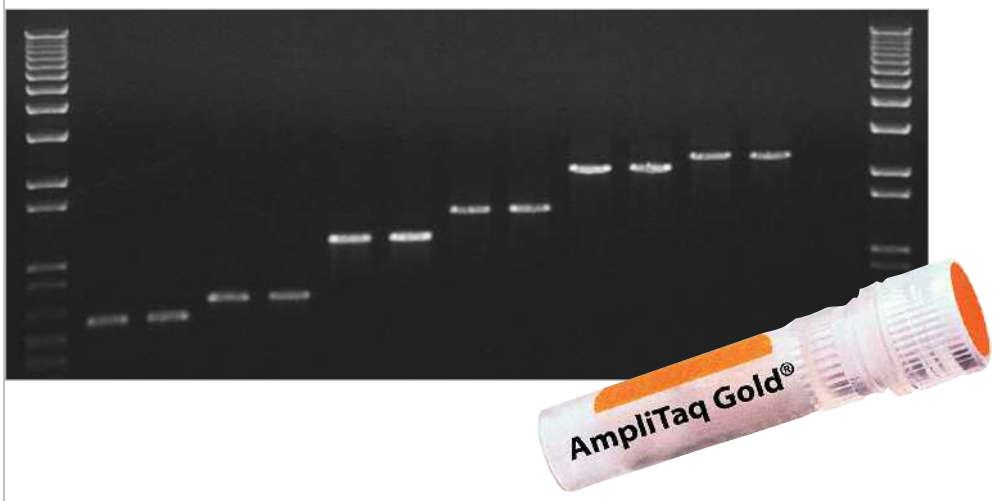
Applied Biosystems

The most referenced brand of DNA Polymerase in the world

AmpliTaq Gold®

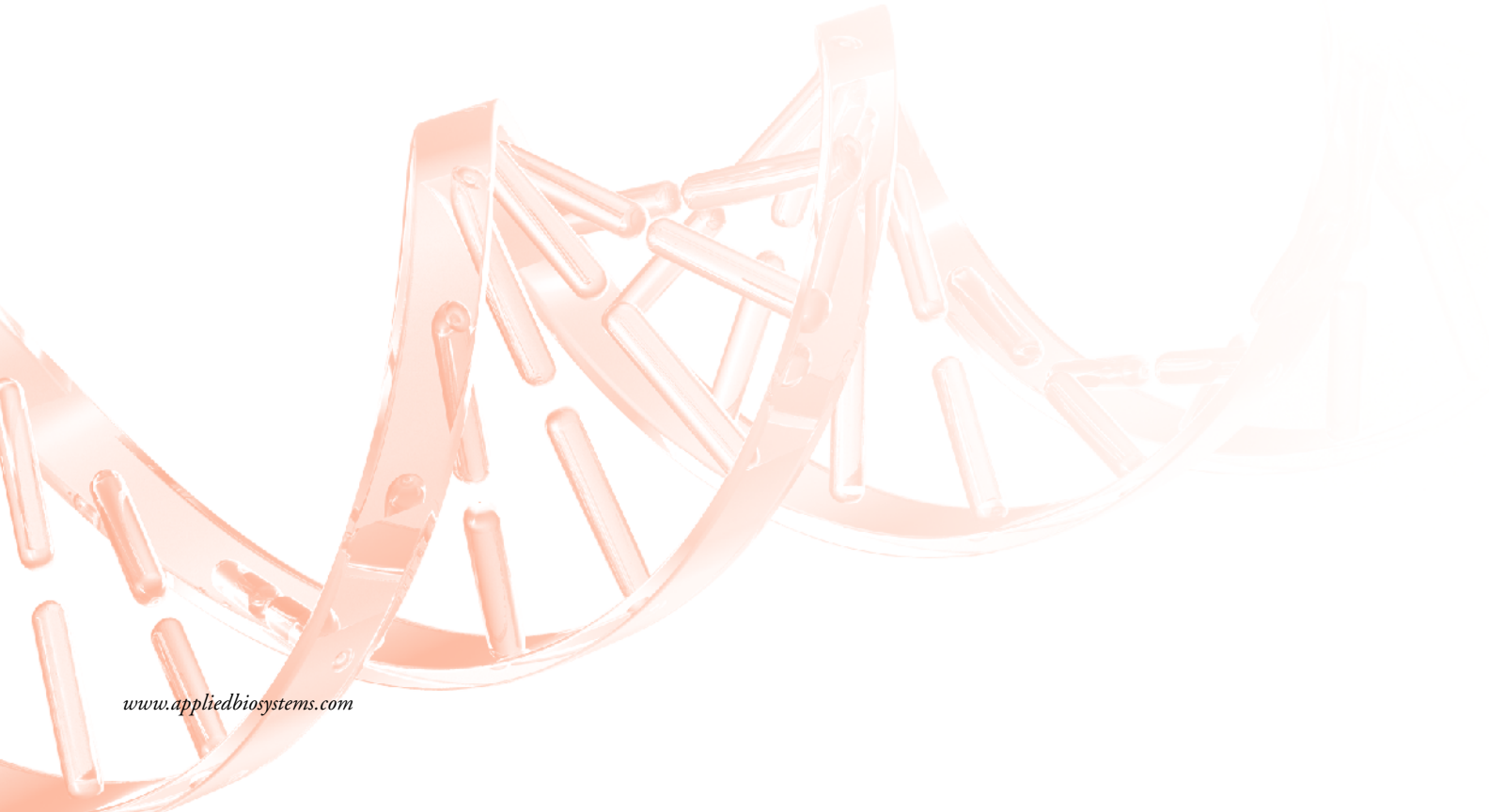
DNA Polymerase

High Specificity and Yield with AmpliTaq Gold® DNA Polymerase



Rely on trusted PCR solutions from Applied Biosystems for:

- High yield and uncompromised specificity
- Chemical hot start – providing better long-term stability than antibody-based hot start
- Time-release enzyme activation – improves sensitivity in low copy number amplification
- Proven and reproducible amplifications (over 9,000 journal references)



Guidelines to Make Your Research More Successful

There are many factors that are important for accurate amplification in the PCR process. The following guidelines are intended to help researchers understand many of the factors that influence the performance of PCR amplifications, allowing for efficient PCR optimization while taking advantage of the unique benefits of using AmpliTaq Gold® enzyme. Since AmpliTaq® and AmpliTaq Gold® DNA Polymerases are the most referenced brands of DNA polymerase in the world, the guidelines have been validated by the research community.

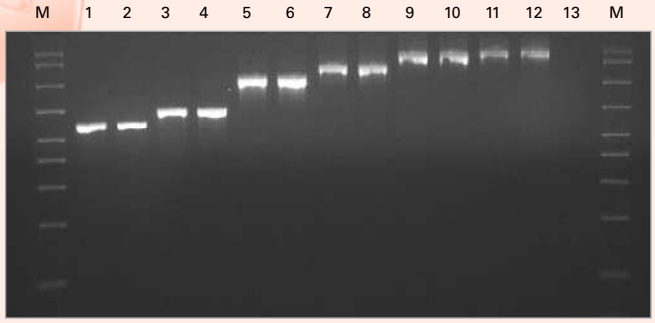
Proven Results for Multiple Applications

