

GoTaq® PCR Core Systems

INSTRUCTIONS FOR USE OF PRODUCTS M7660, M7665, M7650 AND M7655.

GoTaq® PCR Core Systems

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1. Description

The GoTaq® PCR Core Systems I and II^(a,b) provide all of the reagents necessary for the exponential amplification of specific regions of DNA using the polymerase chain reaction (PCR; 1). The GoTaq® PCR Core Systems are supplied with GoTaq® DNA Polymerase, a proprietary formulation of *Taq* DNA polymerase designed for enhanced amplification, and GoTaq® Flexi Buffers, magnesium-free proprietary buffer formulations supplied at pH 8.5. The 5X Green GoTaq® Flexi Buffer increases the density of the sample and contains two dyes (a blue dye and a yellow dye) that separate during electrophoresis, allowing reactions to be loaded directly onto an agarose gel without the addition of loading dye. During electrophoresis in a 1% agarose gel, the blue dye migrates at the same rate as a 3–5kb DNA fragment, and the yellow dye 1.

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1. Description (continued)

migrates slightly faster than primers (<50bp). The 5X Colorless GoTaq® Flexi buffer allows fluorescence or absorbance measurements to be made directly on the amplified DNA without the need to purify it from the amplification reaction.

In addition to all of the reagents necessary for amplification that are provided in GoTaq® PCR Core System I, GoTaq® Core System II also includes positive control primers and template. Please see Section 5 for a discussion on the use of controls in PCR.

Although simple in theory, PCR can benefit from optimization of several parameters. This technical bulletin provides important and convenient information about performing PCR and includes protocols and troubleshooting tips for successful PCR amplifications.

Comprehensive information on the PCR process is available online in the PCR Chapter of the Promega *Protocols and Applications Guide* at: www.promega.com/paguide

2. Product Components and Storage Conditions

Product	Cat.#
GoTaq® PCR Core System I	M7650*
	M7660**

*Cat.# M7650 is only available in Europe or through Distributors supported by Promega European Branch Offices. Each System I contains sufficient reagents for 200 amplification reactions (50µl each).

**Cat.# M7660 For Laboratory Use.

Includes:

- 250u GoTaq® DNA Polymerase
- 2 × 1ml 5X Colorless GoTaq® Flexi Buffer, Mg-Free
- 2 × 1ml 5X Green GoTaq® Flexi Buffer, Mg-Free
- 1.2ml MgCl₂
- 200µl PCR Nucleotide Mix, 10mM

Product	Cat.#
GoTaq® PCR Core System II	M7655*
	M7665**

*Cat.# M7655 is only available in Europe or through Distributors supported by Promega European Branch Offices. Each System II contains sufficient reagents for 200 amplification reactions (50µl each).

**Cat.# M7665 For Laboratory Use.

Includes:

- 250u GoTaq® DNA Polymerase
- 2 × 1ml 5X Colorless GoTaq® Flexi Buffer, Mg-Free
- 2 × 1ml 5X Green GoTaq® Flexi Buffer, Mg-Free
- 1.2ml MgCl₂
- 200µl PCR Nucleotide Mix, 10mM
- 100ng Positive Control Plasmid DNA, 1ng/µl in TE buffer
- 100µl Upstream Control Primer, 15µM
- 100µl Downstream Control Primer, 15µM

Note: The Positive Control Plasmid DNA is provided only in GoTaq® PCR Core System II.

Storage Conditions: Store all components at -20°C. See product label for expiration date.

3. PCR Protocol

To facilitate optimization, troubleshooting and validation of PCR, we strongly recommend performing concurrent positive and negative control reactions as discussed in Section 5.

If working with multiple samples, assemble a master mix of water, MgCl₂, 5X buffer, primers, PCR Nucleotide Mix and GoTaq® DNA Polymerase. Combine the appropriate multiples of these components (except template) and dispense aliquots of the appropriate volume, minus the specific volume to be occupied by the template DNA, to each reaction tube. Initiate the reaction by adding the template. Use individual pipette tips for all additions, being careful not to cross-contaminate the samples.

If possible, start with >10⁴ copies of the target sequence to obtain a signal in 25–30 cycles. Excess template is not beneficial to the reaction. Always ensure that the final DNA concentration is ≤10ng/μl. Less than 10 copies of a target can be amplified (2), but more cycles may be required to detect a signal by gel electrophoresis. Additional cycles may increase nonspecific amplification, evidenced by multiple bands when analyzed by gel electrophoresis.

