

A programmable \$25 thermal cycler for PCR
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ABSTRACT

This article describes the construction of a safe, programmable automatic thermal cycler for PCR which can be easily constructed by persons with basic soldering and mechanical skills for under \$25 in parts and a modest computer such as IBM 486, all of which are readily available. The cycler relies on the heating provided by an incandescent light bulb and cooling by simple convection.

1. Introduction

Because PCR [1] is a fundamental technique in the life science laboratory with numerous applications in other fields, secondary and university educational institutions are strongly motivated to include PCR in their laboratory programs. Furthermore, applications of PCR in the diagnosis and prevention of both heritable and communicable diseases have made the technique indispensable in the clinical and research labs of developing countries. In either case, unfortunately, the cost of commercially available thermal cyclers (\$2000-20,000) is prohibitive to inclusion of PCR in routine laboratory practice. Educators wishing to perform PCR have been encouraged to share equipment among school districts or manually shuttle reaction tubes 90-100 times among water baths set for the appropriate temperatures [2,3,4] as was done in the original method [1].

Our laboratory previously reported a fully automatic two-step thermal cycler that is sufficiently simple, safe, and inexpensive that it can be constructed and incorporated into any laboratory [5]. That design was limited in that the cycling temperature profile was adjustable over only a narrow range, due to the characteristics of the thermostat used. In this paper we describe a fully programmable thermal cycler that incorporates the heating/cooling portion of the earlier design but interfaces it through an A/D converter circuit to a low-end computer.

2. Experimental

Heating/cooling chamber

This programmable thermal cycler relies on the heating of an incandescent light bulb and simple convective cooling. Any cylindrical container that can withstand the temperatures involved can be used. In our prototype we used a stainless steel pot, purchased at a local discount housewares shop for \$8. A series of 8mm diameter holes was drilled equidistant from the center of the pot, as shown in figure 1. Our chamber has a diameter of 22cm, easily accommodating 30 sample wells. As long as the sample

chamber is cylindrical so that all sample tubes are equidistant from the heat source, larger chambers and sample sizes are easily accomplished.

Temperature control

The decision to switch the light bulb on (heating) or off (cooling) is accomplished by wiring the bulb to an A/D converter in a simple circuit; reaction temperature is monitored by the thermistor fixed in a reaction tube in the sample chamber (figure 2).

In our prototype, control of the parameters of the cycling program is accomplished through an interface with the parallel port of an IBM PS/2 (Intel 486, 8 M of RAM), based on the Parapin libraries under Linux. The source code, written in C, would be straightforward to port to other parallel port libraries. Two of the pins of the parallel port are dedicated to sending clock and clear information to the A/D converter, while another pin is used to read the resulting output bits. The source code is available via the internet at: (<http://web.bryant.edu/~bblais/cycler>).

Calibration

Before running a temperature cycling program, it is necessary to calibrate the cycler. A calibration program in the software enables the user to switch the heating "on" until the sample tubes reach the desired denaturation temperature, measured with an independent thermocouple inserted into a dummy reaction mix (typically 50ul water, 30ul mineral oil) in a sample tube in the chamber. At this point the user inputs the measured temperature into the computer. The user then switches the heating "off" until the temperature drops to the desired annealing temperature, and again inputs the actual temperature at that point. In this way, input from the thermistor via the A/D circuit is exactly correlated with actual temperature data measured in a real sample tube.

Running

After calibrating the unit and loading the samples, the user inputs denaturation temperature and time, annealing temperature and time, and extension temperature and time, and number of cycles, into the running program. The cycler will automatically move the samples among the programmed times and temperatures, providing screen data on actual temperature and number of cycles elapsed, and shut off at the conclusion of the program.

3. Results and discussion

Temperature variations among sample positions in the chamber, and temperature variations among repetitive cycles, are both within 1.0°C. Heating and cooling rates depend on the power of the light source, size of the chamber, and ambient conditions but are typically in the ranges 0.5-1°C/sec heating and 0.2-0.3°C/sec cooling. For our prototype, heating occurred at 1°C/sec and cooling at 0.25°C/sec.

Figure 3 shows the results of PCR amplifications performed with this

inexpensive cycler compared with the same reactions performed in an older commercial cycler (Perkin Elmer Model 480). The results are identical except for lanes 1,2 in which the PCR products are the same size (500bp) but lane 2 shows an artifact of overloading. Clearly, our cycler performs adequately in actual PCR experiments.

Teachers and scientists with severe budget restrictions need not do without PCR in their laboratory nor must they resort to manual shuttling of reaction tubes among water baths. This simple thermal cycler is inexpensive and easy to build and use yet performs quite adequately in routine amplifications.