With over 9,000 references and still growing at about 1000 per year, AmpliTaq® and AmpliTaq Gold® DNA Polymerases are used for a wide breadth of applications. As the most referenced brand of DNA polymerase in the world, the results have been published in peer-reviewed journals using the best validation possible, the scientific community. For a list of selected references or recent publications, please see the 'Selected Bibliography by Application.'

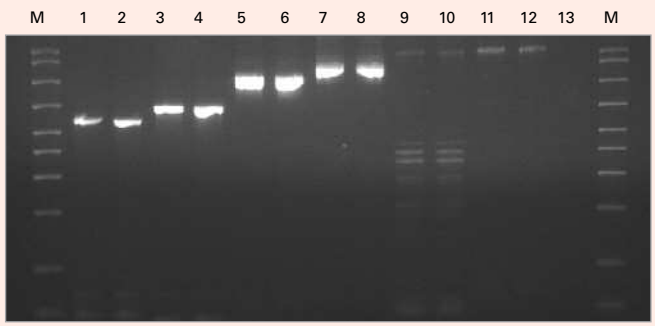
High Specificity and High Yield

In conventional PCR amplifications, where active reaction components are subjected to lower than desired annealing temperatures, non-specific priming can occur. This will result in non-specific products and primer-dimer formation. Once non-specific products are formed, either during set-up or in the initial cycle, they are amplified throughout the remaining PCR cycles. This can reduce the sensitivity and yield of the experiment either by decreasing the desired amplification signal or by obscuring it with high background. In contrast, when non-specific products are avoided, the polymerase is directed by the desired target only. This will increase the specificity and yield of the target by decreasing non-specific annealing (Figure 1).