Materials to Be Supplied by the User

(Solution compositions are provided in Section 8.A.)

- Mineral Oil (Cat.# DY1151)
- Nuclease-Free Water (Cat.# P1193)
- template DNA
- upstream primer
- downstream primer

3.A. Protocol

1. Combine the components, as listed in Table 1, in sterile, 0.5–0.6ml microcentrifuge tubes on ice. Amplification reactions may be scaled up or down as necessary. Use of a master mix, as described above, greatly facilitates the reaction setup and decreases tube-to-tube variability of the reaction components. See Section 5 for guidelines on selecting the optimum amounts of each component (e.g., magnesium concentration) to include in the reaction.
2. If using a thermal cycler **without a heated lid**, overlay the reaction mix with 1–2 drops (approximately 50μl) of Mineral Oil to prevent evaporation during thermal cycling. Centrifuge the reaction mix in a microcentrifuge for 5 seconds.
3. Place the reactions in a thermal cycler that has been preheated to 95°C. We recommend heating the samples at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Incubation longer than 2 minutes at 95°C is unnecessary and may reduce the yield.
4. Start the thermal cycling program. The cycling profile given in Table 2 may be used as a guideline. Optimize the amplification profile for each primer/target combination (see Section 5).

Table 1. Recommended Reaction Volumes and Final Concentrations of the GoTaq® PCR Core System Components.

Component	Component Volume	Final Concentration
MgCl ₂ , 25mM Solution (see Section 4.B)	2.0–8.0μl	1.0–4.0mM
5X Colorless GoTaq® Flexi Buffer		
OR 5X Green GoTaq® Flexi Buffer	10μl	1.0X
PCR Nucleotide Mix, 10mM each	1μl	200μM each
upstream primer	5–50pmol	0.1–1.0μM
downstream primer	5–50pmol	0.1–1.0μM
GoTaq® DNA Polymerase, 5u/μl	0.25μl	1.25u/50μl
template DNA	variable	<0.5μg/50μl
Nuclease-Free Water to a final volume of	50μl	



Thaw the 25mM MgCl₂ solution and GoTaq® Flexi Buffer completely and vortex thoroughly before use. We strongly advise optimizing the MgCl₂ concentration.

Table 2. Thermal Cycling Guidelines for PCR Amplification.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	
Annealing	42–65°C*	0.5–1 minute	25–35 cycles
Extension	72°C	1 minutes/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	indefinite	1 cycle

These guidelines apply to target sequences between 200–2,000bp and may need to be adapted for your thermal cycler.

*The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers. See Section 4.G for discussions on how to determine optimal annealing temperatures for PCR amplification.

3.B. Analysis

1. Analyze PCR products by agarose gel electrophoresis. The products should be readily visible in an ethidium bromide-stained gel under UV light. Reactions containing Green GoTaq® Buffer do not need loading dye added before electrophoresis.
2. Store PCR products at –20°C until needed. The PCR products can be further purified using a number of procedures including the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

4. Optimization of PCR

4.A. Routine PCR

As originally developed, the PCR process amplifies short (approximately 100–500bp) segments of a longer DNA molecule (1). A typical amplification reaction includes the target DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer, magnesium and optional additives. The components of the reaction are mixed and placed in a thermal cycler, an automated instrument that ‘cycles’ the reaction through a predetermined series of specific temperatures and times. One cycle of amplification is defined by the series of temperature and time adjustments. Each cycle of PCR after the first cycle theoretically doubles the amount of targeted template sequence (amplimer). Therefore, ten cycles theoretically multiplies the amplimer by a factor of about one thousand; 20 cycles, by a factor of more than one million. PCR amplification can be completed in as little as 2 hours.

