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Room-temperature-stable PCR Reagents

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PCR has found immediate use in developmental DNA diagnostics procedures and in molecular cloning from genomic DNA.[1] Current protocols for PCR amplification require the dispensing and mixing of aqueous solutions stored at subambient temperatures. In addition, dispensing of such aqueous reagents involving enzymes, buffers, and nucleotides is time consuming. It also may lead to pipetting errors as well as an increased probability of carry over contamination of pristine PCR mixtures. Having considered the need for rapid testing and automation, we have developed an alternate approach to stabilizing PCR reaction mixtures in carbohydrate polymers. This process leads to the formation of glassy matrices that provide room-temperature stability (referred to as stabilized). This paper describes the utility of stabilized PCR reagents and subsets of PCR reagents containing buffered *Taq* DNA polymerase and/or nucleotides for routine PCR applications.

RESULTS AND DISCUSSION

The PCR reagent mixture, containing 2.5 units of *Taq* DNA polymerase (Perkin Elmer Cetus), 0.2 mM each of dNTPs (Pharmacia), and 1× standard PCR reaction buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin (Perkin Elmer Cetus)], was mixed with an equal volume of 20% (wt/vol) carbohydrate polymer and processed to form a stable, glassy matrix.[2] The stabilized PCR mixtures were stored at room temperature for extended periods of time and tested in PCR. Figure 1 shows the amplification of a pBR322 DNA template utilizing PCR reagents prepared from aqueous solutions (stored at −20°C) and stabilized reagents (stored at room temperature for 11 months). The stabilized reagent mixture was rehydrated with 100 μl of water containing the specific primers and DNA template. Equivalent amounts of PCR product were observed with fresh reagents and stabilized reagents (Fig. 1, lanes *1* and *2* vs. *3* and *4*, respectively).

The hot-start method of performing preamplification heating, and deliberately withholding a critical reaction component prior to PCR, has maximized specificity and minimized primer–dimer formations.[3,4] Bearing this application in mind, stabilization of subsets of PCR reagents was attempted by formulating

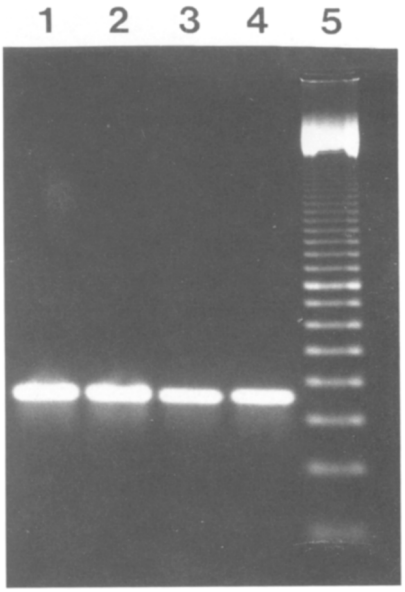

FIGURE 1 Amplification of pBR322 DNA (linearized with *Pvu*II, 1 ng) using 50 pmoles each of complementary primers SF4 (5′-GAT AAG CTT TAA TGC GGT AGT TTA TCA CAG-3′) and SF5 (5′- AGA GGA TCC ACA GGA CGG GTG TGG TCG CCA-3′). PCR was carried out on a Perkin-Elmer Cetus DNA thermal cycler (model 480) and was subjected to 30 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C. Ten microliters of the PCR reactions was analyzed by agarose gel electrophoresis in 1× TBE buffer containing ethidium bromide to visualize the 350-bp PCR product. (Lane *5*) Size marker (100 Base-Pair Ladder, Pharmacia); (lanes *1*, *2*) control, fresh PCR reagents; (lanes *3*, *4*) stabilized PCR reagent mixtures containing 2.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus), 0.2 mM each of dNTPs (Pharmacia), and 1× standard PCR reaction buffer (Perkin-Elmer Cetus), which were stored at room temperature for 11 months.

PCR mixtures containing 1× standard PCR reaction buffer plus dNTPs. The *Taq* DNA polymerase enzyme was not included so that it could be added before amplification. The buffered dNTP PCR subsets processed into stabilized mixtures were rehydrated with 100 μl of an aqueous solution containing fresh *Taq* DNA polymerase, primers, and DNA template. Equivalent amounts of PCR products were observed with fresh reagents and stabilized reagents (Fig. 2, lanes *2, 3, 8, 9,* vs. *6, 7, 12, 13,* respectively). Preparation of stabilized PCR subsets containing reaction buffer plus *Taq* DNA polymerase or *Taq* DNA polymerase plus dNTPs is feasible as well (data not shown).

This approach of stabilizing premixed

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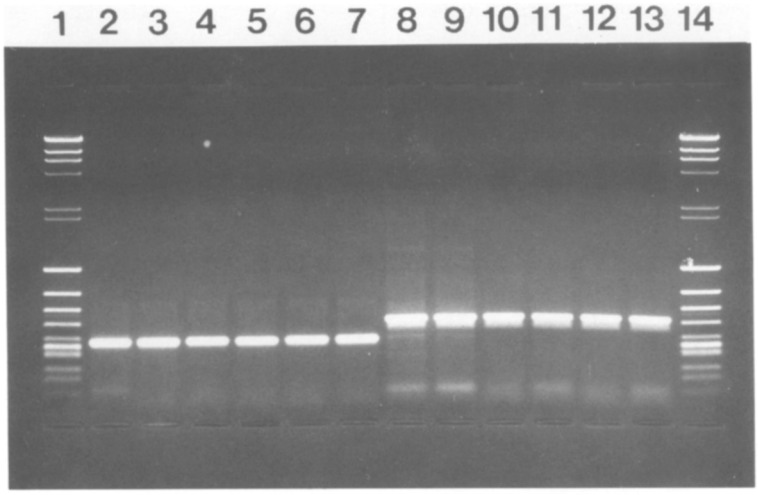

FIGURE 2 PCR-amplified DNA fragments separated on a 1.0% agarose, 1× TBE, gel with ethidium bromide staining. (Lanes *2–7*) pBR322/*Pvu*II template DNA (1 ng) was amplified with 50 pmoles each of primers SF4 and SF5 (see legend to Fig. 1) to generate a 350-bp product. (Lanes *8–13*) λ control DNA template (1 ng) from a GeneAmp PCR reagent kit (Perkin-Elmer Cetus) was amplified using the control primers 1/2 (100 pmoles each) to generate a 500-bp product. Control primer 1 5′-GAT GAG TTC GTG TCC GTA CAA CTG G-3′; Control primer 2 5′-GGT TAT CGA AAT CAG CCA CAG CGC C-3′. PCR reactions contained 1× standard PCR reaction buffer (Perkin-Elmer Cetus), 1 μl of DNA polymerization mix (0.2 mM each dNTPs, Pharmacia), 2.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus) per 100-μl reaction. (Lanes *1,14*) Size markers (λ *Hin*dIII/ϕχ174 *Hin*cII); (lanes *2,3,8,9*) control (fresh PCR reagents); (lanes *4,5,10,11*) stabilized PCR mixtures (*Taq* plus dNTPs plus 1× PCR buffer; stored 6 weeks at 22°C); (lanes *6,7,12,13*) are stabilized buffer plus dNTPs (stored 6 weeks at 22°C) plus fresh enzyme added.

PCR reagents and subsets of PCR reagents would be amenable to automation of routine PCR applications. Added advantages include elimination of pipetting errors and reduction of possible carry over cross contamination. These stabilized PCR reagents are ready to use and are stored at room temperature.

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