Figure 1. Increased Specificity and Yield with AmpliTaq Gold® DNA Polymerase



A. AmpliTaq Gold® DNA Polymerase



B. Competitor hot-start DNA Polymerase

Lane	Description	Size
Lane M	Molecular Weight Standard	
Lane 1,2	KAI1	620 bp
Lane 3,4	ANAT	700 bp
Lane 5,6	CRIM	1 kb
Lane 7,8	p53	1.4 kb
Lane 9,10	USP-36	2.0 kb
Lane 11,12	TPA	2.3 kb
Lane 13	No Template Control	

AmpliTaq Gold® DNA Polymerase eliminates amplification of non-specific products, resulting in a substantial increase in specificity and yield when compared to Competitor hot-start polymerase. A series of six different targets were amplified from human genomic DNA using the GeneAmp® PCR System 9700. Reactions in Figure 1B were performed according to manufacturer's recommendations.

AmpliTaq Gold® DNA Polymerase– The "Gold Standard" in Hot-Start Enzymes.

Hot-start PCR is a simple modification of the original PCR process in which the amplification reaction is started at an elevated temperature. AmpliTaq Gold® DNA Polymerase is a chemically modified form of AmpliTaq® DNA Polymerase. This modification renders the enzyme inactive. Upon thermal activation (i.e., typically 5-10 minutes at 95°C), the modifier is permanently released, resulting in an active enzyme.

Hot-start PCR can increase amplification specificity and yield (Figure 2). However, manual hot start is very inconvenient and can cause problems with reproducibility and contamination. The chemical modification in AmpliTaq Gold® DNA Polymerase allows an 'automated' hot start via the release of the chemical modifier.

The increase of amplification specificity and sensitivity makes AmpliTaq Gold® DNA Polymerase an ideal choice for the majority of PCR applications (Figures 1 & 2).

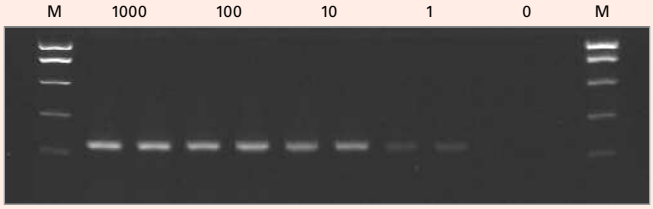
Enhanced Sensitivity

AmpliTaq Gold® Polymerase efficiently amplifies targets present at low copy number (Figure 2), even in the presence of high concentration of complex DNA making it especially suited for low-copy pathogen detection, multiplex PCR, allelic discrimination and amplification of targets from degraded DNA samples.

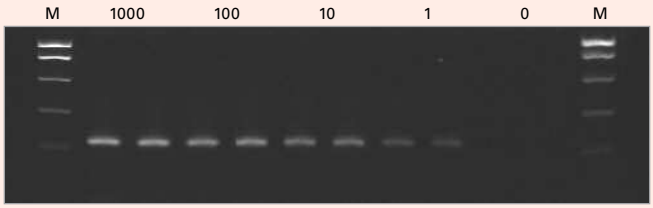
Enhanced Specificity

A high-temperature incubation step is required to activate AmpliTaq Gold® DNA Polymerase, which ensures that the active enzyme is generated only at temperatures in which the DNA is fully denatured and when the primer is not annealed. When AmpliTaq Gold® DNA Polymerase is added to the reaction mixture at room temperature, primer extension does not occur because the enzyme is inactivated. Any low stringency mispriming events that may have occurred will not be enzymatically extended and will not be amplified (Figure 2). Hence, PCR setup on many samples can be performed at room temperature without concern for extension at misprimed sites. The amount of AmpliTaq Gold® DNA polymerase increases in the reaction slowly with each cycle number, and specific product yield increases without buildup of misprimed products.

