

Betaine and DMSO: Enhancing Agents for PCR



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To improve yield and specificity of difficult targets in PCR amplifications, researchers often include enhancing agents in the reaction. We discuss two commonly used PCR enhancing agents, betaine and dimethyl sulfoxide (DMSO), and the identification of betaine in commercially available additives.

INTRODUCTION

PCR is a powerful and extremely robust procedure for most applications and usually requires little optimization. However, there are instances in which a particular DNA region proves difficult to amplify by PCR. The amplification of targets rich in GC content or ones that can form secondary structure often result in little or no yield of expected product. Furthermore, amplification may result in products derived from regions other than the target DNA region, indicated by multiple bands on a stained agarose gel. Optimization of magnesium concentration, buffer pH, denaturing and annealing times and temperatures, and cycle number is useful in some, but not all cases. Targets that are refractory to amplification, despite optimization attempts, can often be amplified if the appropriate additive is included in the amplification mix.

A variety of additives and enhancing agents can be included in PCR amplifications to increase yield, specificity and consistency. Agents include: dimethyl sulfoxide (DMSO), N,N,N-trimethylglycine (betaine), formamide, glycerol, nonionic detergents, bovine serum albumin, polyethylene glycol and tetramethylammonium chloride. These additives have beneficial effects on some PCR amplifications; however, it is not possible to predict which agents might be useful for a particular target. There are reports of PCR amplifications in which specificity was improved by formamide, but not DMSO (1), and reactions in which DMSO was more effective than formamide at increasing yield and specificity (2). Several agents that facilitate product formation in PCR amplifications are now commercially available. These agents alter the melting characteristics of DNA. Their identities, however, are not revealed by the respective suppliers.

ENHANCING AGENTS: BETAINE AND DMSO

Two PCR enhancing agents that deserve particular attention are betaine and DMSO. DMSO is probably the most commonly used enhancing agent and is frequently included as part of a standard optimization of PCR amplifications. Betaine is another agent that has been used successfully for increasing yield and specificity of PCR products (3-6). [Figure 1](#) shows the structural formula of betaine. Both of these agents facilitate strand separation; DMSO disrupts base pairing whereas betaine, an isostabilizing agent, equalizes the contribution of GC- and AT-base pairing to the stability of the DNA duplex (7,8).

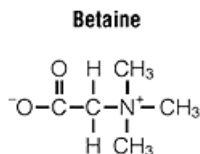


Figure 1. Structural formula of betaine (N,N,N-trimethylglycine).

The human retinoblastoma gene contains a region at its 5'-end that is approximately 75% GC. Bookstein *et al.* (9) reported that amplification of a 180bp fragment, spanning the exon-intron 1 junction, required the addition of 10% DMSO.

RESULTS

We compared the relative efficiencies of betaine and DMSO using the same template, primers and amplification conditions as reported in the Bookstein *et al.* study. As evident in [Figure 2](#), no specific product was produced without additive (lane 1). Although the expected product was seen in the presence of 5% DMSO (lane 2), there were a number of nonspecific bands. Specificity is increased when 1M betaine is added to the reaction mix (lane 3); however, the yield appears to be less than in reactions with DMSO. The combination of

5% DMSO and 1M betaine (lane 4) in the reaction mix shows no improvement with respect to yield or specificity over those reactions in which only 1M betaine is present.

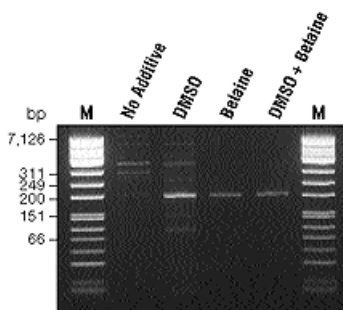


Figure 2. Amplification of a fragment of the human retinoblastoma gene with additives DMSO and betaine. PCR amplifications contained 500ng human genomic DNA, Promega 10X Reaction Buffer diluted to a 1X concentration (50mM KCl, 10mM Tris (pH 8.3), 1.5mM MgCl₂ and 0.1% Triton[®] X-100), 200μM each dNTP, 2 units of *Taq* DNA Polymerase* (Cat.# M1861) and 0.8μM of each primer in 50μl. The retinoblastoma exon 1 primer sequence is 5'-CAGGACAGCGGC-CCGGAG-3' and the intron 1 primer sequence is 5'-CTGCAGACGCTCCGCC-GT-3'. The cycling conditions included a two-minute initial denaturation step at 94°C followed by 30 cycles at 94°C for one minute and 50°C for one minute with a final extension step at 72°C for five minutes in a Perkin-Elmer[®] thermal cycler 480. Reactions containing no additives (lane 1), 5% DMSO (lane 2), 1M betaine (lane 3) and 1M betaine + 5% DMSO (lane 4) are shown. Markers (M) are PhiX174 DNA/*Hinf* I Markers (Cat.# G1751).