Each cycle of PCR amplification consists of a defined number of reaction steps. The steps are designed using temperature and duration time to denature the template, anneal the two oligonucleotide primers and extend the new complementary DNA strands by polymerization. These steps can be optimized for each template and primer pair combination. The target DNA is denatured by heating to 95°C or higher for 15–120 seconds. During denaturation, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA (ssDNA) template for primer annealing and polymerization (extension) by a thermostable polymerase. To anneal the oligonucleotide primers, the temperature of the next step in the cycle is reduced to approximately 40–60°C. At this temperature, the oligonucleotide primers can anneal to the ssDNA strands and serve as primers for DNA synthesis by the polymerase. This step requires approximately 30–60 seconds. Finally, to extend from the primer-bound template DNA, the reaction temperature is raised to the optimum for most thermostable DNA polymerases, which is approximately 72°C. Extension of the primer by the thermostable polymerase requires approximately 1 minute per kilobase to be amplified. Extension completes one cycle, and the next cycle begins by returning the reaction to 95°C for denaturation. After 20–40 cycles, the amplified nucleic acid may then be analyzed (e.g., for size, quantity or sequence) or it may be used in further experimental procedures (e.g., cloning or mutagenesis).

4.B. Magnesium Concentration

Magnesium concentration is a crucial factor affecting the performance of thermostable enzymes. Reaction components, including template DNA, chelating agents present in the sample (e.g., EDTA or citrate), dNTPs and proteins, can affect the amount of free magnesium. In the absence of adequate free magnesium, GoTaq® DNA Polymerase is inactive. Conversely, excess free magnesium reduces enzyme fidelity (3) and may increase the level of

nonspecific amplification (4,5). For these reasons, it is important to empirically determine the optimal MgCl₂ concentration for each reaction. This is accomplished by preparing a series of reactions containing 1.0–4.0mM Mg²⁺, in increments of 0.5mM, by adding 2, 3, 4, 5, 6, 7 and 8µl of a 25mM MgCl₂ stock to 50µl reactions.

The GoTaq® PCR Core Systems include 5X Green GoTaq® Flexi Buffer, Mg-Free; 5X Colorless GoTaq® Flexi Buffer, Mg-Free; and a solution of 25mM MgCl₂. The buffers allow you to adjust the Mg²⁺ concentration to the level that is optimal for each reaction using the 25mM MgCl₂.

Two important steps will ensure the reaction contains the appropriate amount of Mg²⁺: **Magnesium solutions must be thawed completely and vortexed for several seconds prior to use because magnesium chloride solutions can form concentration gradients when frozen.** Thawing and vortexing is required to render the solution uniform with respect to magnesium salts. These two simple steps can eliminate a major source of many failed experiments.

4.C. Buffer Considerations

We recommend using the 5X Green GoTaq® Flexi Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green GoTaq® Flexi Buffer is not recommended for any downstream applications using absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb between 225–300nm, making standard A₂₆₀ readings to determine DNA concentration unreliable. Also, the dyes have excitation peaks at 488nm and between 600–700nm that correspond to the excitation wavelengths commonly used in fluorescence detection instrumentation. However, for some instrumentation, such as a fluorescent gel scanner that uses a 488nm excitation wavelength, there will be minimal interference, since it is the yellow dye that absorbs this wavelength. Gels scanned by this method will have a light gray dye front below the primers corresponding to the yellow dye front. The Green and Colorless GoTaq® Flexi Buffers give approximately equivalent amplification yields. Balanced amplifications between the two buffers may require further optimization.

For reactions going directly from a thermal cycler to an application using absorbance or fluorescence, the 5X Colorless GoTaq® Flexi Buffer is recommended. If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from the Green GoTaq® Flexi reactions using standard PCR clean-up systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or the Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

Both reaction buffers are compatible with common PCR additives such as DMSO and betaine. These additives do not change the color of the Green GoTaq® Flexi Buffer or affect dye migration.

4.D. Enzyme Concentration

We recommend that 1.25 units of GoTaq® DNA Polymerase be used per 50µl amplification reaction. For most applications, enzyme will be in excess; the inclusion of more enzyme will not significantly increase product yield. Increased amounts of enzyme and excessively long extension times increase the likelihood of generating artifacts. Artifacts generally can be seen as smearing of bands in ethidium bromide-stained agarose gels (6–8).

The most frequent cause of excessive enzyme levels is pipetting error. Accurate dispensing of submicroliter (<1µl) volumes of enzyme solutions in 50% glycerol is nearly impossible. We strongly recommend the use of reaction master mixes sufficient for the number of reactions being performed to obviate this problem. A master mix increases the volumes of pipetted reagents and reduces pipetting errors.

4.E. Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. To avoid the production of primer-dimers, the 3' ends of the primers should not be complementary. Primer dimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3' end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal roughly at the same temperature. In any case, the annealing temperature of the reaction is dependent upon the primer with the lowest T_m .

