

PCR Core Systems: Complete Reagent Systems for DNA Amplification



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PCR* - polymerase chain reaction - is a powerful technique widely used in laboratories around the world. Promega continually strives to improve the convenience, performance and reliability of PCR by developing products to aid in using this technique. This article introduces PCR Core Systems** I and II and describes the increased convenience of optimizing amplification reactions with the Control Primers and Positive Control Plasmid DNA.

*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 the PCR Core Systems is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

Introduction

PCR is an extremely sensitive technique that facilitates the amplification of specific DNA sequences obtained from many sources - even with limited DNA. In this technique, an exponential increase of target sequence with minimal amplification of undesired sequences is possible in complex mixtures of nucleic acids (1). Furthermore, PCR products can be manipulated in downstream applications such as cloning into T-tailed vectors (2) or for coupled transcription/translation using *in vitro* transcription/translation systems (3). Promega's new PCR Core Systems I and II can be used for 200 routine 50µl amplification reactions. These systems are quality tested in PCR amplification assays to ensure consistent results.

Format of the PCR Core Systems

PCR Core System I provides the necessary reagents in proper volumes and appropriate concentrations for performing thermal cycled DNA amplifications. PCR Core System II provides the basic components of System I plus Upstream and Downstream Positive Control Primers and Positive Control Plasmid DNA (Table 1). The control reagents can be used to ensure PCR performance under user-defined conditions. The new PCR Nucleotide Mix included in both systems contains dATP, dCTP, dGTP and dTTP at a concentration of 10mM each. PCR Nucleotide Mix is tested for performance in PCR and reverse transcription using avian myeloblastosis virus reverse transcriptase. PCR Nucleotide Mix significantly increases the convenience of reaction setup and accuracy of amplification results by reducing the number of pipetting steps. The Nucleotide Mix is ready for direct addition to a reaction cocktail; one microliter of PCR Nucleotide Mix in a 50µl reaction yields a final dNTP concentration of 800µM, or 200µM per triphosphate, the recommended amount for many PCR applications.

Table 1. Components of PCR Core System I and II.

Component	Size	PCR Core System I	PCR Core System II
Taq DNA Polymerase	250u	*	*
Thermophilic DNA Polymerase	1.2ml	*	*
10X Buffer, MgCl ₂ Free			
MgCl ₂ , 25mM Solution	1.2ml	*	*
Taq DNA Poly 10X Buffer w/15mM MgCl ₂	1.2ml	*	*
PCR Nucleotide Mix, 10mM each	200µl	*	*
Positive Control Plasmid DNA	100ng		*
Upstream Control Primer, 15µM	100µl		*
Downstream Control Primer, 15µM	100µl		*

All reagents are tested for performance in PCR.

Performance of the PCR Core Systems

The components of the PCR Core Systems perform optimally under the conditions recommended in [Table 2](#). The Positive Control Plasmid DNA included with PCR Core System II can be used in several ways to optimize PCR performance. The control template used at a defined concentration or titrated into reactions ensures the sensitivity of the system under the user's reaction conditions. Reaction conditions and parameters indicated in [Table 2](#) and [Figure 1](#) are sufficient to detect the 323bp control amplicon following amplification of as few as 100 copies of the control template (see [Figure 2](#)). Using the control template in this manner, one may deduce how alternative reaction conditions or cycling profile changes might affect amplification efficiency.

Table 2. Recommended Reagent Volumes and Final Concentrations of the PCR Core Systems.

Component	Component Volume	Final Concentration
MgCl ₂ , 25mM	3µl	1.5mM
Thermophilic DNA Polymerase 10X Buffer, MgCl ₂ Free	5µl	1.0X
PCR Nucleotide Mix (10mM each dNTP)	1µl	0.2mM
Upstream Primer, 15µM	0.33-3.3µl	0.1-1.0µM
Downstream Primer, 15µM	0.33-3.3µl	0.1-1.0µM
Taq DNA Polymerase, 5u/µl	0.25µl	1.25u/50µl
Template DNA	variable	<0.5µg/50µl
Nuclease-Free Water to final volume of	50µl	

Optimal reaction conditions, including times, temperatures and reagent concentrations are dependent upon the DNA polymerase, template and primers used. A separate buffer containing MgCl₂ is supplied for convenience. However, we strongly recommend optimizing the MgCl₂ concentration. For a discussion of relevant parameters that may affect the PCR process under user-defined conditions, please refer to the PCR Core Systems Technical Bulletin #TB254 (4) or Promega's PCR Protocols and Reference Guide (5).