References

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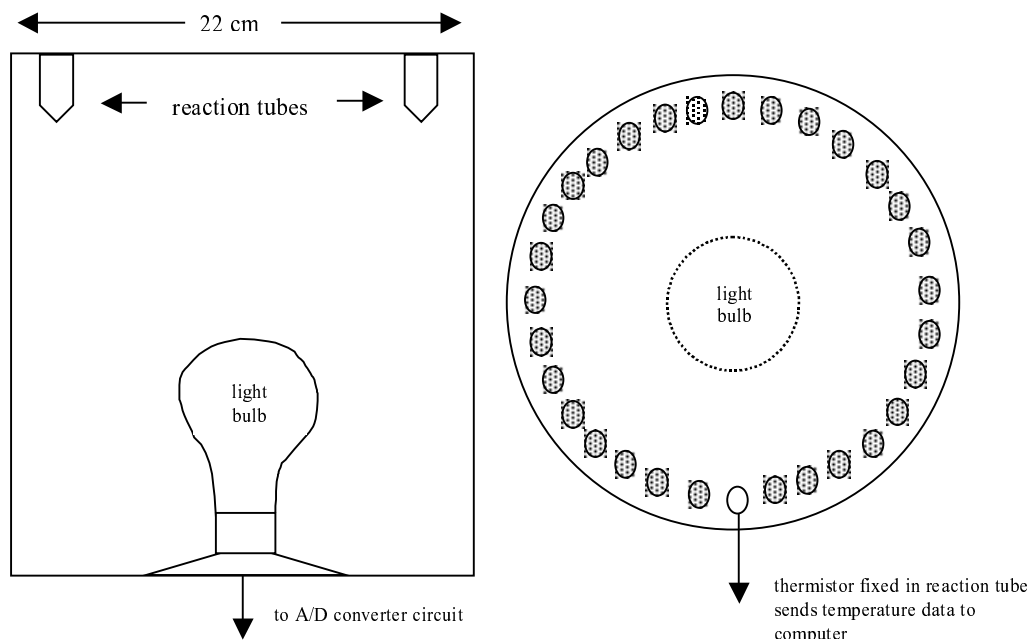


Figure 1. Diagram showing heating chamber with heat source (incandescent light bulb) and relative positions of reaction tubes.

