Specific Amplification of Difficult PCR Products from Small Amounts of DNA Using FastStart Taq DNA Polymerase

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MOLECULAR BIOLOGY

Introduction

The availability of a specific, clean polymerase chain reaction (PCR) product in sufficient amounts prior to cycle sequencing is one important prerequisite for the generation of a good-quality DNA sequence. Since heterozygote samples must be unambiguously identified, excellent DNA quality is particularly crucial for applications such as mutation detection or discovery of single nucleotide polymorphisms (SNPs).

As the PCR efficiency is controlled by many parameters – such as polymerase type, buffer type, primer concentration and stability, dNTP purity and concentration, cycling parameters, as well as complexity and concentration of starting template – it is not possible to establish one standard PCR amplification protocol. Therefore, it is necessary to optimize PCR conditions for each PCR amplicon. Successful amplification of difficult templates (*e.g.*, templates containing GC-rich regions) often requires the addition of cosolvents (*e.g.*, dimethylsulfoxid [DMSO]) to lower the DNA-strand separation temperature. However, this method has the drawback of inhibiting enzyme activity and slowing down the extension rate – which is a problem if only a small amount of DNA is available.

Therefore, we have compared the performance of Taq DNA Polymerase and FastStart Taq DNA Polymerase in PCR amplifications of difficult (GC-rich) DNA templates with different starting amounts of DNA.

Materials and Methods

A 540-bp DNA fragment from 5-hydroxytryptamine receptor 2C (HTR2C) with a GC-content of 65% was amplified in a total volume of 50 µl using Taq DNA Polymerase or FastStart Taq DNA Polymerase on a thermal cycler (MJ Research Tetrad, Watertown, MA).

20 ng of human genomic DNA was amplified using 0.2 mM of each primer (5'-CAGCCATCCGGGACCTGTC-3' and 5'-ACCTGCCGATTGCATATGAAC-3') in the presence of the buffers supplied with the enzyme (10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ for Taq Polymerase; 50 mM Tris pH 8.3, 10 mM KCl, 2.0 mM $MgCl_2$, 5 mM $[NH]_2SO_4$ for FastStart Taq DNA Polymerase), 200 mM of dNTPs, and 1.5 U enzyme. The concentration of DMSO or GC-rich solution (provided with FastStart Taq DNA Polymerase) ranged from 0 – 10%.

The thermocycling protocol consists of an initial incubation at 95 °C for 15 minutes followed by 35 cycles at 94 °C for 1 minute, 60 °C for 30 seconds, 72 °C for 1 minute, and a final extension step of 72 °C for 10 minutes.

The resulting 540-bp PCR products were electrophoresed through a 1.5% agarose gel containing ethidiumbromide, then visualized under UV light.

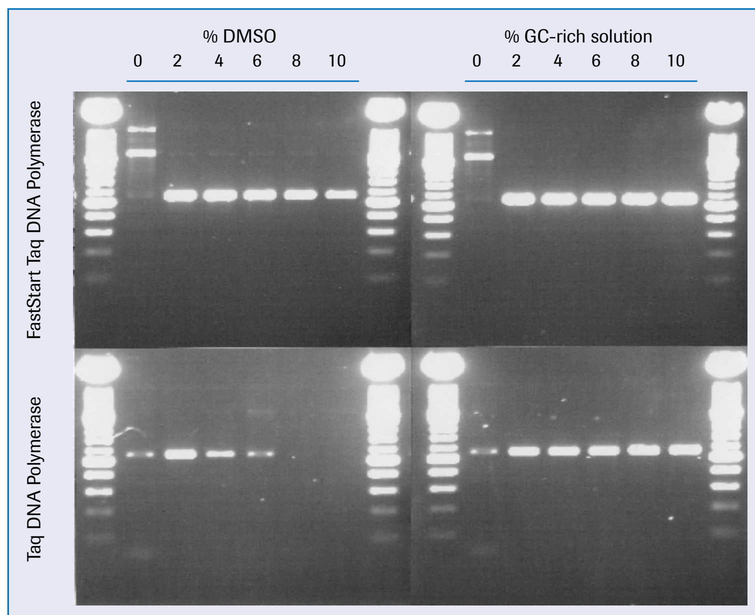

Figure 1: Amplification of a 540-bp fragment of the hydroxytryptamine receptor 2C (with a GC content of 65%) using FastStart Taq DNA Polymerase and Taq DNA Polymerase. Different amounts of DMSO or GC-rich solution were added.