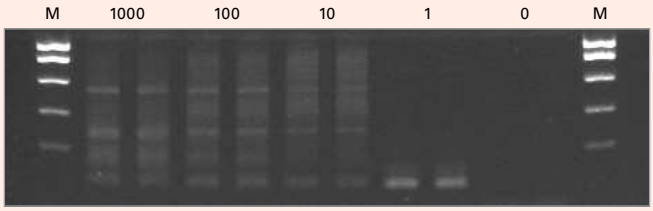
Figure 2. Greater Sensitivity of Low Copy Target DNA in the Presence of a High Background of Nucleic Acid



A. AmpliTaq Gold® DNA Polymerase



B. AmpliTaq® DNA Polymerase with manual hot start



C. Standard Taq Polymerase without hot start

Compared to a manual hot-start technique, AmpliTaq Gold® DNA Polymerase increases amplification sensitivity and specificity of low copy number HIV target DNA detection (10-1000 copies) in the presence of 1 µg of human placental DNA.

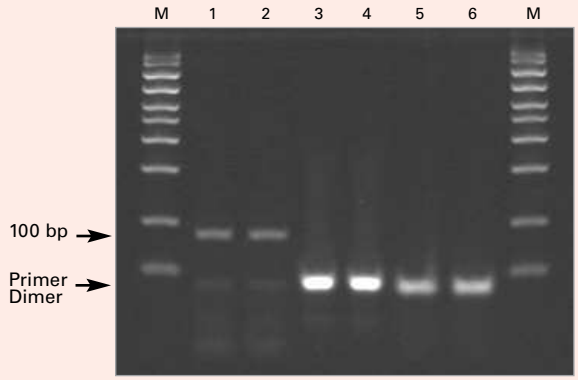
Duplicates of HIV-1 amplification reactions were analyzed after 40 cycles using the enzyme specified above.

High Specificity and Thermostability

Greater Specificity than Antibody-Based Hot-Start Systems

As a result of its stringent chemical modification, AmpliTaq Gold® DNA Polymerase provides greater selectivity and specificity when compared to antibody-mediated hot-start enzymes (Figure 3).

Figure 3. Greater Specificity with AmpliTaq Gold® DNA Polymerase



Lane	Description
Lane M	Molecular Weight Marker
Lane 1,2	AmpliTaq Gold® DNA Polymerase
Lane 3,4	Supplier A Antibody Hot Start
Lane 5,6	Supplier B Antibody Hot Start

AmpliTaq Gold® DNA Polymerase was shown to outperform two antibody-based hot-start enzymes based on amplification of specific PCR product and suppression of formation of non-specific PCR products (primer dimer).

Allelic Discrimination

The hot-start feature of AmpliTaq Gold® DNA Polymerase allows it to discriminate effectively between the amplification of very similar sequences, such as two different alleles of the same gene. Because the enzyme is activated only by temperatures at or above a stringent annealing temperature, assays can be designed so that only primers 100% homologous to the target sequence will hybridize and be extended.

In conventional PCR, primers can hybridize to similar (but not necessarily 100% homologous) sequences during PCR set up and initial temperature ramp up. At lower temperatures, these mismatched primers can be extended by the enzyme. These non-specific products will then contain homologous primer binding sites and be amplified efficiently even at stringent annealing temperatures.

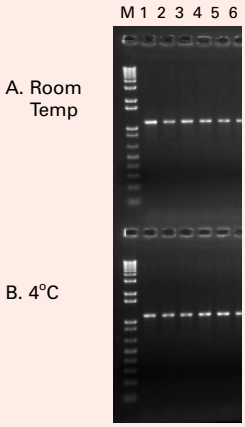


Figure 4. Enhanced Stability Using AmpliTaq Gold® DNA Polymerase

Lane	Description
Lane M	Marker
Lane 1	0 hours
Lane 2	24 hours
Lane 3	72 hours
Lane 4	96 hours
Lane 5	120 hours
Lane 6	1 week

Amplification of a 1.2 kb CRIM target from 20 ng of human genomic DNA using AmpliTaq Gold® DNA Polymerase. All the components for PCR amplification (AmpliTaq Gold® enzyme, primers, template, dNTPs, $MgCl_2$ and Gold Buffer) were combined into one complete master mix. One half of the master mix was left at room temperature and the other was kept at 4°C. 20 µl aliquots were removed and PCR amplifications were performed at various time intervals up to one week. Robust performance of AmpliTaq Gold® DNA Polymerase is seen even after storage at room temperature or 4°C for one week.