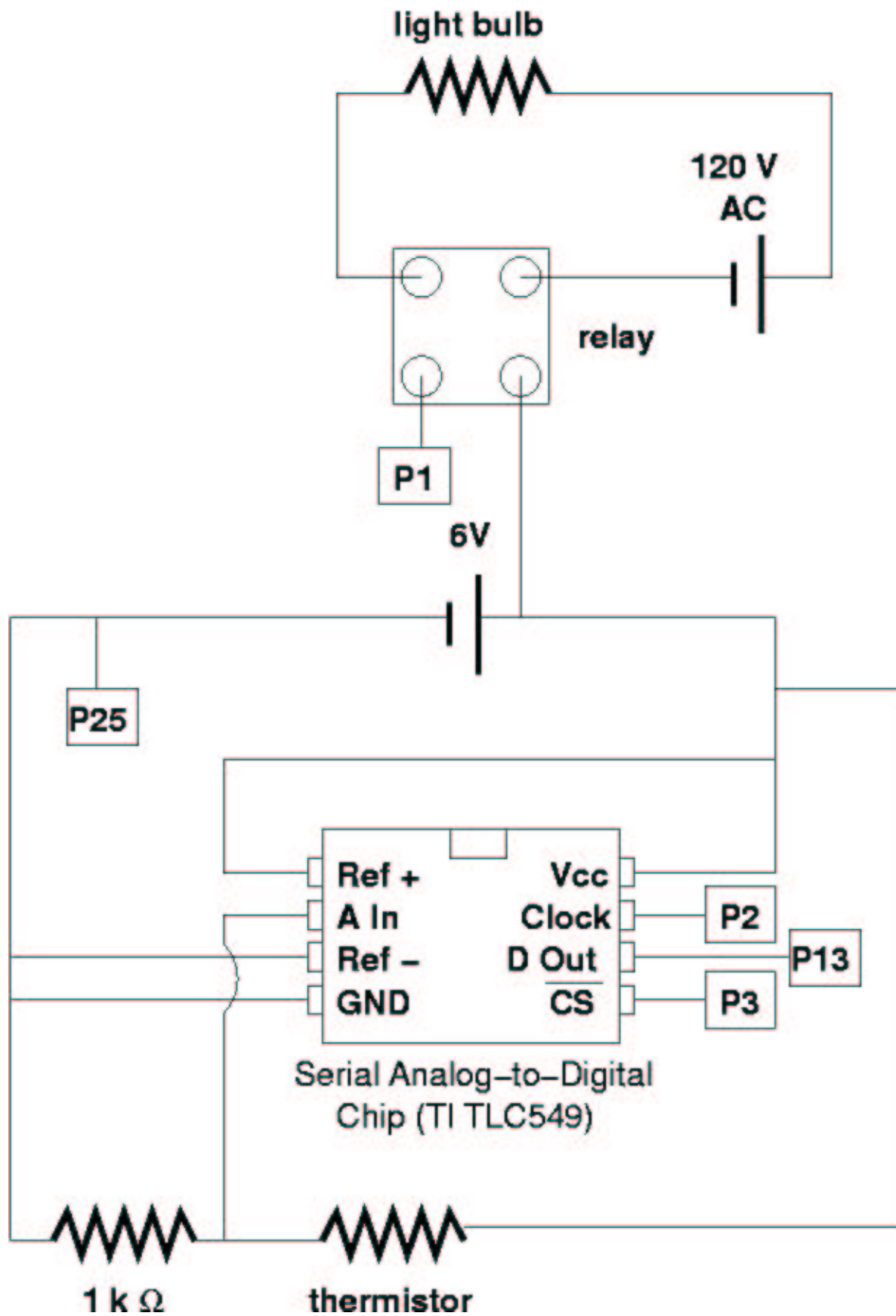


Figure 2. Schematic of A/D circuit interfacing heating chamber with computer.

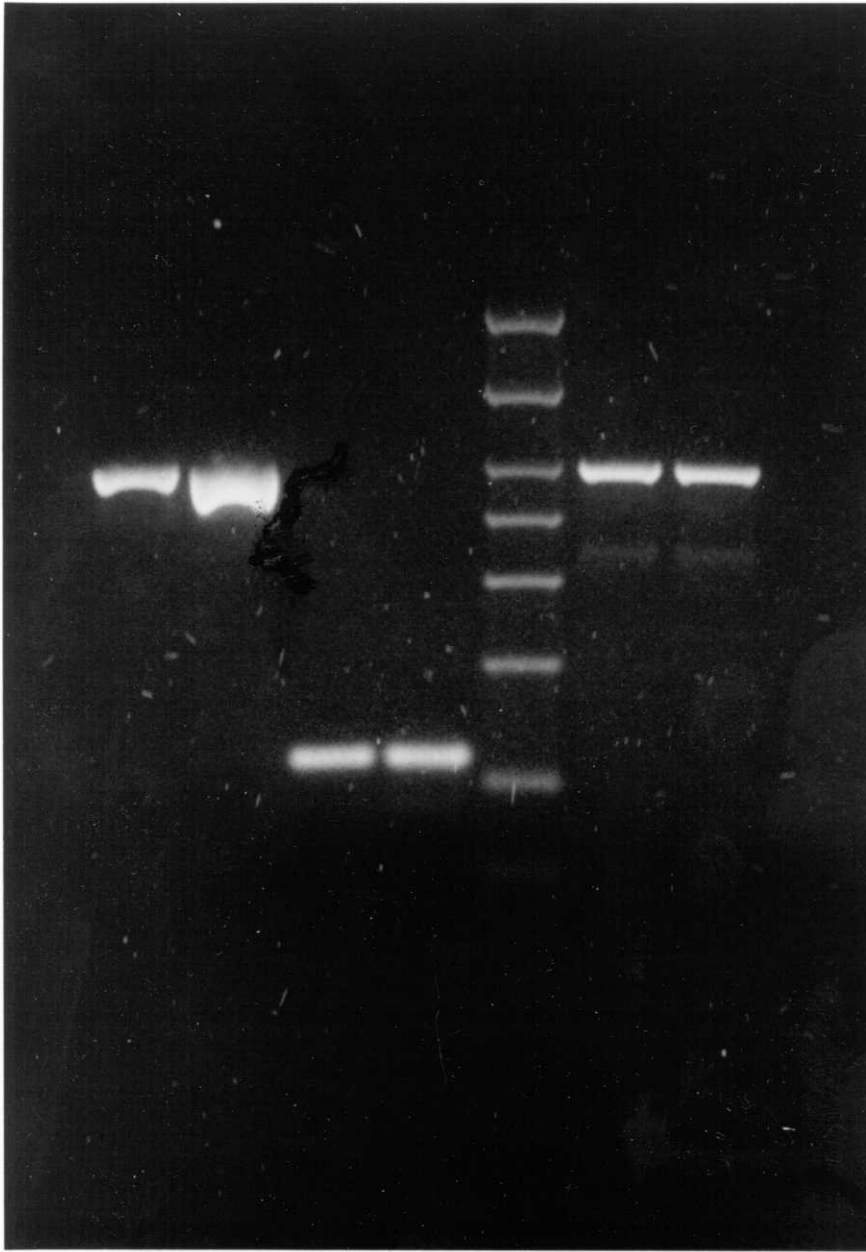


Figure 3. Agarose gel electrophoresis results comparing our prototype with an older Perkin Elmer thermal cycler, model 480. Identical samples containing 5pg of target DNA were run for 30 cycles in each comparison. Each sample lane shows 10ul loaded from a 50ul PCR reaction. DNA is stained with ethidium bromide. Lanes 1,2 – amplification of 500bp fragment from Lambda DNA. Lanes 3,4 – amplification of 110 bp fragment from pUC 19. Lanes 6,7 – amplification of 500bp fragment from Tet gene of pBR322. Lanes 1,3,6 – our \$25 cycler. Lanes 2,4,7 – the Perkin Elmer cycler. Lane 5 – size references 1000, 700, 500, 400, 300, 200, 100, and 50 bp.