Temperature Recording from Thermocyclers Used for PCR

ABSTRACT

Using a simple electronic circuit, a thermocouple can be connected to a chart recorder to measure the actual temperature inside a PCR tube. This allows accurate inspection of the thermocycle program and comparison between thermoprofiles from different thermocyclers. We found that the recording of temperature cycling enabled us to obtain more reliable and reproducible results.

INTRODUCTION

PCR is now a widely used method by which specific DNA fragments located within a high background of DNA sequences can be amplified (6). The reaction requires denaturing of DNA at a high temperature, followed by the annealing of primers to each strand at a lower temperature. The temperature is then adjusted for the actual polymerase reaction which takes place at 72°C. Both commercial and self-made thermocyclers (3) have been created which enable the necessary temperature changes to take place. However, PCR is highly sensitive to slight changes in the parameters of the reaction, including buffer ingredients, primer concentrations and temperature profile. In order to ensure the accuracy of the temperature during each phase of the reaction, one can use a device external to the thermocycler which will measure the temperature during each step of the process.

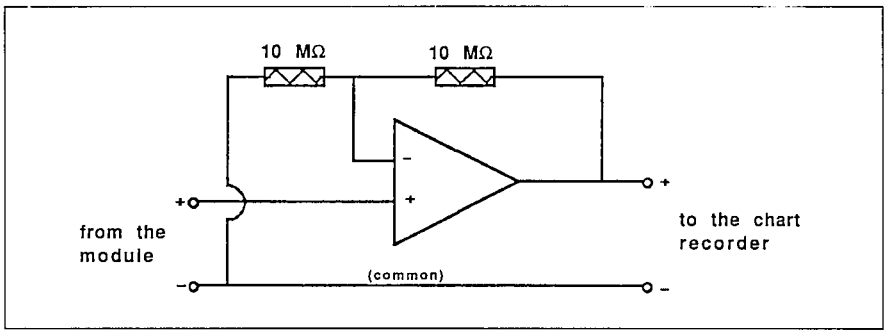


Figure 1. Circuit that serves as a resistance buffer between the thermocouple module and the chart recorder. Standard electronic symbols were used. This circuit multiplies the voltage signal from the module twofold.

This report will discuss a device created to measure temperature within the PCR tube containing the buffer and record the temperature profile on a chart recorder.

MATERIALS AND METHODS

Temperature Measurement

Temperature was measured with commercially available type K thermocouples. A thermocouple consists of two dissimilar metals joined together at a junction that produces a small thermoelectric voltage that is temperature dependent. The thermocouple has the advantage of fast response and low heat capacity. For comparison, four different thermocouples were tested, a commercially available thermocouple probe for thermocyclers PC0705 (USA Scientific Plastics, Ocala, FL), a hypodermic probe (Omega, Stamford, CT) and an uncoated and epoxy-coated general-purpose bead thermocouple (Fluke Manufacturing, Everett, WA). To prevent corrosion, the general-purpose probe was coated with epoxy according to manufacturer's protocol (Devcon, Danvers, MA; available in hardware stores). The hypodermic probe and the bead probes were put into a 50-µl PCR reaction mixture (16.6 mM $(NH_4)_2SO_4$, 200 µM deoxynucleoside triphosphates [dNTPs], 67 mM Tris-HCl, pH 8.8, 170 µg/ml bovine serum albumin, 3.5 mM $MgCl_2$) and overlaid with mineral oil in a 0.5-ml microcentrifuge tube. The small thermoelectric voltage generated by the probes was amplified to 1 mV/°C using an 80 TK thermocouple module (Fluke Manufacturing). The output of the module can be given directly to a voltmeter having a high input impedance, thus allowing direct temperature measurement (for example, Fluke handmeter 8024 B). Prior to use, the thermoprobes were tested for accuracy in water baths of 0°C, 45°C and 65°C. The errors were found to be less than 1% of the reading, which was within the manufacturer's specification of ±1°C. For all subsequent experiments, we used only the PC0705 probe to ensure comparability of the temperature measurements.

