AmpliTaq® 360 DNA Polymerase

Protocol

Applied Biosystems

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Contents

Preface

This preface covers:

Safety information

Note: For general safety information, see this Preface and Appendix C on page 23. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "Obtaining MSDSs" on page 26.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

How to use this guide

Purpose of this guide

The *AmpliTaq® 360 DNA Polymerase Protocol* provides all the information you need to perform PCR over a wide range of DNA templates, including some of the most challenging GC-rich sequences.

Audience

This guide is intended for biologists who have had some experience performing PCR.

Assumptions

This guide assumes that your thermal cycler has been installed by an Applied Biosystems technical representative.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

AmpliTaq® 360 DNA Polymerase Protocol

This chapter covers:

Product information

Purpose of the product

Use the AmpliTaq® 360 DNA Polymerase to amplify a wide range of DNA sequences using PCR.

About AmpliTaq® 360 DNA Polymerase

AmpliTaq® 360 DNA Polymerase is AmpliTaq DNA Polymerase purified by an additional proprietary separation process to reduce contaminating bacterial DNA sequences from the enzyme preparation. This ultra-pure enzyme reduces false positives and amplifies bacterial templates and very low target sequences. The enzyme is quality-control tested to ensure that, in a 5-unit aliquot of the enzyme, bacterial 16S ribosomal RNA gene sequences are present in low copy number.

About the 360 GC enhancer

The 360 GC Enhancer is used for difficult-to-amplify templates, especially for templates with high GC content. You can adjust the amount of 360 GC Enhancer to optimize the PCR reaction. Amplicons that generate nonspecific products may require small amounts of enhancer to improve specificity. Amplicons with high GC-content require more enhancer.

About this protocol

This protocol provides:

- Procedures and guidelines on PCR using the AmpliTaq® 360 DNA Polymerase
- Information on troubleshooting PCR results
- A list of equipment and materials required for using the AmpliTaq® 360 DNA Polymerase

For more information, refer to the documents shipped with your Applied Biosystems PCR System.

Contents

AmpliTaq® 360 DNA Polymerase contains:

- AmpliTaq® 360 DNA Polymerase
- AmpliTaq® 360 Buffer, 10×
- 25 mM Magnesium Chloride
- 360 GC Enhancer

Note: The 360 GC Enhancer, AmpliTaq® 360 Buffer, 10×, and 25 mM Magnesium Chloride are also available without enzyme.

Available kit packaging

AmpliTaq® 360 DNA Polymerase is available in the following packaging:

Quantity	Part number
Reagents sufficient for 80 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 100 U • AmpliTaq® 360 Buffer, 10X, 1 × 1.5 mL • 360 GC Enhancer, 1 × 1.5 mL • 25 mM Magnesium Chloride, 1 × 1.5 mL	4398808
Reagents sufficient for 200 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 250 U • AmpliTaq® 360 Buffer, 10X, 1 × 1.5 mL • 360 GC Enhancer, 1 × 1.5 mL • 25 mM Magnesium Chloride, 1 × 1.5 mL	4398818
Reagents sufficient for 800 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 1000 U • AmpliTaq® 360 Buffer, 10X, 4 × 1.5 mL • 360 GC Enhancer, 4 × 1.5 mL • 25 mM Magnesium Chloride, 4 × 1.5 mL	4398828
Reagents sufficient for 1200 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 6 × 250 U • AmpliTaq® 360 Buffer, 10X, 6 × 1.5 mL • 360 GC Enhancer, 6 × 1.5 mL • 25 mM Magnesium Chloride, 6 × 1.5 mL	4398891
Reagents sufficient for 2400 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 2 × 1500 U • AmpliTaq® 360 Buffer, 10X, 12 × 1.5 mL • 360 GC Enhancer, 12 × 1.5 mL • 25 mM Magnesium Chloride, 12 × 1.5 mL	4398893
Reagents sufficient for 4000 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 5 × 1000 U • AmpliTaq® 360 Buffer, 10X, 20 × 1.5 mL • 360 GC Enhancer, 20 × 1.5 mL • 25 mM Magnesium Chloride, 20 × 1.5 mL	4398895
Reagents sufficient for 20,000 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 25 × 1000 U • AmpliTaq® 360 Buffer, 10X, 100 × 1.5 mL • 360 GC Enhancer, 100 × 1.5 mL • 25 mM Magnesium Chloride, 100 × 1.5 mL	4398897
Reagents sufficient for 20,000 × 50-µL reactions: • AmpliTaq® 360 DNA Polymerase, 1 × 25,000 U • AmpliTaq® 360 Buffer, 10X, 1 × 150 mL • 360 GC Enhancer, 1 × 120 mL • 25 mM Magnesium Chloride, 1 × 150 mL	4398899
AmpliTaq® 360 DNA Polymerase—standalone, 25,000 U, 1 × 5 mL	4398838

