

Master Your PCR Domain!

Performance Advantages Designed into Promega's PCR Master Mix

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Abstract

Promega's PCR Master Mix is designed for the rapid and convenient amplification of many common genomic and cDNA templates. PCR Master Mix has been optimized to produce PCR products up to 2kb in size, and it exhibits superior stability. PCR Master Mix is provided as a 2X solution that contains all the necessary reaction components except primers and template DNA, thus greatly reducing setup time and pipetting steps.

...PCR Master Mix...contains all the common reaction components required at 2X concentration for amplification.

Introduction

PCR amplification^(a) is routinely used in molecular biology. Typically, the common reaction components (e.g., buffer, dNTPs, MgCl₂ and DNA polymerase) are mixed together to create a "master mix", which is then aliquoted to individual reaction tubes. Primers and template DNA are added just prior to performing PCR. Promega's new PCR Master Mix^(a) is truly a master mix solution that contains all the reaction components required at 2X concentration for amplification. One need only add reaction-specific primers, DNA template and the provided Nuclease-Free Water to achieve 1X working concentration.

Sensitivity

Increasingly, scientists are required to analyze minute amounts of DNA, and thus PCR amplification sensitivity has become critical. To demonstrate the sensitivity of amplifications performed with PCR Master Mix, we amplified a fragment of the α 1-antitrypsin gene from serial dilutions of Human Genomic DNA (Cat.# G3041). α 1-antitrypsin is a single copy per genome gene. As evident in Figure 1, we were able to amplify a 360bp fragment from dilutions containing as few as two copies per reaction of this gene using PCR Master Mix.

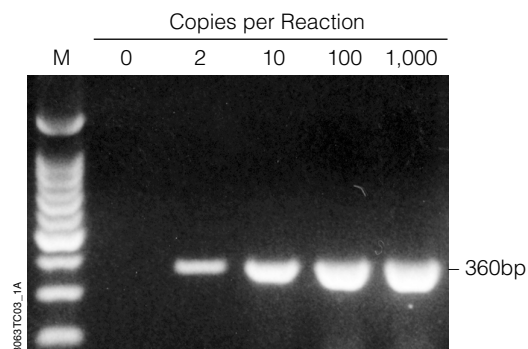


Figure 1. Detection of low copy number templates using PCR Master Mix. A 360bp fragment of the human α 1-antitrypsin gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lane M, 100bp DNA Ladder (Cat.# G2101).

Template Versatility

DNA templates come from species as varied as bacteria, plants and humans. As seen in Figure 2, we tested PCR Master Mix using serial dilutions of DNA from many such sources including bacteria, lambda phage, soybean, human and viral ssRNA (as a template to amplify a fragment of the 5'-UTR).

Stability

We used two approaches to test the stability of PCR Master Mix: i) time in storage and ii) number of freeze-thaw events. First, PCR Master Mix was thawed at room temperature and then stored at 4°C for up to 15 months. PCR amplification of a 360bp fragment of the human α 1-antitrypsin gene was performed following storage. In Figure 3, PCR Master Mix amplified the target with no noticeable changes in PCR quality even after 15 months in storage.