Temperature Recording on a Chart Recorder

Like other thermocouple modules, the 80 TK module has an output impedance of 10 MΩ. However, the input impedance of standard laboratory chart recorders is 1 MΩ. Connecting the module output directly to the chart recorder input results in shorting the output signal from the module. To overcome this problem, a simple, low-cost electronic circuit was added. This circuit acts as a resistance buffer and allows the module to be connected to the chart recorder (Figure 1).

The circuit consists only of a standard field effective transistor (FET)-input high-impedance (>10^{12} Ω) operational amplifier (Burr-Brown Research Corporation, Tucson, AZ) and two 10-MΩ resistors (1% accuracy) mounted on a dual IC board (Radio

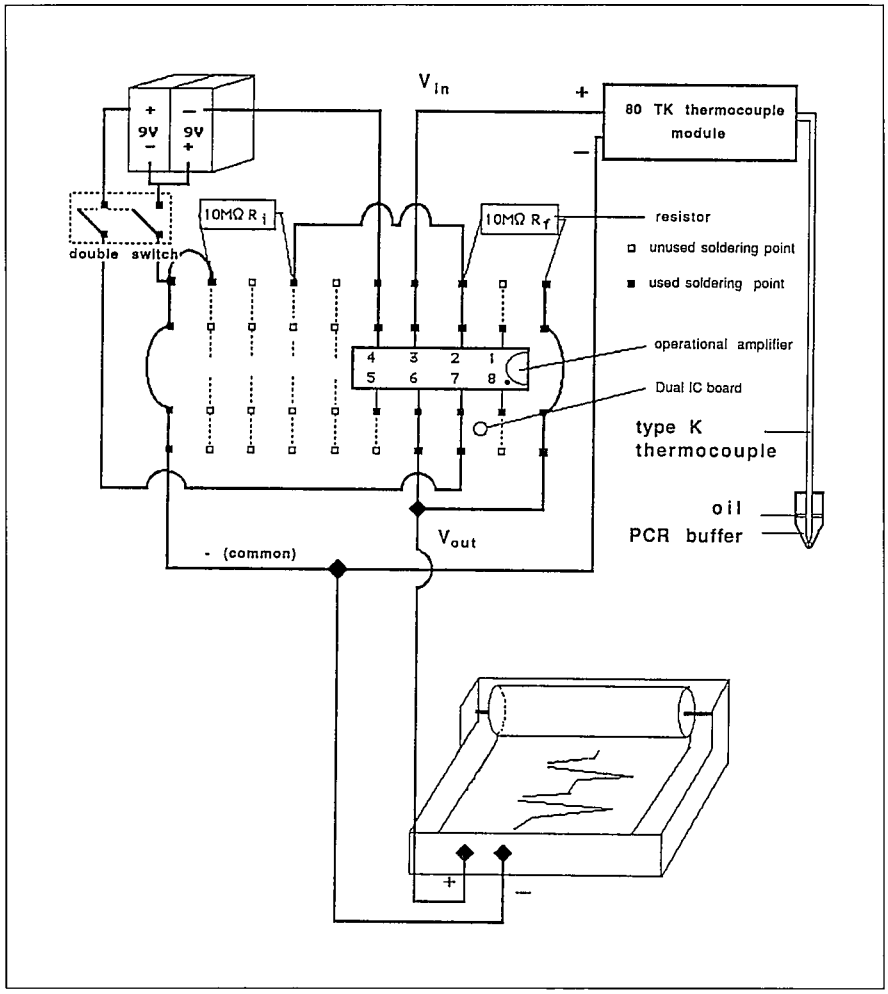


Figure 2. Detailed description of the resistance buffer circuit and the experimental design. The position of the socket for an operational amplifier is indicated. Dashed lines are unused connections; solid lines are used connections and wires. Drawing is not to scale.