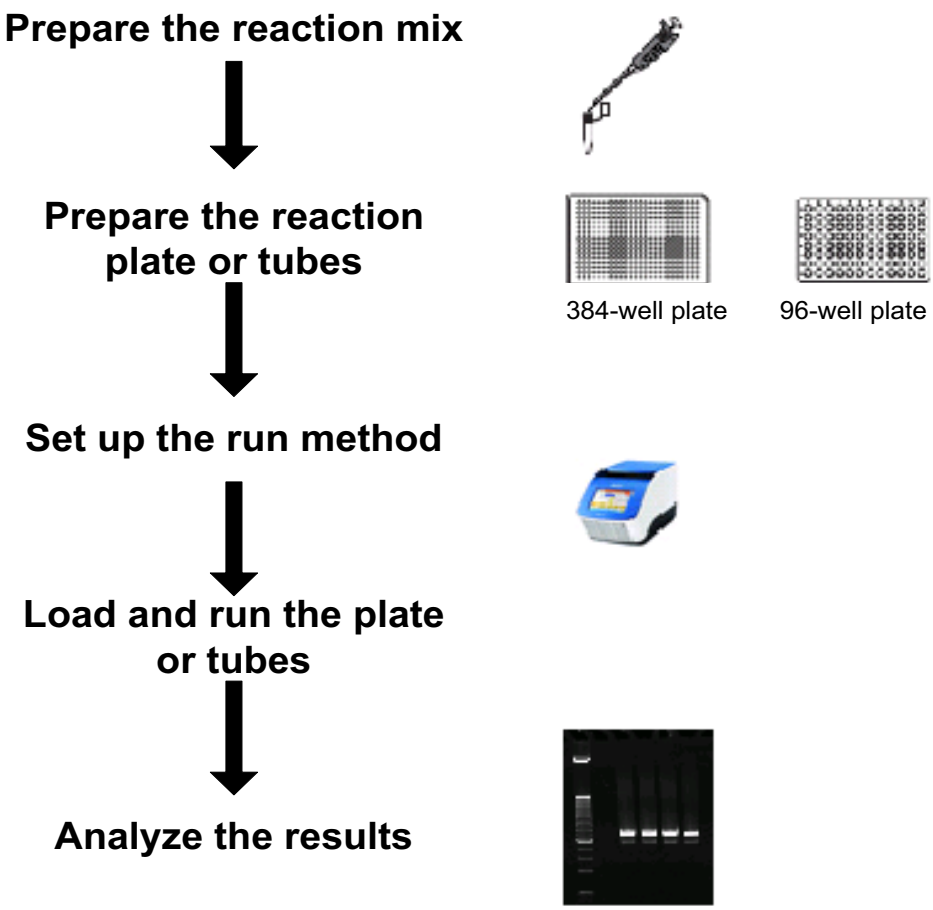
Quantity	Part number
AmpliTaq® 360 Buffer Kit: • AmpliTaq® 360 Buffer, 10X, 1 × 1.5 mL • 360 GC Enhancer, 1 × 1.5 mL • 25 mM Magnesium Chloride, 1 × 1.5 mL	4398848
AmpliTaq® 360 Buffer Kit: • AmpliTaq® 360 Buffer, 10X, 6 × 1.5 mL • 360 GC Enhancer, 6 × 1.5 mL • 25 mM Magnesium Chloride, 6 × 1.5 mL	4398858
AmpliTaq® 360 Buffer Kit: • AmpliTaq® 360 Buffer, 10X, 1 × 150 mL • 360 GC Enhancer, 1 × 120 mL • 25 mM Magnesium Chloride, 1 × 150 mL	4398868

Storage

Store all components at −15 to −25 °C.

Workflow

The workflow for PCR using the AmpliTaq® 360 DNA Polymerase:



Before you perform PCR

Prevent contamination

Review "PCR good laboratory practices" on page 18.