The sequence of the primers can also include regions at the 5' ends that may prove useful for downstream applications. For example, restriction enzyme sites can be designed into the primer pair for ease in downstream manipulations such as cloning. Regardless of primer choice, the final concentration of the primer in the reaction must be optimized. We recommend adding 50pmol of each primer (1µM final concentration in a 50µl reaction) as a starting point for optimization. Generally, nanograms of primer DNA equivalent to 50pmol is: $16.3\text{ng} \times b$, where b is the number of bases in the primer.

4.F. Template Considerations

Successful amplification of the region of interest is dependent upon the amount and quality of the template DNA. Reagents commonly used to purify nucleic acids (e.g., salts, guanidine, proteases, organic solvents and SDS) are potent inhibitors of DNA polymerases. A final ethanol precipitation of the nucleic acid sample will eliminate most of the inhibitory agents. Spiking a

control DNA fragment and the appropriate primer pair into the DNA preparation may be useful in verifying the purity of the DNA sample.

The amount of template required for successful amplification depends upon the complexity of the DNA sample. For example, whereas a 4kb plasmid containing a 1kb insert equates to 25% of the DNA being the target of interest, a 1kb gene in human genomic DNA (genome of $3.3 \times 10^9\text{bp}$) represents approximately 0.00003% of the input DNA. Therefore, approximately 1,000,000-fold more human genomic DNA is required to maintain the same number of target copies per reaction.

Two common mistakes encountered when trying to amplify target DNA are using too much plasmid DNA and too little genomic DNA. Table 3 lists the correlation of molecules per microgram of nucleic acids from some common RNA and DNA targets. Table 4 shows typical yields of genomic DNA from a variety of source materials. As a general guide for how much template DNA to use, start with a minimum of 10^4 copies of the target sequence to obtain a signal in 25–30 cycles, but keep the final DNA concentration of the reaction $\leq 10\text{ng}/\mu\text{l}$.

Table 3. Conversion of Nucleic Acids from Microgram Amount to Number of Molecules.

Nucleic Acid	Amount	# of Molecules
1kb RNA	1µg	1.8×10^{12}
1kb dsDNA	1µg	9.18×10^{11}
pGEM® Vector DNA	1µg	2.9×10^{11}
lambda (λ) DNA	1µg	1.9×10^{10}
<i>E. coli</i> genomic DNA	1µg	2×10^8
human genomic DNA	1µg	3.0×10^5

Table 4. DNA Yields from Different Human Tissue Sources.

Amount of Material	Source of DNA	
	Typically Used	Typical Yield
Whole blood	30µl	0.5–1µg
Blood spot	1/2 of a 5mm spot	1–3µg
Cell suspension	5×10^5 cells	2–5µg
Buccal cells	Single mouth rinse	0.1–1µg
Chorionic villus biopsy	Small frond	1–3µg
Semen	30µl	5–10µg
Hair root	Single root	10–200ng
Tissue block	50mg	0.1–10µg

4.G. Primer Annealing Temperature

The sequences of the primers are a major consideration in determining the optimal temperature of the PCR amplification cycles. For primers with a high T_m , it may be advantageous to increase the annealing temperature. Higher temperatures minimize nonspecific primer annealing, increase the amount of specific product produced and reduce the amount of primer-dimer formation.

Numerous formulas exist to determine the theoretical T_m of nucleic acids (9,10), and these may serve as a starting point for annealing conditions. However, it is best to optimize the annealing conditions by performing the reaction at several temperatures starting approximately 5°C below the calculated T_m (visit our web site for a T_m calculator that can calculate melting temperatures for primers in a GoTaq® reaction at: www.promega.com/biomath/). The formula below can be used to estimate the melting temperature for any oligonucleotide:

$$T_m = 81.5 + 16.6 \times (\log_{10}[\text{Na}^+]) + 0.41 \times (\%G+C) - 675/n,$$

where $[\text{Na}^+]$ is the molar concentration of monovalent cations and n = number of bases in the oligonucleotide.

Example: To calculate the melting temperature of a 22mer oligonucleotide with 60% G+C in 50mM KCl:

$$\begin{aligned} T_m &= 81.5 + 16.6 \times (\log_{10}[0.05]) + 0.41 \times (60) - 675/22 \\ &= 81.5 + 16.6 \times (-1.30) + 24.60 - 30.68 \\ &= 53.84^\circ\text{C} \end{aligned}$$

4.H. Extension Temperature

During the extension step, allow approximately 1 minute for every 1kb to be amplified (minimum extension time of 1 minute). Generally, 25–40 cycles are sufficient for most reactions.