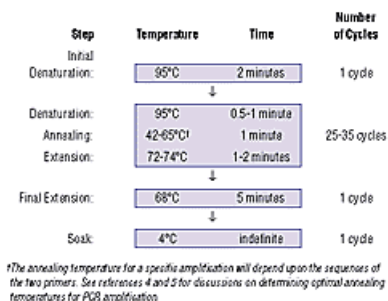


Figure 1. Recommended starting thermal cycling conditions for PCR amplification. These guidelines apply to target sequences between 200 and 2,000bp and are optimal for the Perkin-Elmer Thermal Cycler Model 480 or comparable thermal cyclers.

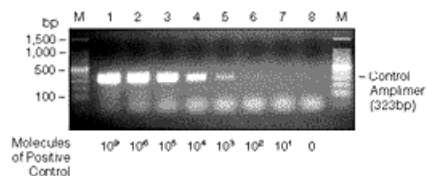


Figure 2. Amplification of the Positive Control Plasmid DNA as a standalone control. PCR reactions containing the indicated number of molecules of control DNA template and primers supplied with Promega's PCR Core System II (lanes 1-8) were performed using the standard protocol (4). The amplification reaction consisted of an initial denaturation (94°C for 2 minutes), followed by 30 cycles consisting of denaturation (94°C for 30 seconds), annealing (60°C for 60 seconds), extension (72°C for 90 seconds), and a single final extension cycle (68°C for 7 minutes). Equivalent amounts of each reaction were analyzed on a 1.5% agarose gel in TAE 1X buffer. The specific 323bp amplicon obtained with the control template is indicated. Lane M, Promega's 100bp DNA Ladder (Cat.# G2101); lanes 1-8, indicated amounts of target molecules.

An alternative use of the Positive Control Plasmid is to test for the presence of inhibitory agents in a specific DNA sample under fixed PCR conditions. To accomplish this, the control template is titrated into reactions containing a fixed amount of genomic DNA (Figure 3). Successful amplification of the control template indicates that the test reaction conditions are compatible with the genomic DNA sample (i.e., inhibitory agents are absent). This control is especially useful when attempting to amplify DNA isolated with high concentrations of denaturants. A decrease in the PCR amplification of control template, from a mixture of control *and* sample DNA, suggests that suboptimal conditions exist; inhibitory agents may be in the sample and further purification then is required. Additional discussions of parameters that affect PCR amplification are available (4,5).

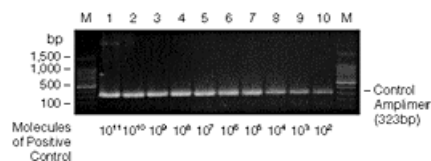


Figure 3. Positive Control Plasmid DNA as a template compatibility test. Genomic DNA was isolated from mouse liver using the Wizard[®] Genomic DNA Purification System (Cat.# A1120). Amplifications contained 150ng genomic DNA spiked with the indicated amount of PCR Control DNA Plasmid, supplied with PCR Core System II (lanes 1-10). The amplification reaction consisted of an initial denaturation (94°C for 2 minutes), followed by 40 cycles consisting of denaturation (94°C for 30 seconds), annealing (60°C for 60 seconds), extension (68°C for 2 minutes), and a single final extension cycle (68°C for 7 minutes). Equivalent amounts of each reaction were analyzed as in Figure 2. The 323bp control amplicon is indicated. Lane M, Promega's 100bp DNA Ladder (Cat.# G2101); lanes 1-10, indicated amounts of target molecules.

Summary

Promega's PCR Core Systems allow for consistent amplification of many target templates by providing all of the required PCR-qualified reagents in a convenient package. The two systems differ only in that PCR Core System II contains a control template and primers. These positive control reagents can be used as a standalone PCR control, as a test control for the compatibility of sample target DNA or as an internal coamplification control in a PCR containing two targets. The PCR Core Systems serve as valuable tools for successfully amplifying target DNA for a variety of downstream applications.

References

1. d'Aquila, R.T. *et al.* (1991) *Nucl. Acids Res.* **19**, 3749.
2. *pGEM[®]-T and pGEM[®]-T Easy Vector Systems Technical Manual* #TM042, Promega Corporation.
3. *TNT[®] T7 Quick Coupled Transcription/Translation Technical Manual* #TM045, Promega Corporation.
4. *PCR Core Systems Technical Bulletin* #TB254, Promega Corporation.
5. *PCR ACCESS! PCR Protocols and Reference Guide* #BR070, Promega Corporation.

Ordering Information

Product	Cat.#
PCR Core System I	M7660
PCR Core System II	M7665

Each system contains sufficient reagents for 200 amplification reactions (50µl each).

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