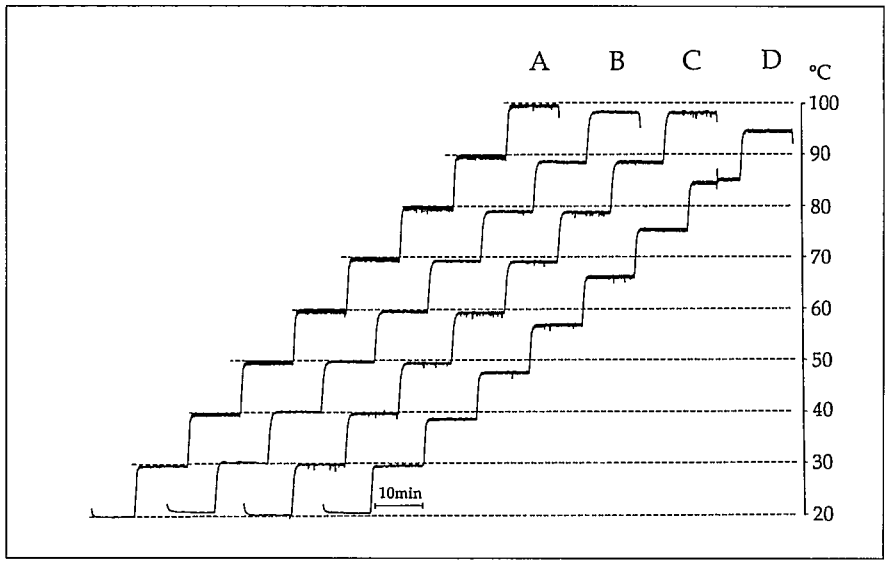
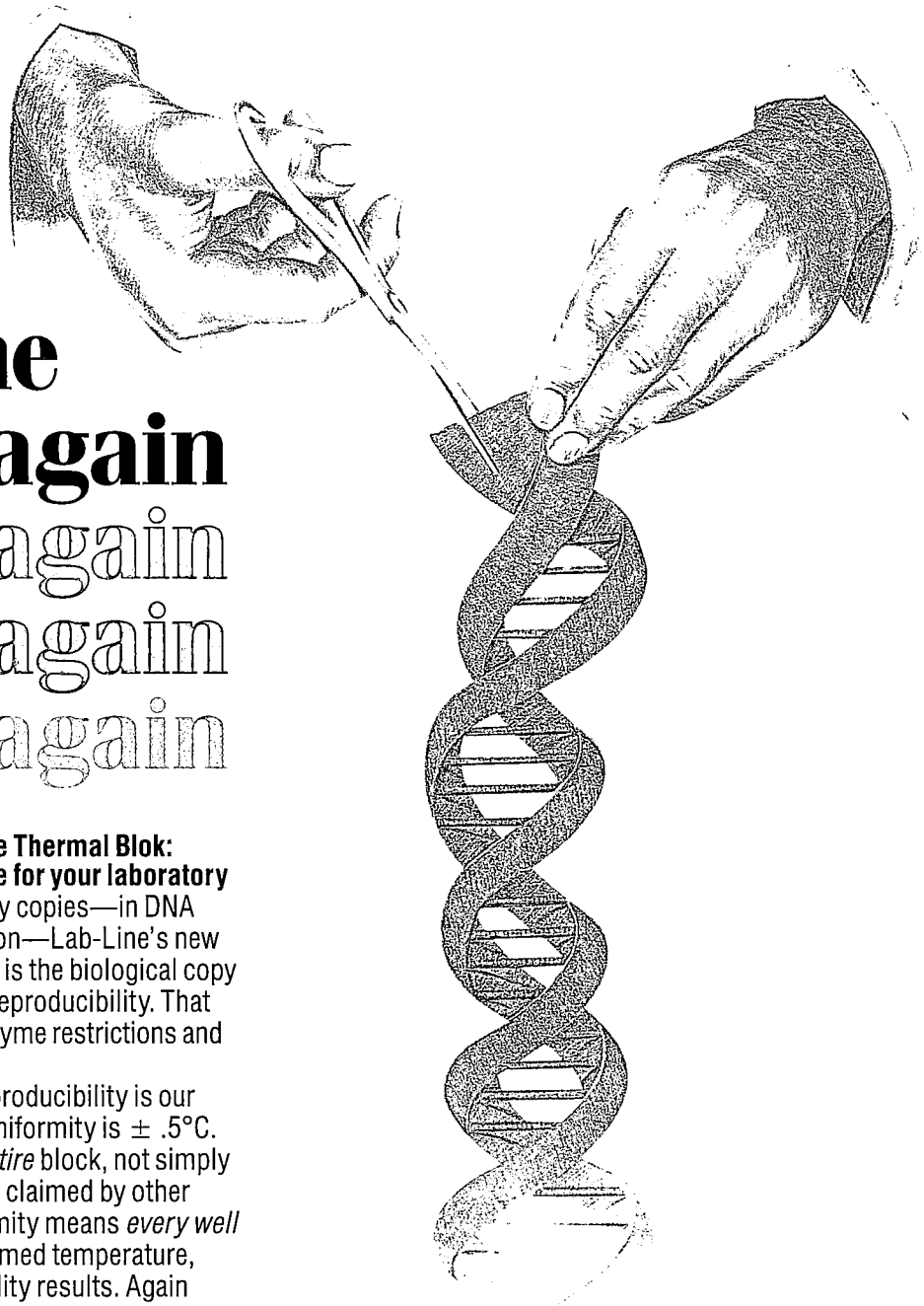