4.I. Nucleic Acid Cross-Contamination

It is important to minimize cross-contamination between samples and prevent carryover of RNA and DNA from one experiment to the next. Use separate work areas and pipettors for pre- and post-amplification steps. Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. Wear gloves and change them often. Consider using a contamination control technique (11) to prevent DNA carryover to subsequent reactions.

5. Using Controls in PCR

The Positive Control Plasmid DNA template included with the GoTaq® PCR Core System II can be used in several formats to ensure PCR performance. The control template can be used at a defined concentration or it may be titrated into reactions to ensure the sensitivity of the system under reaction conditions of the user's choice. Using the reaction conditions and parameters suggested in the system and the cycling profile indicated in Table 6, the expected 323bp amplicon can be detected following amplification of as few as 1×10^2 copies of the control template. Using the control template in this manner, one can deduce how alternative reaction conditions or cycling profile changes might affect amplification efficiency.

An alternative use of the Positive Control is to test the compatibility of a DNA sample with PCR conditions. In this manner, the control template is titrated into reactions that contain a fixed amount of test or sample DNA. Successful amplification of the control template will indicate that the genomic DNA sample is compatible with the reaction conditions used (i.e., inhibitory agents are not present). This control is especially useful when attempting to amplify DNA isolated in the presence of high concentrations of denaturants. A decrease in the PCR amplification of the control template in the presence of sample DNA more than likely suggests that inhibitory agents are in the sample and that further purification is required.

Table 5 lists component volumes for setting up a control reaction. These conditions should serve as an excellent starting point. For a complete discussion on using internal controls in PCR, see reference 12.

Table 5. Recommended Reaction Volumes and Final Concentrations When Using the GoTaq® PCR Core System II Controls.

Component	Final Volume	Component Concentration
MgCl ₂ , 25mM Solution	3µl	1.5mM
5X Colorless GoTaq® Flexi Buffer		
OR 5X Green GoTaq® Flexi Buffer	10µl	1.0X
PCR Nucleotide Mix, 10mM	1µl	0.2mM
Upstream Control Primer, 15µM	3.3µl	1.0µM
Downstream Control Primer, 15µM	3.3µl	1.0µM
GoTaq® DNA Polymerase, 5u/µl	0.25µl	1.25u/50µl
Positive Control Plasmid DNA, 1ng/µl	1µl*	1ng/50µl
Nuclease-Free Water to a final volume of	50µl	

*One nanogram of the Positive Control Plasmid is equivalent to 2.2×10^8 molecules. The 323bp control amplification product is routinely observed when starting with $<10^5$ molecules.


 Completely thaw and thoroughly vortex the MgCl₂, 25mM Solution, the buffers and any frozen solutions containing DNA.

Table 6. Thermal Cycling Guidelines for the GoTaq® PCR Core System II Controls.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5-1 minute	
Annealing	60°C	1 minute	25-35 cycles
Extension	72°C	2 minutes	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	indefinite	1 cycle

These guidelines may need to be adapted for your thermal cycler.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

Symptoms	Causes and Comments
Low yield or no amplification	<p>Insufficient number of cycles. Return reactions to thermal cycler for 5 or more cycles.</p> <p>Template degraded. Verify the integrity of the DNA by electrophoresis after incubation in the presence of Mg²⁺.</p> <p>Thermal cycler programmed incorrectly. Verify that times and temperatures are correct. Use step cycles, not hold segments.</p> <p>Temperature too low in some positions of thermal cycler. Perform a set of control reactions to determine whether certain positions in the thermal cycler give low yields.</p> <p>Top of thermal cycler is open. The top must be closed for correct heating and cooling.</p> <p>Inhibitor present in DNA template. Reduce the volume of sample in the reaction. Ethanol precipitate template DNA to remove inhibitor.</p> <p>Improper reaction conditions. Reduce the annealing temperature and/or allow longer extension times for longer amplifiers. Also, optimize Mg²⁺ concentration.</p> <p>Missing reaction component. Check the reaction components and repeat the reaction.</p> <p>Mineral oil problem. The reaction must be overlaid with high-quality, nuclease-free light mineral oil. Do not use autoclaved mineral oil.</p>