Enhanced Stability

AmpliTaq Gold® DNA Polymerase is stable at room temperature even after one week. This superior stability provides you better flexibility allowing you anywhere from two hours to two days to set up and run your PCR reactions, while preventing any non-specific products from forming (Figure 4).

Buffer Selection

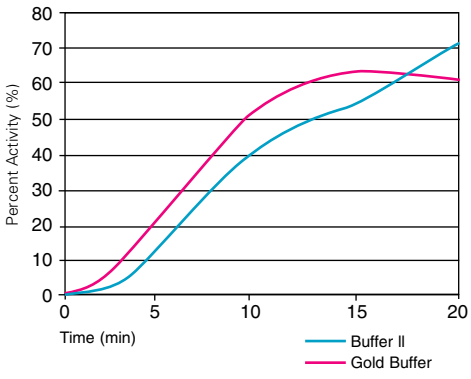
AmpliTaq Gold® DNA Polymerase activation is a process dependent on time, temperature and pH. It can be precisely controlled to deliver the optimum amount of enzyme activity throughout the PCR process. AmpliTaq Gold® DNA Polymerase is provided with 3 different buffer options:

Buffer	Composition			
	Tris-HCL, pH	KCl	$MgCl_2$	Gelatin
10X PCR Buffer I	100 mM, pH 8.3	500 mM	15 mM	0.01%
10X PCR Buffer II	100 mM, pH 8.3	500 mM	Separate Vial*	- - - - -
10X PCR Gold Buffer	150 mM, pH 8.0	500 mM	Separate Vial*	- - - - -

*$MgCl_2$ is sold separately at a concentration of 25 mM.

Buffer I and II provide the same Tris and KCl concentration and pH. Buffer I has a fixed $MgCl_2$ concentration while Buffer II contains a separate vial of $MgCl_2$. Gold Buffer is formulated to provide flexible, efficient activation of AmpliTaq Gold® DNA Polymerase resulting in greater specificity and robustness.

Figure 5. Quicker Activation of AmpliTaq Gold® DNA Polymerase at 95°C Using Gold Buffer Compared to Buffer II.



Gold Buffer allows for quicker activation time of AmpliTaq Gold® DNA Polymerase and increased rate of the PCR reaction.

Advantages of Gold Buffer

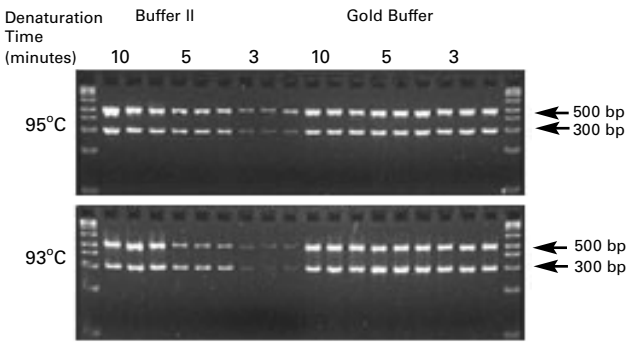
Gold Buffer enhances the activation of AmpliTaq Gold® DNA Polymerase. The use of Gold Buffer allows for quicker activation time and increased rate of the PCR reaction (Figure 5). Gold Buffer also provides a broader time and temperature range for enzyme activation (Figure 6).

This combination of enzyme and buffer improves the initial PCR success rate. The increased range for time and temperature also compensates for variations in thermal cycler temperatures.

Multiplex PCR

Multiplex PCR is an amplification technique in which multiple primer sets are used to amplify multiple specific targets simultaneously (Figure 6). This saves both time and reagents because a single reaction can yield multiple desired products. Applications that previously required two or more reactions can now be performed in a single reaction. The unprecedented specificity of AmpliTaq Gold® DNA Polymerase makes successful multiplexing possible.