Figure 3. Response of different thermocouples to a step profile. A thermocycler was programmed for 10 min at 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C. This step profile was recorded with different probes. Only probes with low heat capacity gave accurate readings. A: PC0705; B: coated bead thermocouple; C: uncoated bead thermocouple; D: hypodermic probe.



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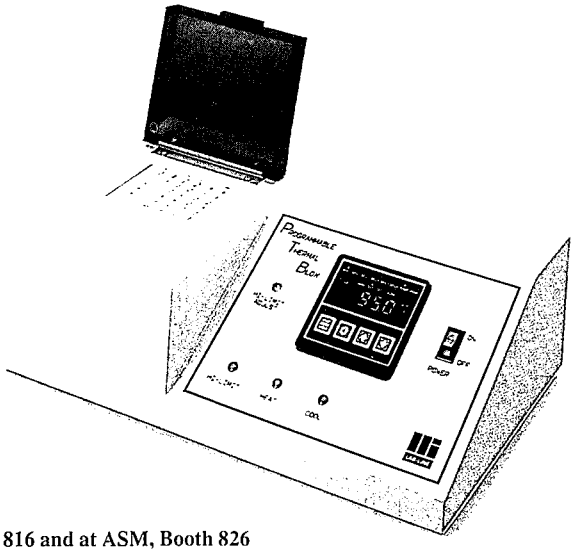

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Shack, Catalog No. 276-159A). Since the circuit resistors are matched with the module output resistance, there is no need to trim the voltage offset. This makes the circuit extremely simple. In the proposed circuit, the signal output from the module (1 mV/°C) is multiplied twofold; thus the chart recorder reads 2 mV/°C. The electronic circuit is simple and can be easily built, even by inexperienced individuals. Figure 2 shows all the circuit connections in detail. Except for the operational amplifier, all components can be purchased in any electronics supply store.

RESULTS AND DISCUSION

Influence of Different Thermocouples on the Measurement of Temperature Profiles

To investigate the influence of a particular thermocouple probe on the recording of temperature, the temperature was measured in a step profile (10 min at 30°C, 40°C, 50°C...100°C) with four different thermocouples (Figure 3). In the step profile, long soak times (10 min) were programmed to ensure that the machine reached the required temperature. The temperature recording attained with the thermocouple probe PC0705 coincided best with the programmed profile. The bead probes gave less accurate measurements, with the coated and uncoated thermocouple bead probes yielding the same results. However, at temperatures higher than 70°C, where the probes were not fully submerged in a thermocycler (machine I, Figure 3), these probes gave readings that were lower than the programmed temperature (-1°C to -1.5°C between 70°C and 90°C, -2°C at 100°C). In contrast, in machine II where the probes were fully submerged in the heating block, the readings of the bead probe were identical to the PC0705 thermocouple probe (data not shown). The hypodermic probe also gave lower readings, starting from -1.5°C at 40°C to -5°C at 100°C when the probe was not submerged (Figure 3) and readings 1°–2°C too low when the probes were submerged. The deviations in temperature were probably due to the high heat capacities of the bead and hypodermic probe. Thus, when constructing a thermocouple probe for measurement of the PCR tube temperature, it is important to ensure that the heat capacity of the probe used is as low as possible.