Advantages of PCR Master Mix...continued

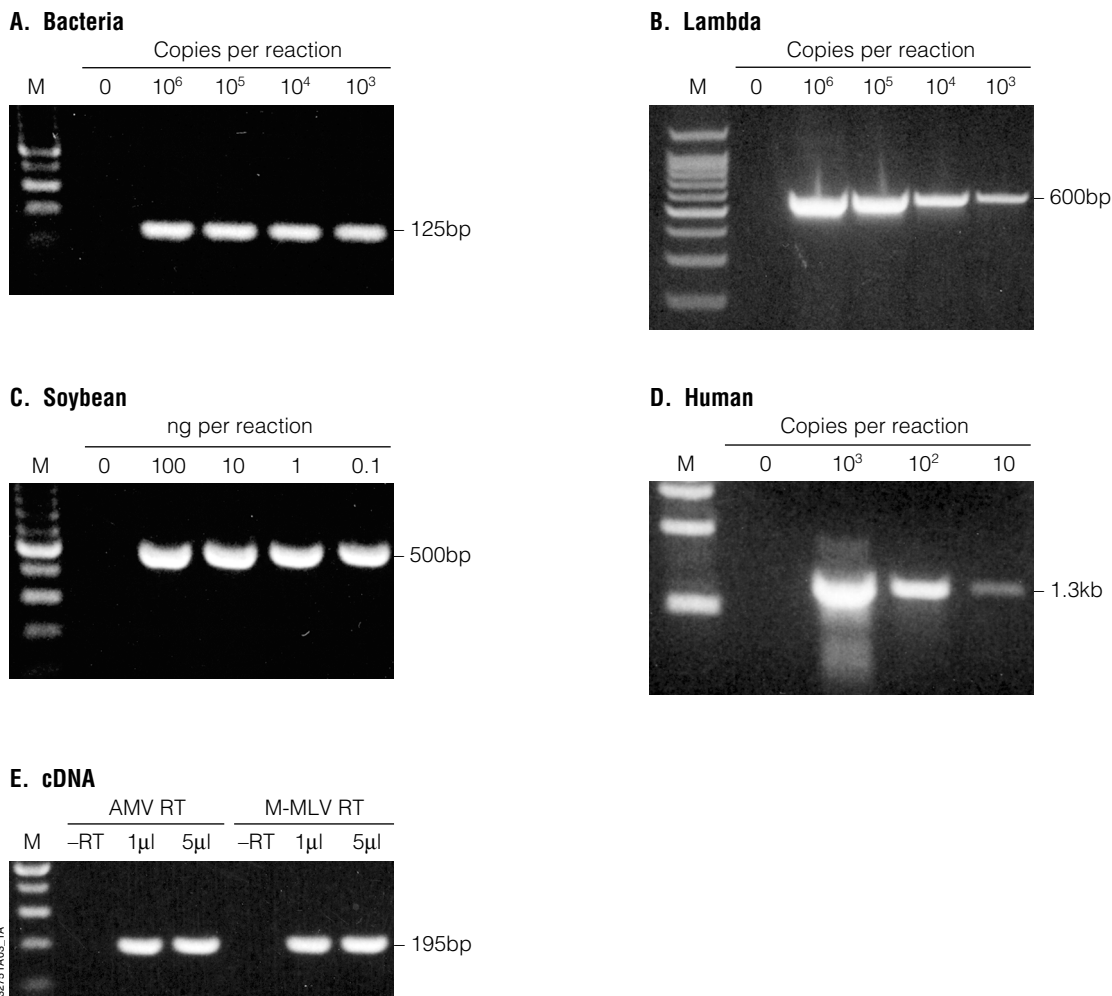


Figure 2. PCR amplification using DNA from different sources. Panel A: A 125bp fragment of the *hemL* gene from *E. coli* (1). Panel B: A 600bp fragment from lambda phage DNA. Panel C: A 500bp fragment of the *rbcl* gene from Soybean DNA (2). Panel D: A 1.3kb fragment of the β -globin gene from Human Genomic DNA. Panel E: A 195bp fragment from the 5'-NTP using cDNA from ssRNA virus. Lane M in Panels A, B, C and E are the 100bp DNA Ladder; lane M in Panel D is the 1kb DNA Step Ladder.

PCR Master Mix amplified the target with no noticeable changes in PCR quality even after 15 months in storage.

Second, PCR Master Mix was thawed and refrozen up to 10 times before use. Two rates of freeze-thaw events were performed, "slow" and "fast" thaws. For the slow thaw, PCR Master Mix was removed from storage at -20°C and allowed to thaw at room temperature (-25°C); for the fast thaw, it was removed from -70°C and placed directly in a 50°C water bath until thawed. PCR amplification was performed after each thaw event had been repeated five or 10 times. As evident in Figure 4, there was no noticeable drop in amplification product after 10 freeze-thaw cycles.

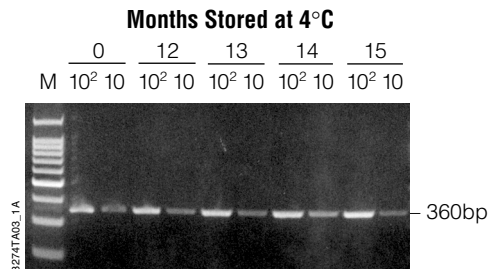


Figure 3. Stability of PCR Master Mix at 4°C. PCR Master Mix was thawed at room temperature and then stored at 4°C for up to 15 months. Following storage, it was used to amplify a 360bp fragment of the human α 1-antitrypsin gene from the indicated number of molecules of Human Genomic DNA. Lanes M, 100bp DNA Ladder.

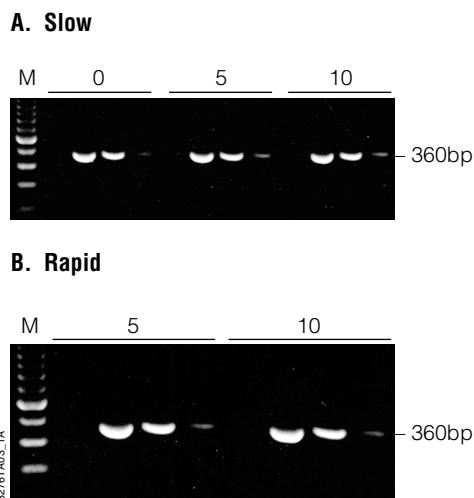


Figure 4. Stability of PCR Master Mix through multiple freeze-thaw events. Both slow (**Panel A**) and rapid (**Panel B**) freeze-thaw events were performed, by allowing PCR Master Mix to thaw from -20°C to room temperature ("slow") or thawing from -70°C to 50°C ("rapid"). A 360bp fragment of the human $\alpha 1$ -antitrypsin gene was amplified using the PCR Master Mix after 5 or 10 freeze-thaw cycles.