Select an instrument and reaction plate

You can perform PCR amplification with any of the instruments listed under "Thermal cyclers" on page 14. Use MicroAmp™ Optical 96-Well Reaction Plates (PN N8010560). Other recommended equipment and consumables are listed beginning on page 14.

Calculate the number of required reactions

Calculate the number of reactions to perform for each assay. In your calculations, include extra reactions (approximately one extra reaction for every 10 required reactions) to provide excess volume for the volume lost during reagent transfers. For example, for a 96-well plate, prepare enough volume for approximately 110 reactions.

Note: You can run multiple PCRs on one reaction plate. Include controls for each run on the plate.

Perform PCR using AmpliTaq® 360 DNA Polymerase

Prepare the reaction mix

For the following hazards, see the complete safety alert descriptions in "Specific chemical alerts" on page 29.

⚠ **CHEMICAL HAZARD. AmpliTaq® 360 DNA Polymerase, AmpliTaq® 360 Buffer, 10X.**

IMPORTANT! Prepare the reagents on ice. Avoid generating bubbles when mixing the enzyme.

1. Thaw AmpliTaq® 360 Buffer, 10X, 25 mM Magnesium Chloride, dNTP mix, primers, template, and (optional) 360 GC Enhancer on ice, then vortex the reagents before use.
2. Thaw AmpliTaq® 360 DNA Polymerase on ice. Mix the enzyme by gently pipetting it up and down, then put the enzyme on ice.
3. Combine the following components on ice in an appropriate tube according to the volumes that are shown in Table 1. Multiply the volume for one reaction component (Table 1) by the total number of reactions, then add that volume to the tube.

Table 1 PCR reaction mix

Component	Volume Per 25-µL reaction (µL)‡	Volume per 50-µL reaction (µL)	Final concentration
PCR-grade water	Variable	Variable	—
AmpliTaq® 360 Buffer, 10X	2.5	5	1X
25 mM Magnesium Chloride	1 to 4	2 to 8	1.0 to 4.0 mM§
dNTP mix	2#	4#	200 µM each
(Optional) 360 GC Enhancer	0.5 to 5‡‡	1 to 10‡‡	N/A
AmpliTaq® 360 DNA Polymerase	0.125	0.25	1.25 Units per 50-µL reaction§§

‡ If the DNA is difficult to amplify in a 25-µL reaction, performing the PCR in a 50-µL reaction may give better results.

§ The optimal magnesium chloride concentration may vary depending on the primer and template that are used and must be determined by experiment. In most cases, a final concentration of magnesium chloride at 1.8 mM in the reaction mix works well.

Contains a 10-mM solution of dNTP (2.5 mM each of dATP, dCTP, dGTP, and dTTP).

‡‡ For targets with 65 to 75% GC, start with 2.5 µL in a 25-µL reaction or 5.0 µL in a 50-µL reaction (10% (v/v) of the reaction). For targets with >75% GC, start with 5 µL in a 25-µL reaction or 10 µL in a 50-µL reaction (20% (v/v) of the reaction). In general, if increased specificty is required, add 0.5. to 1 µL 360 GC Enhancer per 25-µL reaction or add 1 to 2 µL 360 GC Enhancer per 50-µL reaction (2 to 5% (v/v) of the reaction).

§§ For some difficult-to-amplify targets, up to 5.0 U per 50 µL of reaction can be added.

4. Cap the tube.
5. Gently vortex the tube on a low setting for no more than 5 seconds to mix the components.
6. Centrifuge the tube briefly to spin down the contents and to eliminate air bubbles from the solution.
7. Dispense equal volumes of the PCR reaction mix to the reaction plate or into PCR tubes (see Table 1).
8. Place the plate in a MicroAmp™ Splash-Free 96-Well Base or place the tubes in a MicroAmp™ 96-well Base. Keep the plate or tubes in their respective bases throughout the remainder of the protocol.
9. Seal the plate with MicroAmp™ Clear Adhesive Film or cap the tubes with MicroAmp™ 8-Cap Strips.
10. Centrifuge the plate or tubes to collect the liquid at the bottom of the wells.
11. Put the plate or tubes on ice.

Prepare the reaction plate or tubes

1. Prepare primers and DNA to their appropriate working dilutions (see Table 2). For multiple PCR assays, prepare a master mix of components.
2. With the plate or tubes in the appropriate base, remove the seal from the plate or open the tubes.
3. Add primers and DNA to the appropriate wells or tubes according to Table 2. Include the no-template controls.