Table 1. Description of the Temperature Profiles Used

#	Machine	Soak 94°C	Ramp 94°C→55°C	Soak 55°C	Ramp 55°C→72°C	Soak 72°C	Ramp 72°C→94°C
A	I	30	78	60	34	120	44
B	I	30	78	30	34	90	44
C	II	30	30	30	30	90	60
D	II	30	78	60	34	120	44
E	II	30	30	60	30	120	60

The different ramp and soak times are indicated in seconds. Note that profiles A and D should be identical, but were generated on different machines.

Influence of Different Temperature Profiles on PCR Products

The amplification of rat BC1 DNA from plasmid DNA was chosen as an example of how different temperature profiles influence the PCR products. We used the same PCR mixture for each reaction under different thermoprofile conditions. The mixture contained 16.6 mM $(NH_4)_2SO_4$, 200 µM dNTPs, 170 µg/ml bovine serum albumin, 67 mM Tris-HCl, pH 8.8, 3.5 mM $MgCl_2$, 1 U AmpliTaq® DNA Polymerase (Perkin-Elmer Cetus, Norwalk, CT) and 1 ng plasmid (pΔBF1) containing the target DNA. The primers spanning the rat BC1 RNA gene (1) were SS021 5′-GGGGTTGGGGA TTTAGC-3′ and SS022 5′-AAAGG TTGTGTGTGCCAG-3′. The melting points of these oligonucleotides under

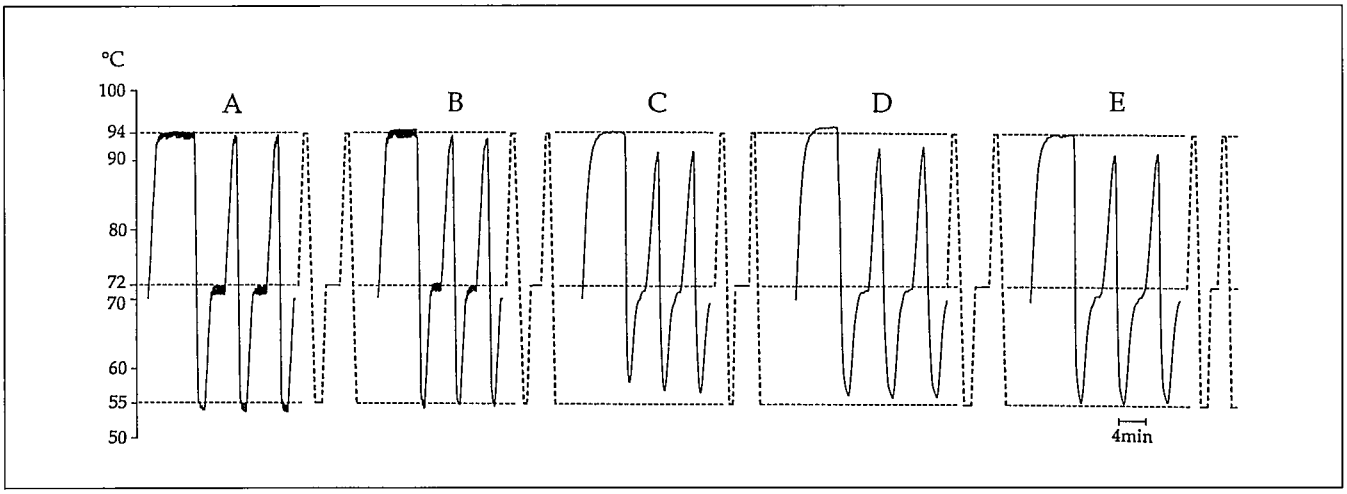

Figure 4. Chart recording of the temperature profiles. Two cycles are shown. In front of the first cycle is a 4-min 94°C step, showing that both machines do not reach 94°C during the regular cycles. After the second cycle, the 'theoretical,' programmed cycle is drawn in (dashed), assuming a linear temperature response during ramping. The profile was recorded using the PC0705 probe. The profiles are described in Table 1; products generated with them are shown in Figure 5.