*The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

Amplification of a GC-rich region of the human insulin-like growth factor receptor II (IGFR II) gene also requires additives for successful amplification by PCR. A 510bp fragment of the IGFR II gene was cloned using the pGEM[®]-T Easy Vector System I^(a,b) (Cat.# A1360). **Figure 3** shows the effect of adding 1M betaine to the amplification reaction for this fragment compared to PCR additives of unknown composition from two different suppliers. In the absence of additives, there is no 510bp product when amplification is carried out using Promega's *Taq* DNA Polymerase (Cat.# M1861) and 10X Reaction Buffer (lane 1) or polymerases and buffers from the two other commercial sources, Supplier B (lane 3) and Supplier C (lane 5). Supplier C's buffer contains 5% DMSO. In the presence of either 1M betaine and 5% DMSO (lane 2), or the commercially-available PCR additives and 5% DMSO (lanes 4 and 6), the expected product is seen.

^(a)Licensed under one or both U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

^(b)U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

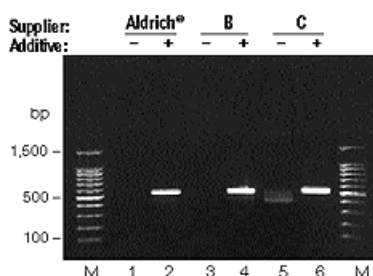


Figure 3. Amplification of a fragment of human IGFR II using betaine and two commercial PCR additives. PCR amplifications contained approximately 20ng plasmid DNA, 200μM each dNTP and 0.4μM of each primer. The buffer and polymerase were used as directed by the manufacturers (10,11). The final volume of the reactions was 50μl. The primer sequences are: 5'-TCCCGCTCCGTCTCCACCTCCGC-3' and 5'-ACAGGAAGGCAATGCTGCTCTGGA-3'. The cycling conditions were a one-minute denaturation step at 94°C followed by 30 cycles at 94°C for 30 seconds and 68°C for two

minutes with a final extension step at 68°C for five minutes in a Perkin-Elmer® thermal cycler 480. Reactions with no additives (lanes 1, 3 and 5), 1M betaine + 5% DMSO (lane 2), additive used as directed by the manufacturers (10,11) and 5% DMSO (lanes 4 and 6) are shown. The buffer provided by Supplier C contains 5% DMSO, so it was present in the reaction shown in lane 5, and no extra DMSO was added to the reaction in lane 6. Marker lanes (M) contain 100bp DNA Ladder (Cat.# G2101).

Since the IGFR II amplification reactions with either 1M betaine or the commercial additives are remarkably similar with respect to yield and specificity, the identity of the commercial additives was investigated. [Figure 4](#) shows the nuclear magnetic resonance (NMR) analysis of a solution of 5M betaine (Aldrich®; Cat.# 21,906-1) in water without pH adjustment ([Panel A](#)) compared to the two commercially available additives ([Panels B and C](#)). The positions of the peaks indicate that the three solutions are the same with respect to chemical composition; the sizes of the peaks suggest that the three solutions are present in approximately the same concentration. This analysis clearly demonstrates that the PCR-enhancing reagent from these suppliers is betaine.

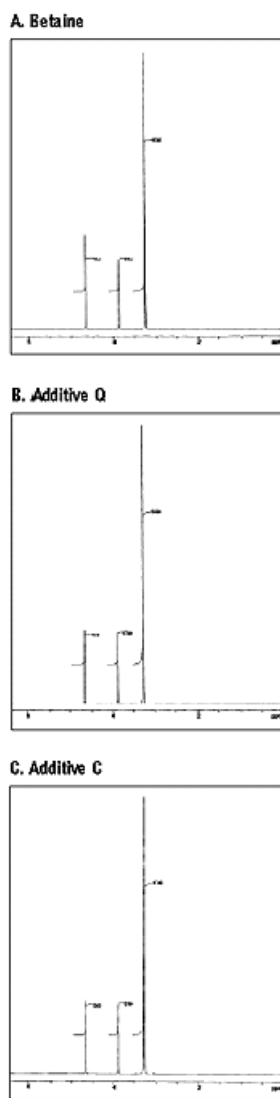


Figure 4. NMR analyses of betaine and two commercial PCR additives. [Panel A](#): NMR analysis of 5M betaine from Aldrich® (Cat.# 21,906-1) in water without pH adjustment. [Panel B](#): Additive provided with Supplier B's *Taq* PCR core kit. [Panel C](#): Additive provided with Supplier C's PCR kit.

CONCLUSIONS

In summary, there are instances in which standard PCR amplification conditions do not produce acceptable results. In those cases there are a number of additives that can be used to increase yield and specificity of a reaction. With the information presently available it is not possible to predict which enhancing agent is best for any particular target, so it may be necessary to test several different additives. Betaine and DMSO are two frequently used PCR additives that are effective separately or in combination. Both of these reagents are readily available from chemical supply companies. It should be noted that, although these agents can be useful for increasing efficiency

and specificity of PCR amplifications, their effects on the melting temperature of DNA may alter the optimal annealing conditions for a particular reaction. Therefore, it may be necessary to empirically determine the optimal annealing temperature if adding one or both of these agents to a particular amplification reaction.

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Related Products		
Product	Size	Cat.#
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PCR Core System II	200 reactions	M7665

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