Robustness

Thermal cycling instruments may exhibit a temperature deviation of 1–2 degrees. PCR Master Mix contains a specialized buffer to keep the *Taq* DNA Polymerase^(a) stable at elevated temperatures. This can be especially important in cases when the amplification profile requires an elevated denaturation temperature or an increased number of cycles (3).

We tested the tolerance of PCR Master Mix at three elevated denaturation temperatures—95, 97 and 99°C —by amplifying a 360bp fragment of the $\alpha 1$ -antitrypsin gene from Human Genomic DNA (Cat.# G3041). In Figure 5, Promega's PCR Master Mix was compared to another commercially available PCR mix under the same conditions.

Supplier L's PCR master mix produced no detectable product at denaturation temperatures $>95^{\circ}\text{C}$. Promega's PCR Master Mix amplified product with equal efficiency at all denaturation temperatures.

Scalability

Finally, we assessed scalability in terms of reaction volume. Different applications require PCR to be performed in different volumes, typically in the range of 10 to $50\mu\text{l}$. PCR Master Mix produces consistent amplification results when used in reactions with final volumes between 10 and $50\mu\text{l}$ (Figure 6).

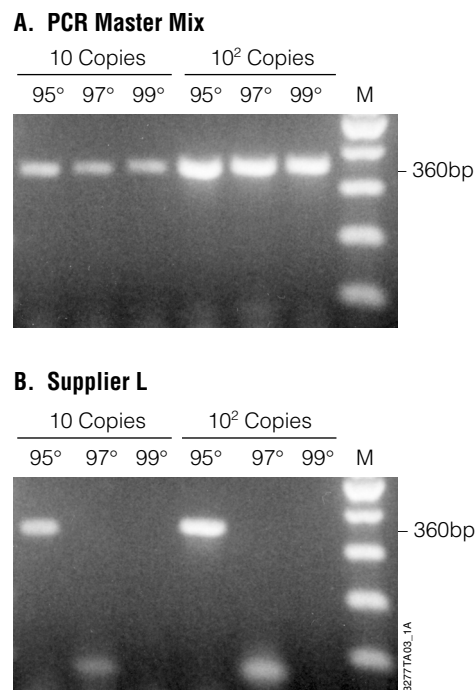


Figure 5. Stability of PCR Master Mixes at high denaturation temperatures. Using the denaturation temperatures indicated, Promega PCR Master Mix (**Panel A**) was compared to supplier L's (**Panel B**) in amplifying a 360bp fragment of the human $\alpha 1$ -antitrypsin gene from 10 or 100 molecules of Human Genomic DNA. Lanes M, 100bp DNA Ladder.

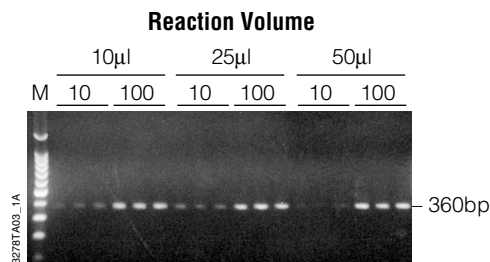


Figure 6. Scalability of the PCR Master Mix. A 360bp fragment of the human $\alpha 1$ -antitrypsin gene was amplified in triplicate from 10 or 100 molecules of Human Genomic DNA in the indicated reaction volumes. Lane M, 100bp DNA Ladder.

Advantages of PCR Master Mix...continued

Conclusions

In this article, we show that the PCR Master Mix offers improved sensitivity, amplifying a target sequence from as few as two copies of starting template. PCR Master Mix was shown to effectively amplify sequences in sizes ranging from 125bp to 1.3kb. PCR Master Mix amplified DNA isolated from mammalian, plant, bacterial and viral sources with equal success. In addition, stability is a key feature of PCR Master Mix. We describe here experiments that show that the Master Mix is fully scalable and sensitive enough to detect low copy number templates and is also stable when stored for long periods at 4°C even after multiple freeze-thaw cycles. In addition, Nuclease-Free Water is included in a convenient separate vial to ensure high-quality results.

As PCR amplification has become central to molecular biology research, methods to streamline reaction setup are required. Promega's PCR Master Mix, formulated as a 2X solution, offers single-tube format for reaction setup, reducing pipetting times, steps and errors as well as greatly reducing reagent waste.



We highly recommend the use of barrier pipet tips for assembling components for PCR or RT-PCR to prevent contamination of reactions and reagents with template DNA, RNA or primers.

References

1. Ilag, L.L. *et al.* (1991) *J. Bacteriol.* **173**, 3408–3413.
2. Lin, J.-J. *et al.* (2000) *BioTechniques* **28**, 346–350.
3. Ruano G. *et al.* (1992) *BioTechniques* **13**, 266–274.

Protocol

- ◆ PCR Master Mix Product Information #9PIM750, Promega Corporation. (www.promega.com/tbs/9pim750/9pim750.html)



Ordering Information

Product	Size	Cat.#	Price (\$)
PCR Master Mix ^{(a)*}	1,000 reactions	M7505	490
	100 reactions	M7502	65
	10 reactions	M7501	10

^{*}For Laboratory Use.

^(a)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.

Technical Questions?

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