in paper and soft forms, and mechanical/electronics workshop in which components are fabricated or altered. MERADD outsource a part of the work to institutions and factories mainly in Lahore and Islamabad. MERADD also develop/manufacture other medical equipments which are not described here.

MERADD manufacturing strategy

MERADD develops/manufactures medical equipment in interest of the scientific community. Clients are charged for the manufacturing cost and R&D expenditure. Salaries of the employees are being paid by the PAEC. They are not included in the price of the equipment being sold to the universities and institutes in Pakistan. Repair and maintenance after the warranty period are provided to the clients on no profit no loss basis. MERADD understand the implications of the wastes on the environment. MERADD aims to keep its activities harmless to the environment. MERADD manufacturing strategy model emphasizes on the indigenization, flexibility of the equipment use in biomedical laboratories, result reliability, consumer choice and the quality.

R&D work related to MERADD PCR machines

MERADD started basic R&D work on the development of PCR machines in 2001. Extensive literature and equipment survey was conducted. Experimental work on the development of first PCR machine was started in 2004. Peltier heating and cooling system was developed in 2005. During the development of Peltier heating and cooling pumping system, several basic experiments were conducted. PCR machine power and control systems were developed. These two systems were developed simultaneously. They were finalized in 2007. Last task was the development thermal cycling of DNA mixtures. The volume of the DNA mixture tube finalized as 0.2 milliliter after experiments. DNA mixture used in experiments ranged from microliters to a fraction of a milliliter. First PCR machine was developed in 2008. Its name was Programable Thermal Cycler PTC-06.

Specifications/Features of the MERADD PCR machine PTC-15

The latest model of the MERADD PCR machines is Amplicon PTC-15. Its specifications are given in Table 1. It is a locally manufactured, high-tech Peltier cell based, auto adjustment for sample plate and heater plate contact, reproducible and trustworthy results, low cost and low power consumption technology. Quick maintenance services are provided to the clients around the country.

Manufacturing of MERADD PCR machines

Manufacturing is transforming the raw materials into a usable product using labour, machines and tools. It is a wide spread term

S. No.	Features	Specifications
1.	Thermal Range	0°C to 99°C
2.	Accuracy	± 0.4°C of programmed target at 60°C ± 0.1 s of programmed target at 30 min
3.	Thermal Uniformity	± 0.5°C well-to-well within 30 s of arrival at 60°C
4.	Ramping Rate	2.5°C per s
5.	Peltier Cells	02 No.
6.	Sample Capacity	96-well block; 96 × 0.2 ml tubes
7.	No. of Cycles	999 (Max)
8.	Memory	64 Kb
9.	Line Input Voltage	220 VAC
10.	Frequency	50 Hz single phase
11.	Power	360 Watts (maximum)
12.	Fuses	2.5 Amp (main), 10 Amp (power supply card)
13.	Display	4 × 40 LCD character alpha-numeric
14.	Operating Environment	25°C Ambient
15.	Equipment Life	10 years, under normal conditions
16.	Hot Bonnet Lid Temperature	105°C

Table 1: Features and specifications of MERADD PCR Machine Amplicon PTC-15.

which covers making or fabrication of material things of all types/sizes at all scales. It has direct impact on life style and modern facilities available to citizens of a state. Briefly, it is considered backbone of a state. Pakistan is a developing country and has limited infrastructure for manufacturing modern equipment. MERADD is a small factory (of the PAEC) where medical equipments are developed for the research and clinical purposes. MERADD members have multiple duties in MERADD groups. MERADD is architectured as design group, materials selection group, purchasing group, process planning group, marketing group and customer services group.

MERADD is manufacturing PCR machines for the last 15 years. Consumption of MERADD PCR machines is in local universities and research institutes. Warrant period of the produced PCR machines is one year while maintenance to the clients is provided at cheaper rates for any period of the use. Our engineers/technicians visit the universities and institutes around the country for installation and maintenance. Manufactured PCR machines have both mechanical and electronic parts. Machines used in mechanical parts include CNC machines, press machines, dye moulding systems and Lathe machines. Electronic parts are fabricated using P-Cad designing, PCB manufacturing, soldering machines and screen printing machines. Figure 3 shows MERADD PCR machine PTC-15, (left) in open condition and (right) in ready to use condition.

Quality control

MERADD has developed a quality control procedure for manufactured PCR machines. One person from the MERADD serves as a quality inspector. All the manufactured parts are inspected through naked eye and magnifying glass to detect any defect or fault. Only fault free components are selected for the manufacturing purpose. Quality control guidelines have been prepared and are followed strictly. Components found defective during inspection are corrected or rejected depending upon the nature of the defect.

Production and cost analysis

Now, MERADD PCR machines are in active use in Pakistani universities and institutes around the country. Table 2 gives the yearly detail of PCR machine sales. On the average we are selling 8 PCR machines per year. Prices of international and MERADD PCR machines are given in Table 3. Machine models, manufacture name, and manufacture countries are also given. MERADD PCR machines have price lower than 40% of the relevant imported machines. The imported machines become further costlier due to higher transportation charges for the imported machines.

