Shortened PCR Cycles in a Conventional Thermal Cycler


The demand created by the wide and expanding range of applications for polymerase chain reaction (PCR) is straining the capacity of existing thermal cyclers in many research settings. This situation motivated us to reevaluate the necessity of the duration prescribed by conventional PCR protocols for each of the three steps in a PCR cycle. Typically, denaturation and annealing range from 20 to 90 s, and extension times are often several minutes long. A 30-cycle PCR can take 4 h or longer.

Recently, new techniques have been developed to amplify DNA within 1 h or less. These techniques require specialized thermal cyclers, designed to increase the rate of thermal equilibration (3,4). Primer extension by a thermostable polymerase, at the high temperatures at which PCR occurs, proceeds at the rate of 35–100 bp/s (1). Primer annealing, driven by a large primer excess, also requires less than 1 s (2,3). The only purpose for prolonging PCR steps to more than a few seconds would be to allow thermal equilibration to occur from the heat source (or sink) to the heating block, to the tube wall and to the reaction solution. It has been assumed that shortening the time of PCR cycles over those conventionally used would require a specialized PCR apparatus and tubes (3,4). It is not clear how rigorously this assumption has been tested. For this reason, we examined the limits to which the duration of PCR steps could be shortened in two conventional block-type thermal cyclers, DNA Thermal Cycler 480 (Perkin-Elmer, Norwalk, CT, USA) and PTC-100™ Thermal Cycler (MJ Research, Watertown, MA, USA). We were able to drastically reduce the duration of all steps in the PCR amplification of a number of different templates with no loss of quality or yield. We describe the procedure and its validation.

We used both genomic and first-strand cDNA as reaction templates. The reaction was performed in 0.5-mL tubes of regular wall thickness. Mineral oil was layered over the reaction to prevent evaporation and between the tube and the block well to promote heat transfer. The total reaction volume was 25 µL, containing 2.5 µL 10X PCR buffer, 0.5 µL of 40 mM MgCl₂, 0.5 µL of 10 mM dNTP. 10 pmol of each primer and 0.75 U of one of three polymerases: Taq (Life Technologies, Gaithersburg, MD, USA), ID-zyme™ or ID-pol™ (ID Labs, London, ON, Canada). Reaction buffers came from the corresponding suppliers. We started with denaturation for 2 min at 94°C, followed by 30–35 cycles at 94°C for 2 s, 55–60°C for 5 s and 72°C for 8 s. No ramp times were programmed. Block cooling proceeded at a mean rate of 1.00%/s and heating at 0.43%/s in the Thermal Cycler 480, and a mean rate of

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0.64°/s and heating at 0.69°/s in the PTC-100 Thermal Cycler. Thus, the transition from 94° to 55°C took 39 and 61 s, respectively, in each cycler, and the transition from 72° to 94°C took 51 and 32 s, respectively.

This cycling program was successful in amplifying most genomic and cDNA templates in both conventional cyclers. Amplification was consistently successful with short templates (<500 bp), whereas success with longer fragments (>1 kb) was less predictable. However, we were able to amplify a 1.2-kb fragment of the IGF2 gene (Figure 1B) without extensive optimization. Multiplex PCR worked without difficulty, even in one case in which our conventional protocol failed for the same combination of templates. Shortening the denaturation and annealing steps to 0–2 s and the extension step to 2–7 s did not significantly alter the results in most reactions.

With such short cycles, it can be assumed that the effective reaction temperatures did not reach their programmed annealing and denaturation value. We did not specifically correct for the difference between nominal and actual annealing temperatures, but successful PCR did not require more elaborate optimization, including slight annealing temperature adjustments, than did conventional protocols. An annealing temperature higher than that programmed might partly explain the fewer extraneous bands we obtained with the shorter cycles (Figure 1, E and F).

It is possible that the programmed denaturation temperature of 94°C was not attained. There is evidence that considerably lower denaturation temperatures are adequate (5). To confirm this, we modified a successful short-step protocol by trying lower denaturation temperatures while extending the time to 25 s to assure equilibration. The minimal denaturation temperatures that gave amplification were 86° and 88°C for the two templates evaluated.

Using these protocols, we were able to reduce total cycling time of most PCR amplifications done in our laboratory from 4–5 h to as little as 75 min. In addition to improved efficiency, another benefit is the economy in polymerase, because exposure to denaturing temperatures and consequent loss of activity is minimized. Shorter annealing has also been shown to minimize mispriming (3), an additional possible explanation for less extraneous bands (Figure 1, E and F).

We did not measure the difference between nominal and actual temperatures, because our only endpoint was successful amplification. Because these differences can vary between instruments, even those of the same make and model, we recommend that the protocol given here be used only as a starting point. Optimization for each specific apparatus and each template may be needed. However, our experience using five different Thermal Cycler 480s, indicates that any optimization required would be trivial.

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