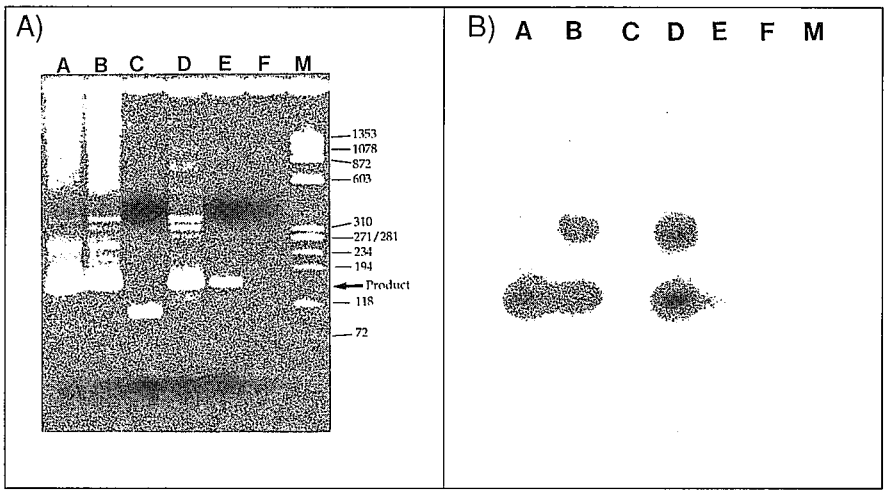

Figure 5A. Ethidium bromide staining of the PCR products generated from identical PCR mixtures with the different profiles. 1/5 of the total reaction was loaded on a 2% SeaKem (FMC BioProducts, Rockland, ME) gel in TBE. A–E: reactions were done using temperature profiles A–E, respectively. F: negative control with no plasmid DNA. M: ϕX174 RF DNA, *Hae*III digest (New England Biolabs, Beverly, MA). The expected size of the PCR product is 152 bp. Only profile E gives a clean product of the expected size. **Figure 5B. Southern blot of the gel in 5A.** The DNA was electroblotted onto Genescreen® (Du Pont, Wilmington, DE) and probed with an internal ^{32}P-end labeled probe (RB010) according to the manufacturer's protocol.

similar salt conditions (50 mM KCl, 1.5 mM $McCl_2$, 0.5% Triton X-100, 0.1% gelatin) were 54°C for SS021 and 52°C for SS022, based on the formula of McGraw et al. (5). The internal oligonucleotide used for Southern blotting was RB010 5′-GGTTCGGTCCT CAGCTCCGA-3′. Five different profiles on two machines from different manufacturers gave us different products (Table 1; Figures 4 and 5). It is interesting that in all cases the temperature profile measured in the PCR mixture of a 500-µl microcentrifuge tube was different from the theoretically programmed profile. Most notably, during the cycles both machines did not reach 94°C. Furthermore, theoretically identical profiles (A and D) on machines of different manufacturers gave different temperature profiles in the test tube and different by-products. Only profile E gave a clean product; profiles A, B and D gave too many by-products due to mispriming. Profile C gave no signal in the Southern blot (Figure 5B). The band appearing in the ethidium bromide-stained gel is thus most likely a primer artifact. Different positions in machine I gave identical profiles. Machine II differed between corner and center positions by maximally 2°C. However, holes next to each other showed identical profiles. Thus, we used the same spot in machine II for our experiments.

The above results indicate that the sensitive nature of PCR requires the temperature profile be controlled by an external source. In addition, it is also important to routinely record temperature profiles from thermocyclers in order to verify their proper function. The Peltier junctions used in most machines tend to get worn down, resulting in improper temperature levels. Proper function is crucial since identical temperature profiles are necessary to reproduce the results of a particular PCR.

While this manuscript was under review, two other papers reporting problems with the reliability of thermocyclers were published (2,4). This additionally underlines the importance of an external temperature control for thermocyclers.

CONCLUSION

Temperature profiles have an important influence on PCR products and by-products. Thermocyclers are not always reliable concerning the programmed temperature profiles. Thus, recording of temperature profiles might be important to reliably reproduce PCR runs within the same and among different laboratories. We describe here a simple and affordable way to achieve this goal by any PCR users.

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