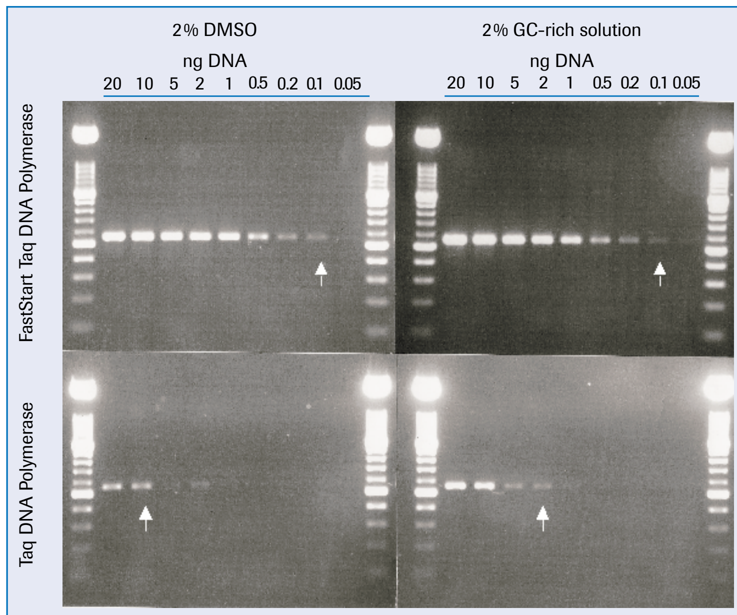

Figure 2: Dilutions of human genomic DNA were amplified in the presence of 2% DMSO or 2% GC-rich solution. FastStart Taq DNA Polymerase showed a lower threshold of detection (*i.e.,* a visible PCR product was obtained from only 100 pg of DNA).

Results and Discussion

It was not possible to obtain the desired 540 bp amplicon in sufficient amounts without the addition of DMSO or GC-rich solution using Taq DNA Polymerase or FastStart Taq DNA Polymerase.

The addition of DMSO and GC-rich solution improved the reactions with respect to specificity and yield (Figure 1). 2% DMSO or 2–10% GC-rich solution resulted in the amplification of a specific PCR product using both enzymes, but with a higher yield using FastStart Taq DNA Polymerase. Therefore, FastStart Taq – in contrast to Taq Polymerase – does not react to DMSO or GC-rich solution by slowing down its extension rate. DMSO in concentrations higher than 2% inhibits the activity of Taq Polymerase, but does not inhibit the activity of FastStart Taq DNA Polymerase. FastStart Taq DNA Polymerase, in combination with GC-rich solution, which has been especially developed and recommended by Roche Applied Science, seems to be most suitable for templates with an even higher GC-content of 65%, without any inhibition of enzyme activity.

To determine the sensitivity of both enzymes, dilutions of human genomic DNA (50 pg, 100 pg, 200 pg, 500 pg, 1 ng, 2 ng, 5 ng, 10 ng, 20 ng) were amplified in the presence of 2% DMSO or 2% GC-rich solution (Figure 2). The threshold of detection is defined as the amount of DNA (present in the dilution) that shows the PCR signal of the 540-bp fragment on the gel (see arrows). Taq DNA Polymerase amplified a visible PCR product in 2% DMSO reactions with ≥10 ng template DNA, and in 2% GC-rich solution reaction with ≥2 ng of DNA. FastStart Taq DNA Polymerase showed at least 10 times higher sensitivity, generating visible PCR products in reactions with only 100 pg of DNA template. These results are in agreement with the results obtained with different concentrations of cosolvents: The higher sensitivity of FastStart Taq DNA Polymerase can be explained by its robustness in the presence of additives (*e.g.*, DMSO or GC-rich solutions). FastStart Taq DNA Polymerase did not seem to be inhibited by these cosolvents and did not slow its extension rate.

Summary

We have successfully used FastStart Taq DNA Polymerase to obtain clean and specific PCR products from GC-rich regions of small amounts of template DNA. In addition to the advantage of hot start for specificity, FastStart Taq Polymerase is not inhibited even by high amounts of cosolvents, such as DMSO or GC-rich solutions, and provides clean PCR products for DNA sequencing from DNA-template amounts as little as 100 pg. Therefore it is superior to Taq DNA Polymerase for this application.

Order INFO

Product	Pack Size	Cat. No.
FastStart Taq DNA Polymerase	50 units	2 158 264
	100 units	2 032 902
	500 units	2 032 929
	1,000 units	2 032 937
	2,500 units	2 032 945
	5,000 units	2 032 953