Table 2 Primer and DNA mix for PCR reactions

Component	Volume per 25-µL reaction (µL)‡	Volume per 50-µL reaction (µL)	Final concentration
Primer 1	0.5 to 2.5	1 to 5	0.2 to 1.0 µM§
Primer 2	0.5 to 2.5	1 to 5	0.2 to 1.0 µM§
DNA	Variable#	Variable#	<1 µg/reaction‡‡
Total PCR volume	25	50	—

‡ If the DNA is difficult to amplify in a 25-µL reaction, performing the PCR in a 50-µL reaction may give better results.
§ Lowering the primer concentration reduces potential secondary products.
# For a no-template control, add an equivalent volume of water.
‡‡ Preferably >10^4 copies of template but <1 µg DNA/reaction

4. Seal the plate with MicroAmp™ Clear Adhesive Film or cap the tubes with MicroAmp™ 8-Cap Strips.
5. Centrifuge the plate or tubes to collect the liquid at the bottom of the wells or the tubes. Ensure that the wells are uniformly filled.
6. Put the plate or tubes on ice.

Set up the run method

Set the:

- Thermal cycling conditions (Table 3):

Table 3 Three-temperature thermal cycling on a Veriti™, GeneAmp® PCR System 9700, or 2720 Thermal Cycler

Stage	Step	Temp.	Time
Holding	Initial denaturation	94 °C	3 min‡
Cycling (25 to 40 cycles)	Denature	95 °C	30 sec
	Anneal	Primer T_m§	30 sec#
	Extend	72 °C	60 sec/kb
Holding	Final Extension	72 °C	7 min
Holding	Final hold	4 °C	∞

‡ For easy-to-amplify targets, the initial denaturation can be reduced to 2 minutes.
§ Although any primer can be used with this product, Applied Biosystems recommends using primers with T_ms >55 °C. Use the Primer T_m calculator on an Applied Biosystems thermal cycler, or go to **www.appliedbiosystems.com/support/techtools/calc**.
# Thirty seconds for denaturation and annealing is adequate when you use Veriti™ or GeneAmp® PCR System thermal cyclers that display a calculated sample temperature. Some models of thermal cyclers may require longer times.

- Ramp speed or mode: **Standard**
- Reaction volume (µL): **25** or **50**

Load and run the plate or tubes

1. Remove the plate or PCR tubes from the base.
2. Use a MicroAmp™ Optical Film Compression Pad when you use a MicroAmp™ Clear Adhesive Film.
3. Load the reaction plate or tubes into a PCR instrument.
4. Start the run.
5. Unload the reaction plate or tubes after the run is complete.
6. Store the plate or tubes at 4 °C or at −15 to −25 °C for long-term storage.

For more information

Refer to the user guide or getting started guides for your PCR system for more information about setting up the experiment, using and maintaining the instrument, and performing instrument calibrations.

Analyze the results

WARNING! CHEMICAL HAZARD. Ethidium bromide.

Check the purity of the PCR product

Analyze the PCR amplification products by agarose gel electrophoresis.

IMPORTANT! To prevent contamination, never bring amplified PCR products into the PCR setup area.

1. Obtain a 1% agarose gel with ethidium bromide stain. You can use a gel of up to 3% agarose with ethidium bromide stain. Set up the electrophoresis apparatus and running buffer according to the manufacturer's instructions.
2. Add an aliquot of the PCR product to a well of a new plate or to an appropriate, new tube. Add an appropriate volume of gel-loading buffer to the PCR-product aliquot. For example, add 1 µL of 10× gel-loading buffer to a 9-µL aliquot of PCR reaction.
3. Mix the PCR-product aliquot and buffer in the wells by pipetting up and down or briefly vortex the samples in the tubes. Spin the plate or pulse-spin the tubes.
4. Dispense the entire volume of the buffer-PCR product aliquot from each well, or tube into a well of the gel.
5. Into one well of the gel, load a DNA-ladder marker appropriate to the PCR product length.
6. Run the gel at the voltage or time appropriate to amplicon length and agarose percentage so that the samples run 1/3 to 1/2 the length of the gel. Do not run the dye off the gel.
7. Place the gel on a UV transilluminator. Verify that each lane with a PCR-product aliquot contains one distinct band.

For more information

Refer to the getting started guides for your PCR system for information about analyzing PCR results.