Figure 6. Robust performance of AmpliTaq Gold® DNA Polymerase with Gold Buffer Using 93°C and 95°C Activation Temperatures



Multiplex Lambda targets were amplified using AmpliTaq Gold® DNA Polymerase with Buffer II and Gold Buffer with variations of time and temperature during the initial denaturing step of the PCR. The combination of AmpliTaq Gold® DNA Polymerase and Gold Buffer gave similar yield at both 93°C and 95°C even when the denaturation time was lowered from 10 minutes to 3 minutes. Hence, Gold Buffer provides a broader denaturation time and temperature range allowing for quicker activation and better yield.

Conclusion

When used as recommended, AmpliTaq Gold® DNA Polymerase increases amplification specificity and sensitivity, which improves product yield. This increased specificity makes AmpliTaq Gold® DNA Polymerase ideal for standard PCR reactions or challenging applications such as multiplex PCR or allelic discrimination. The enzyme efficiently amplifies low-copy targets even in the presence of high concentrations of complex DNA. Thus, it is especially useful for pathogen detection and the amplification of degraded DNA sample. Furthermore, because AmpliTaq Gold® DNA Polymerase is completely inactive at room temperature (unlike antibody-mediated hot-start enzymes), reactions can be set up in advance without the fear of amplifying nonspecific sequences. This saves time without compromising performance. These conclusions have been validated in the greater scientific community since AmpliTaq Gold® DNA Polymerase is the most referenced brand of DNA polymerase in the world.

Other PCR Enzymes

For longer targets (>5Kb), or for higher fidelity use GeneAmp® High Fidelity PCR System. It is a blend of AmpliTaq Gold® DNA Polymerase and a proofreading enzyme. GeneAmp® High Fidelity Polymerase offers you the advantages of:

- Higher yield generates more DNA for your research
- Longer targets (up to 10Kb) provides greater flexibility
- Higher fidelity (up to 3 times greater fidelity than Taq alone)

For faster PCR results use GeneAmp® Fast PCR Master Mix. It offers you:

- Time Savings – up to 66% reduction in PCR cycling time
- High Specificity – due to quick-activating hot-start chemistry
- Convenience of a PCR master mix

Enzyme Selection Guide

Enzyme	Amplicon Size	Specificity	Sensitivity	Yield	Fidelity	Convenience	Fast
AmpliTaq® DNA polymerase	≤5Kb	••	••	••	•••	••	••
AmpliTaq Gold® DNA polymerase	≤5Kb	••••	••••	••••	•••	•••	•
AmpliTaq Gold® PCR Master Mix	≤5Kb	••••	••••	••••	•••	•••••	•
AmpliTaq® DNA polymerase,LD	≤5Kb	•••	•••	••	•••	••	••
AmpliTaq Gold® DNA polymerase, LD	≤5Kb	•••••	•••••	••••	•••	•••	•
AmpliTaq® DNA polymerase, Stoffel Fragment	≤2Kb	•••	•••	•••	•••	••	•••
GeneAmp® Fast PCR Master Mix	≤2Kb	•••	••••	••••	•••	•••••	•••••
GeneAmp® High Fidelity PCR System	≤10Kb	••••	•••	•••••	•••••	•••	•
True Allele® PCR Premix	≤5Kb	••••	••••	••••	•••	••••	•
rTth DNA polymerase	≤5Kb	•••	•••	••••	•••	••	••
rTth DNA polymerase, XL	≤40Kb	••••	•••	••••	••••	••	••

Reagent Optimization Guidelines

About Reagent Optimization

Optimizing reactions for each primer-template pair may be necessary. One can achieve optimization by following the suggested guidelines for primer design and by varying the concentrations of the following reagents:

• Template • Primer • $MgCl_2$ • dNTPs • Enzyme

The effect of the above variables can be monitored by examining the intensity and distribution of amplification products after electrophoresis on agarose gel followed by visualization with ethidium bromide staining of the gel.

Optimizing the Template Concentration

- Start with enough copies of the template to obtain better yield after 25 to 30 cycles. Preferably use more than 10^4 copies and less than 1 µg of human genomic DNA per 50 µl reaction.
- If the target DNA concentration is low, more than 35 cycles may be required to produce sufficient product for analysis. As few as 1 to 10 target copies can be amplified. Validation for low copy number amplification is best done for an average of 5 to 10 target molecules per sample to avoid statistically arising dropouts (false negatives).