Performance and Results

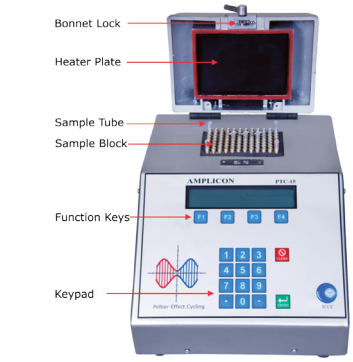
There are two types of optimization of MERADD PCR machines. One is done in the Optimization/Testing Laboratory. Other is in the user laboratory according to the experimental conditions of the user. In optimization of a PCR machine, different functions of the PCR machines are tested and optimized. One important step is temperature calibration of the PCR machine before delivery to a client. This calibration is shown in Figure 4 for typical MERADD PCR machine. This is done with the help of the temperature calibrator Alpha Technics 4500. Calibration curve/line is a relationship between set temperatures and corresponding achieved temperature or actual temperature of the

Year	No. of MERADD Machines Sold
2010-12	21
2013	11
2014	9
2015	10
2016	6

Table 2: Year wise sales of MERADD PCR machines.

S. No.	PCR Machine Model	Manufacturer	Price (US$)
1.	ABI9700, 96-well	Applied Biosystems, USA	5500/-
2.	T100, 96-well	Bio-Rad, USA	5000/-
3.	AMLICON (PTC-15), 96-well	MERADD, ICCC, Pakistan	2070/-

Table 3: Price comparison of MERADD PCR machine with imported PCR machines.



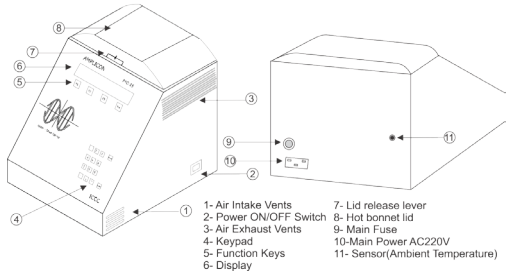


Figure 3: MERADD PCR machine picture of the model PTC-15 (top) and its drawings (bottom). Left bottom is front view while right bottom is back view with explanation of different keys and indicators.

PCR machine. The 2nd optimization is done by the user in his/her laboratory under the conditions of the planned experiment.

PCR machine amplifies a set of a specific DNA segments present in the DNA solution used in the machine. Under ideal conditions, after each cycle, the number of target DNA fragments is doubled. PCR amplification of DNA fragments is shown in Figure 5. The rise of DNA fragment amount shows exponential rise. In the subset of Figure 5, the same amplification rate is plotted on the logarithmic scale for 35 cycles. Panel A in Figure 6 show the comparative amplifications of MERADD (ICCC) and Bio-Rad PCR machines (A) while Panel B compares the amplificationsof ABI2700 and MERADD PCR machines (B).

Conclusions and Future Direction

Development and manufacturing PCR machines by the PAEC, Pakistan have been reported. Performance of MERADD PCR machines is as good as that of imported machines, but price of MERADD PCR machines is less than 40% of the imported machines. Higher import charges increase the price of the imported machines in comparison with MERADD PCR machines. In case of imported machines, repair and maintenance charges are very high in comparison with MERADD PCR machines. MERADD provides quick and cheap repair and maintenance to its clients throughout the country. Conventional PCR machines as described above provide only qualitative DNA amplification results. R&D on the advanced PCR machine, called Real Time PCR, is underway at MERADD. Real Time PCR machines give the quantitative results of DNA amplification. It is expected that R&D phase of Real Time PCR machine will be complete in two years of period.

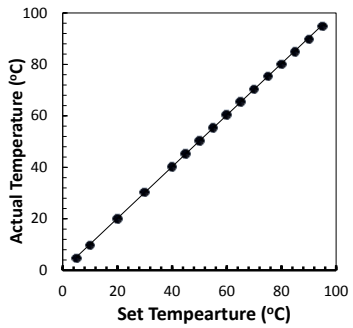


Figure 4: Temperature calibration of the MERADD PCR machine using Alpha Technics 4500 (Oceanside, US) temperature calibrator.

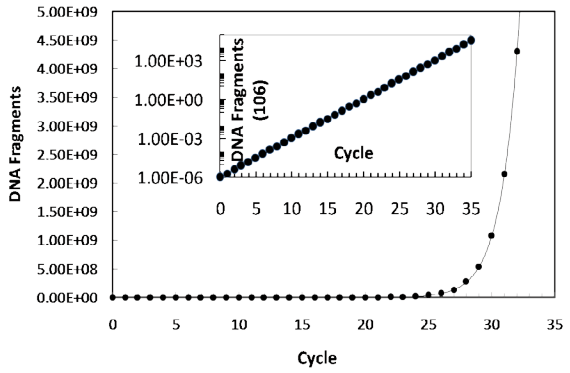


Figure 5: Amplification of PCR under ideal conditions. The inset shows the same plot on logarithmic scale.

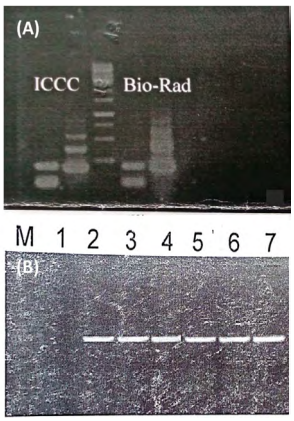


Figure 6: Comparison of amplification Agarose gel results of MERADD and Bio-Rad PCR machines (A) and comparison of amplifications of ABI2700 and MERADD PCR machines (B) using 100 bp DNA ladder. Lane in 1 lower panel (B) is -ve PCR sample, lanes 2-4 are positive PCR samples with ABI2700 and lanes 5-7 are positive PCR samples with MERADD PCR machine.

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