Symptoms	Causes and Comments
Low yield or no amplification (continued)	<p>Poor primer design. Make sure primers are not self-complementary or complementary to each other.</p> <p>Incorrect primer specificity. Verify that the primers are complementary to the appropriate strands.</p> <p>Primer concentration too low. Verify primer concentration in the reaction and increase the concentration as needed.</p> <p>Suboptimal reaction conditions. Optimize Mg²⁺ concentration, annealing temperature and extension time. Always vortex the Mg²⁺. Verify that primers are present in equal concentrations.</p> <p>Degraded nucleotides or primers. Keep nucleotides and primers frozen in aliquots, thaw quickly and keep on ice once thawed. Avoid multiple freeze-thaw cycles.</p> <p>Target sequence not present in target DNA. Redesign experiment or try other sources of target DNA.</p>
Low yield or no amplification in mouse tail genotyping applications	<p>Adjust annealing temperature. The reaction buffer composition affects the melting properties of DNA. See the BioMath Calculator to calculate the melting temperature for primers in the GoTaq® reaction (www.promega.com/biomath). The salt-adjusted and base-stacking T_ms are lower in GoTaq® reaction buffer than in Taq DNA polymerase reaction buffer.</p> <p>Reduce volume of template DNA in reaction or dilute template DNA prior to adding to reaction. Many DNA preparations for genotyping applications result in the copurification of amplification inhibitors. Reducing the amount of inhibitors present in the reaction may improve results. Diluting samples even 1:10,000 has been shown to be effective, depending on initial DNA concentration.</p> <p>Ethanol precipitate template DNA. Include an ethanol precipitation and wash step prior to amplification to remove inhibitors that copurified with the DNA.</p> <p>Add PCR enhancers. Adding PCR-enhancing agents may improve yields. General stabilizing agents such as BSA (Sigma Cat.# A7030; final concentration 0.16mg/ml) may also help to overcome amplification failure.</p>

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Multiple, nonspecific amplification products	<p>Suboptimal reaction conditions. Optimize MgCl₂ concentration, annealing temperature, size, extension time and cycle number to minimize nonspecific priming.</p> <p>Poor primer design. Make sure primers are not self-complementary or complementary to each other, especially near the 3' ends. Try a longer primer. Avoid use of three consecutive G or C nucleotides at the 3' end of the primers.</p> <p>Primer concentration too high. Verify primer concentration in the reaction. Try a lower concentration in the reaction.</p> <p>Contamination by another target DNA.</p> <ul style="list-style-type: none"> Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. Use a separate work area and pipettor for pre- and post-amplification. Wear gloves and change them often. Use UNG (11) or another contamination control technique to prevent DNA carryover to subsequent reactions. <p>Multiple target sequences exist in target DNA. Design new primers with higher specificity to target sequence.</p>

7. References

- Saiki, R. *et al.* (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**, 1350-4.
- Saiki, R.K. *et al.* (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487-91.
- Eckert, K.A. and Kunkel, T.A. (1990) High fidelity DNA synthesis by the *Thermus aquaticus* DNA polymerase. *Nucleic Acids Res.* **18**, 3739-44.
- Williams, J.F. (1989) Optimization strategies for the polymerase chain reaction. *BioTechniques* **7**, 762-9.
- Ellsworth, D.L., Rittenhouse, K.D. and Honeycutt, R.L. (1993) Artfactual variation in randomly amplified polymorphic DNA banding patterns. *BioTechniques* **14**, 214-7.
- Longley, M.J., Bennett, S.E. and Mosbaugh, D.W. (1990) Characterization of the 5' to 3' exonuclease associated with *Thermus aquaticus* DNA polymerase. *Nucleic Acids Res.* **18**, 7317-22.

- Sardelli, A.D. (1993) Plateau effect—understanding PCR limitations. *Amplifications* **9**, 1-6.
- Bell, D.A. and DeMarini, D.M. (1991) Excessive cycling converts PCR products to random-length higher molecular weight fragments. *Nucleic Acids Res.* **19**, 5079.
- Baldino, F., Chesselet, M.F. and Lewis, M.E. (1989) High-resolution in situ hybridization histochemistry. *Methods Enzymol.* **168**, 761-77.
- Rychlik, W., Spencer, W.J. and Rhoads, R.E. (1990) Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Res.* **18**, 6409-12.
- Longo, M.C., Berninger, M.S. and Hartley, J.L. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* **93**, 125-8.
- Clementi, M. *et al.* (1993) Quantitative PCR and RT-PCR in virology. *PCR Methods Appl.* **2**, 191-6.