Designing the Primers

Use the following guidelines when designing your primers:

- The single-stranded DNA primers should be 15-30 bases in length.
- The %G+C of the primers should be near 50% to maximize specificity.
- To avoid potential problems, primers should be purified by gel electrophoresis or HPLC ion-exchange chromatography.
- Primer sequences should not complement within themselves or to each other, particularly at the 3' ends. This avoids template independent amplification of primer sequence (primer-dimer). Primer-dimer may occur to some extent even without an apparent overlap.

Optimizing the Primer Concentrations

Use the following guidelines to optimize the primer concentration

- Optimal primer concentration can be determined empirically by testing concentrations in the range of 0.1 to 1 µM.
- Primer concentrations that are too low will result in little or no PCR product. Primer concentrations that are too high may result in amplification of non-target sequences, which are evidenced by secondary bands and/or smearing when viewed on a gel.
- Primer concentrations in the range of 0.2 to 0.5 µM will work for most PCR amplifications.

Optimizing the $MgCl_2$ Concentrations

The magnesium ion concentration required for optimal PCR amplification is dependent on the specific set of primers and template. Too much or too little $MgCl_2$ reduces amplification efficiency or results in amplification of non-target sequences. The optimal $MgCl_2$ concentration must be determined empirically. $MgCl_2$ concentration should also be adjusted when the concentrations of sample DNA and/or dNTPs change significantly.

Optimizing the dNTP Concentrations

The dNTP concentration in the reaction mix is balanced. If the blend is altered and the concentration of any one dNTP is significantly different from the rest, then AmpliTaq Gold® DNA Polymerase will tend to misincorporate bases and slowdown the reaction leading to lower yields.

Optimizing the Enzyme Concentrations

Increasing the AmpliTaq Gold® DNA Polymerase concentration up to 2X the recommended amount may improve the yield of amplification product.

Selected Bibliography by Application

AmpliTaq Gold® DNA Polymerase is the most referenced brand of DNA polymerase in the world.

Allele-Specific PCR

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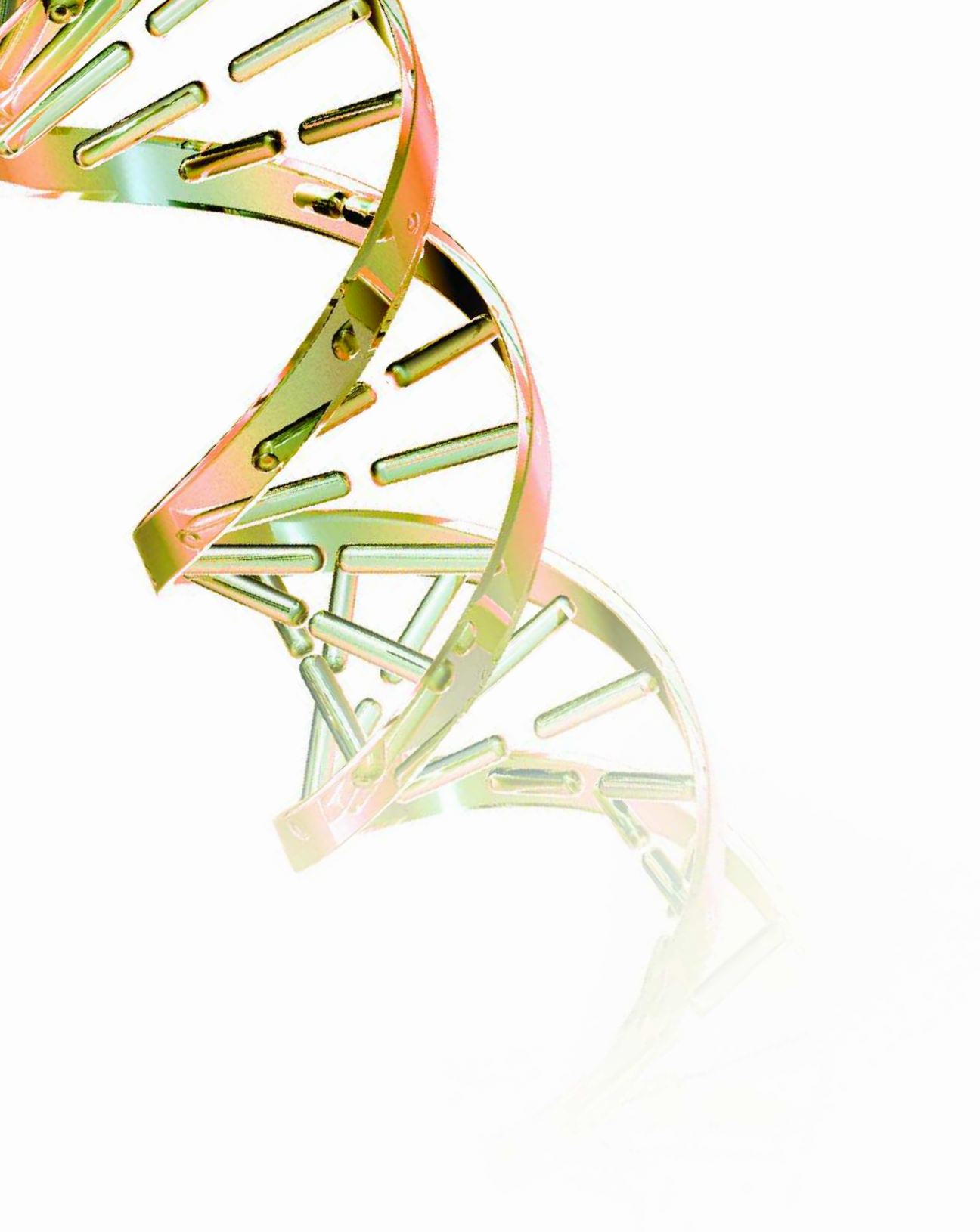
PCR Enzymes

