PCRboost™: A Novel PCR Amplification Enhancer

Introduction

Polymerase chain reaction (PCR) is one of the most performed molecular techniques not only in the academic and industrial research world but also in the forensics, veterinary and health industries. In PCR, DNA is exponentially amplified using Taq DNA polymerase from Thermus *aquaticus*, a bacterium found in hot springs. PCRboost™ was designed to enhance the amplification of DNA without the need for time consuming optimizations. PCRboost™ allows for a decrease in the number of PCR cycles and use of Taq DNA polymerase, resulting in significant time and money savings.

Materials and Methods

PCR reactions

A) Serial Dilution Using PCRboost™: 50ng human genomic DNA (gDNA) was amplified by PCR using 2.5 U Taq DNA polymerase (NEB), 3µl 10x thermopol reaction buffer (NEB), 0.5 µl dNTPs (10µM each nucleotide), 0.5 µl each human β-actin forward (5'ctacctcatgaagatcctcacc3') and β-actin reverse (5'gtacttgcgctcaggaggagc3';10µM each) in a final volume of 30 µl. The PCRboost™ volume was serially diluted and replaced by water*. Cycling parameters were: 94°C for 5 min followed by 40 cycles of 94°C for 15 sec, 55°C for 30 sec and 72°C for 30 sec. 10 µl of each PCR reactions was run on a 0.8% agarose/ethidium bromide gel.

B) Identical Reactions in Water Versus PCRboost™: 100, 50, 20, 10 or 4 ng gDNA was used in PCR reactions where the reaction was brought up to volume in either water or PCRboost™. Samples were used to amplify the fibroblast growth factor 13 (FGF13) gene by PCR using 2.5 U Taq DNA polymerase (NEB), 3µl 10x thermopol reaction buffer (NEB), 0.5 µl dNTPs (10µM each nucleotide), FGF13 forward (5'gaatgttaacaacatgctggc3') and FGF13 reverse (5'agaagctttaccaatgttttcca3') (kind gift of Dr. D. Cohn) in a final volume of 30 µl*. Cycling parameters were: 94°C for 5 min followed by 40 cycles of 94°C for 15 sec, 55°C for 30 sec and 72°C for 30 sec. 10 µl of each PCR reaction was run on a 0.8% agarose/ethidium bromide gel.

C) Comparison of PCRboost to Other PCR Enhancer: PCR enhancers from 3 different companies were tested alongside PCRboost™ and compared to a non-enhanced water control* using 1 ng gDNA (conditions used were the same as A, above).

D) Using Different Taq Polymerases: Taq DNA polymerases from 3 different suppliers were tested alongside PCRboost™ using 10 ng gDNA* (conditions used were the same as A, above).

* PCR reactions were set up using cocktails of common elements.

A

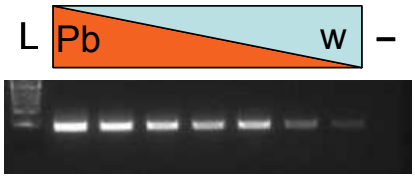

Figure 1: 50ng gDNA was amplified using human β-actin primers in decreasing PCRboost™ (Pb) and increasing water (w). -: no template control. L: ladder

B

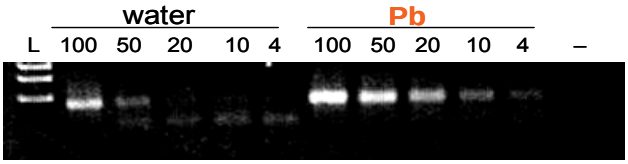

Figure 2: 100, 50, 20, 10 or 4 ng gDNA were used for FGF13 gene PCR amplifications in water or PCRboost™ (Pb). –: no template control. L: ladder.

C

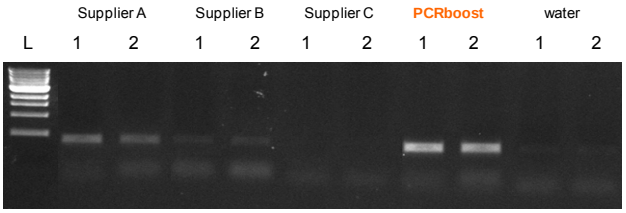

Figure 3: 1 ng gDNA amplified using human β-actin primers with PCR enhancers from 3 different companies (A,B,C) or PCRboost™ in duplicate compared to water. L: ladder.

D

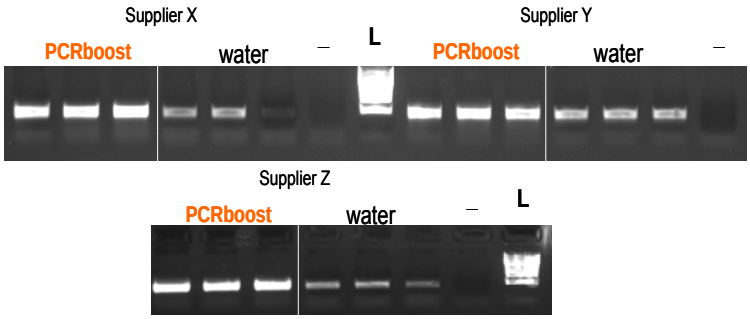

Figure 4: 10ng gDNA were amplified using human β-actin primers in PCRboost™ or water using Taq DNA polymerases from 3 different suppliers (X,Y,Z). -: no Taq control. L: ladder

RTPCR reactions

E) Testing in RTPCR: RTPCR reactions were set up using the gene specific reverse primer for the single-copy RNase P gene (5'agaccatcctggctaacacg3'). Small amounts of total RNA (100ng) extracted from 293 cells (human adenocarcinoma cell line) were used for first strand cDNA generation, using manufacturer's instructions for the AffinityScript™ (Stratagene/Agilent Tech.) RTPCR kit. Reactions were set up using either water or replacing the water component with PCRboost™. 1µl of the cDNA was then used in a subsequent second strand PCR reaction using forward (5'ttcactgcttcatgcctacg3') and reverse primers for the RNase P gene (conditions used were the same in A). Reactions were set up using either water or replacing the water component with PCRboost™.

E

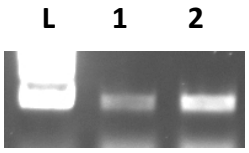

Figure 5: RTPCR amplification for RNase P (510bp) transcript using 1 µl of cDNA generated from 100 ng total RNA using PCRboost™ (lane 2) or water (lane 1). 10 µl of PCR reaction were run on 0.8% agarose gel.

Results and Discussion

PCRboost™ is a novel amplification enhancer. Genomic DNA was used to test the enhancing capacity of PCRboost™ in PCR or RTPCR reactions. We serially diluted PCRboost™ in PCR reactions to show increased amplification when compared to water (Figure 1). Human genomic DNA at various concentrations also amplifies to higher quantities when PCRboost™ is used (Figure 2). Comparison to other PCR enhancers shows that PCRboost™ has more consistent and better enhancing quality under identical conditions (Figure 3). Furthermore, PCRboost™ is compatible with various taq DNA polymerases available on the market (Figure 4). When conducting RTPCR reactions we went to the lower limits and started with 100ng of RNA for first strand cDNA generation. The reactions that contained PCRboost™ in first and second strand reactions resulted in amplification products, whereas reactions containing water yielded very little to no amplicons were obtained (Figure 5).

PCRboost™ is an enhancer that can be used without the need for long optimization reactions. Simply bring your final volume of your PCR reaction up in PCRboost™ and run your reaction using current protocols and equipment. The use of PCRboost™ allows increased amplification, as well as for a reduction in cycles and significant cost savings per reaction.