8. Appendix

8.A. Composition of Solutions

PCR Nucleotide Mix, 10mM
 Composed of 10mM each of dATP, dCTP, dGTP and dTTP in water.

8.B. Related Products

For more detailed product information, please visit: www.promega.com/catalog/

Real-Time Quantitative Amplification

Product	Size
Plexor® qPCR System	200 reactions
Plexor® One-Step qRT-PCR System	200 reactions
Plexor® Two-Step qRT-PCR System	200 reactions

For Research Use Only. Not for use in diagnostic procedures. For more information, go to: www.promega.com/plexorresources/

Thermostable Enzymes

Product	Size
GoTaq® Hot Start Polymerase	100u
	500u
	2,500u
	10,000u

Contents include 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and Magnesium Chloride Solution, 25mM. Reaction buffers are magnesium-free.

8.B. Related Products (continued)

Thermostable Enzymes

Product	Conc.	Size
GoTaq® Green Master Mix	5X	100 reactions
	5X	1,000 reactions

GoTaq® Green Master Mix is a premixed solution of GoTaq® Polymerase, GoTaq® Green Reaction Buffer, dNTPs and Mg²⁺. One reaction is 50µl.

Product	Conc.	Size
GoTaq® Colorless Master Mix	5X	100 reactions
	5X	1,000 reactions

GoTaq® Colorless Master Mix is a premixed solution of 5X Colorless GoTaq® Flexi Buffer, dNTPs and Mg²⁺. One reaction is 50µl.

Product	Conc. (u/µl)	Size
GoTaq® Flexi DNA Polymerase	5	100u
	5	500u
	5	2,500u
	5	5,000u
	5	10,000u

Contents include 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and Magnesium Chloride Solution, 25mM. Reaction buffers are magnesium-free.

Product	Conc. (u/µl)	Size
GoTaq® DNA Polymerase	5	100u
	5	500u
	5	2,500u

Contents include 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer. Both buffers provide 1.5mM MgCl₂ in the final 1X concentration.

Product	Size
TaqBead™ Hot Start Polymerase, 1.25 units/bead, Nonbarrier	100 reactions

Product	Conc. (u/µl)	Size
Tfi DNA Polymerase	5	100u
	5	1,000u
Tli DNA Polymerase	3	50u
Tth DNA Polymerase	5	100u
	5	500u
Pfu DNA Polymerase*	2-3	100u
	2-3	500u

*Not available in Canada and United States.

Reverse Transcription and RT-PCR

Product	Size
Access RT-PCR System	100 reactions
Access RT-PCR Introductory System	20 reactions
AccessQuick™ RT-PCR System	20 reactions
	100 reactions
	500 reactions
Reverse Transcription System	100 reactions
AMV Reverse Transcriptase	300u
M-MLV Reverse Transcriptase	10,000u
M-MLV Reverse Transcriptase, RNase H Minus*	10,000u
M-MLV Reverse Transcriptase, RNase H Minus, Point Mutation*	10,000
	50,000

*Not available in Canada and United States.

Product	Size
Recombinant RNasin® Ribonuclease Inhibitor	2,500u
RQ1 RNase-Free DNase	1,000u
RNasin® Plus RNase Inhibitor	2,500u
	10,000u

Markers

Product	Size
BenchTop 1kb DNA Ladder	600µl (100 lanes)
BenchTop 100bp DNA Ladder	300µl (50 lanes)
100bp DNA Ladder	250µl (50 lanes)
1kb DNA Ladder	500µl (100 lanes)

PCR Clean-Up System

Product	Size
Wizard® SV Gel and PCR Clean-Up System	50 preps
	250 preps

PCR Cloning

Product	Size
pGEM®-T Easy Vector System I	20 reactions

⁽⁴⁾Use of Cat.# M7660 and M7665 in the US for basic PCR is outside of any valid US patents assigned to Hoffman La-Roche or Applera. These products can be used in the US for basic PCR in research, commercial or diagnostic applications without any license or royalty fees.

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⁽⁵⁾U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175 and European Pat. No. 1088060 have been issued to Promega Corporation for enzyme stabilization by cationic surfactants. Other patents are pending.

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