DESCRIPTION	PACK SIZE	PART NUMBER
For the convenience and specificity of Hot-Start Taq DNA Polymerase:		
AmpliTaq Gold® with GeneAmp® PCR Gold Buffer	250 Units	4311806
	1000 Units	4311816
	6 X 250 Units	4311814
	12 X 250 Units	4311820
	5 X 1000 Units	4311818
	25 X 1000 Units	4317742
	25,000 Units	4311858
AmpliTaq Gold® with 10X Buffer I	250 Units	N8080240
	1000 Units	N8080246
	6 X 250 Units	N8080242
	12 X 250 Units	N8080244
	5 X 1000 Units	N8080248
	25 X 1,000 Units	N8080258
	25,000 Units/vial	N8080255
AmpliTaq Gold® with 10X Buffer II and $MgCl_2$	250 Units	N8080241
	1000 Units	N8080247
	6 X 250 Units	N8080243
	12 X 250 Units	N8080245
	5 X 1000 Units	N8080249
	25 X 1000 Units	N8080259
	25,000 Units/vial	N8080256
For the value of Taq DNA Polymerase:		
AmpliTaq® with 10X Buffer I	250 Units	N8080160
	1000 Units	N8080171
	6 X 250 Units	N8080166
	12 X 250 Units	N8080152
	5 X 1000 Units	N8080155
	25 X 1000 Units	N8080185
	25,000 Units/vial	N8080195
AmpliTaq® with 10X Buffer II and $MgCl_2$	250 Units	N8080161
	1000 Units	N8080172
	6 X 250 Units	N8080167
	12 X 250 Units	N8080153
	5 X 1000 Units	N8080156
	25 X 1000 Units	N8080186
	25,000 Units/vial	N8080196
For an enzyme with low DNA and very high purity:		
AmpliTaq Gold® DNA Polymerase, LD with 10X Gold Buffer & $MgCl_2$	250 Units	4338856
	4 x 250 Units	4338857
AmpliTaq® DNA Polymerase, LD with 10X Buffer I	250 Units	N8080157
AmpliTaq® DNA Polymerase, LD with 10X Buffer II and $MgCl_2$	250 Units	N8080158
For high yield and moderate fidelity:		
GeneAmp® High Fidelity PCR System	100 Units	4328216
	2 x 250 Units	4328217
For faster amplification:		
GeneAmp® Fast PCR Master Mix (2X) without protocol	250 Reactions	4359187
GeneAmp® Fast PCR Master Mix (2X) with protocol	250 Reactions	4362070



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