Troubleshooting

Observation	Possible cause	Recommended action
Nonspecific amplification with or without a product band	Carryover contamination	• Use the GeneAmp® PCR Carry-Over Prevention Kit (PN N8080068). • Dispose of reagents, make fresh reagents, then repeat the PCR. • Use 1 to 2 µL of 360 GC Enhancer in a 50 µL reaction. Use the 360 GC Enhancer only if there are nonspecific products or products with ≥65% GC (see "Prepare the reaction mix" on page 7).
	Denaturation temperature is too low or too high	Adjust the temperature in increments of 1 degree Celsius. If you have a Veriti™ thermal cycler, adjust the VeriFlex™ Block in increments of 1 degree Celsius for up to six different temperatures.
	Nonspecific priming	Increase the annealing temperature in increments of 1 to 2 degrees Celsius.
	Primer design is not optimal	Review primer design and composition.
Low levels of PCR product or no product band visible	Template concentration is too low	Increase the sample concentration.
	Experimental sample DNA is damaged or degraded	Add more DNA or use sample that has been processed to minimize shearing and nicking.
	Denaturation time is too short or too long	Adjust the time in increments of 5 seconds.
	Denaturation temperature is too low or too high	Adjust the temperature in increments of 1 degree Celsius. If you have a Veriti™ thermal cycler, adjust the VeriFlex™ Block in increments of 1 degree Celsius for up to six different temperatures.
	Annealing/extension temperature is too high	Lower the temperature in increments of 2 degrees Celsius. If you have a Veriti™ thermal cycler, adjust the VeriFlex™ Block in increments of 2 degrees Celsius for up to six different temperatures.
	Annealing/extension time is too short	Increase the time in increments of 15 seconds.
	Cycle number is too low	Increase the cycle number in increments of three cycles.
	Primer design is not optimal	Review primer design and composition.

Observation	Possible cause	Recommended action
Product band is smeared	Carryover contamination	• Use the GeneAmp® PCR Carry-Over Prevention Kit (PN N8080068). • Dispose of reagents, make fresh reagents, then repeat the PCR.
	Denaturation time is too short or too long	Adjust the time in increments of 5 seconds.
	Denaturation temperature is too low	Increase the temperature in increments of 1 degree Celsius.
	Annealing/extension time is too long	Shorten the time in increments of 15 seconds.
	Cycle number is too high	Shorten the cycle number in increments of 3 cycles.
	Experimental sample DNA is degraded	Test a new aliquot of sample.

Appendix A Ordering Information

This appendix covers:

Materials and equipment not included

In addition to the reagents supplied, the items listed in the following table are required:

Thermal cyclers

Item‡	Applied Biosystems PN
Veriti™ 60-Well Thermal Cycler	4384638
Veriti™ 96-Well Fast Thermal Cycler	4375305
Veriti™ 96-Well Thermal Cycler	4375786
2720 Thermal Cycler	4359659
Aluminum 96-Well GeneAmp® PCR System 9700, Sample Block Module	4314445
Gold-plated 96-Well GeneAmp® PCR System 9700	4314878

‡ Only one thermal cycler or one PCR system is required.

Other equipment and consumables

Item	Source
MicroAmp™ Optical 96-Well Reaction Plates	Applied Biosystems (PN N8010560)
MicroAmp™ Splash-Free 96-Well Base	Applied Biosystems 4312063
MicroAmp™ 8-Tube Strip, 0.2 mL	Applied Biosystems (PN N8010580)
MicroAmp™ 8-Cap Strip	Applied Biosystems (PN N8010535)
MicroAmp™ 96-well Base	Applied Biosystems N8010531
dNTP Mix	Applied Biosystems (PN N8080260)
MicroAmp™ 96-Well Tray/Retainer Set, 10 sets	Applied Biosystems (PN 403081)
Nuclease-free water (not DEPC-treated), 500 mL	Applied Biosystems (PN AM9930)
MicroAmp™ Clear Adhesive Film	Applied Biosystems (PN 4306311)
MicroAmp™ Optical Film Compression Pad‡.	Applied Biosystems (PN 4312639)
Centrifuge with plate adapter	Major laboratory supplier (MLS)§

Item	Source
Pre-cast agarose gels, 1% up to 3% with ethidium bromide stain	MLS
Agarose	MLS
Disposable gloves	MLS
Electrophoresis apparatus	MLS
Microcentrifuge	MLS
Pipettes, positive-displacement or air-displacement	MLS
Pipette tips with filter plugs	MLS
Polypropylene tubes	MLS
TBE buffer	MLS
TE buffer	MLS
Vortex	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
1.5-mL microcentrifuge tubes	MLS
Tris-EDTA (TE) buffer, pH 8.0	MLS
Vortexer	MLS

‡ See instrument manual for compatibility.

§ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

For more product recommendations, visit the PCR technology page at:
www3.appliedbiosystems.com/applicationstechnologies/PCR/index.htm?newGlobalNav=true

Appendix B Guidelines for Designing PCR Assays

This appendix covers:

PCR good laboratory practices

General PCR practices

When preparing samples for PCR amplification:

- When using a non-hot-start DNA polymerase, keep PCR reagents on ice. Prepare the reactions on ice.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-operating techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Select the amplicon site

Using Primer Express® Software, select an *amplicon site* within the target sequence (refer to the *Primer Express® Version 3.0 Getting Started Guide* and *Software Help*).

Guidelines

- Design primer pairs according to Primer Express Software guidelines.
- Use a primer pair that is specific to the target gene and does not amplify pseudogenes or other related genes.
- Test the primer pairs, then select the primer pair that produces the largest amount of specific product and the least amount of non-specific product. To determine specific product, compare migration through an agarose gel of amplicons to that of the DNA bands of known length in a DNA ladder.

Adjust thermal cycling

Adjusting denaturation conditions

- In the early cycles, make sure that your DNA template is completely denatured.
- Do not exceed a denaturation temperature of 95 to 96 °C (Gelfand and White, 1990).
- A denaturation period of 30 seconds is adequate when using Veriti™ and GeneAmp® PCR System thermal cyclers with a calculated in-tube temperature. Some models of thermal cyclers may require longer denaturation times.

Adjusting annealing conditions

- For increased product specificity, use annealing temperatures higher than 45 °C (Saiki, Gelfand, and Stoffel, 1988; Rychlik, Spencer, and Rhoads, 1990).
- Determine the optimum annealing temperature by testing at increments of 5 or fewer degrees Celsius until the maximum specificity is reached.
- Computer programs that calculate primer melting temperatures (T_m) can help you narrow the range of annealing temperatures to test. For such a T_m calculator, go to **http://www.appliedbiosystems.com**, then select **Services & Support ▸ Technical Tools ▸ T_m Calculator**. The GeneAmp® PCR System 9700 Thermal Cycler also contains a T_m calculator.
- Thirty seconds is adequate annealing time when using the Veriti™ and GeneAmp PCR System thermal cyclers with a calculated in-tube temperature. Some models of thermal cyclers may require longer annealing times.

Adjusting extension conditions

- The length of the target sequence affects the required extension time. Longer targets require increased extension times. In general, allow an extension time of approximately 60 seconds per 1000 bases at 72 °C.
- As the amount of DNA increases, the number of DNA polymerase molecules may become limiting. Compensate for this limitation by increasing the extension time in later cycles.

Optimize the PCR conditions

Optimizing template concentration

- The DNA segment to be amplified from the template can be up to 4 kb long (Jeffreys *et al*., 1988), although 100 to 1000 bases are more typical and easier to amplify.
- Start with enough copies of the template to obtain a signal after 25 to 30 cycles. More than 10^4 copies, but less than 1 µg of human genomic DNA per 50-µL reaction is the recommended range.
- If the target DNA concentration is low, you may need more than 35 cycles to produce sufficient product for analysis. As few as 1 to 10 target copies can be amplified (Saiki, Gelfand, Stoffel, 1988; Chou *et al*., 1992). Validation for low-copy-number amplifications is best done for an average of 5 to 10 target molecules per sample to avoid statistical false negatives.

Optimizing enhancer concentration

The 360 GC Enhancer helps amplify amplicons that are difficult to amplify, including amplicons that are GC-rich or have GC-repeats or that generate nonspecific products.

In a 50-µL reaction, for targets with:

- 65 to 75% GC, start with 5 µL.
- >75% GC, start with 10 µL.

In general, if increased specificity is required, add 1 to 2 µL per 50-µL reaction.

The 360 GC Enhancer can reduce nonspecific amplification and improve the yield of specific products. However, excessive use of the 360 GC Enhancer can reduce yield, particularly for non-GC-rich amplicons.

Optimizing Magnesium Chloride concentration

The magnesium ion concentration that is required for optimal PCR amplification depends 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 by experiment.

To determine the optimum $MgCl_2$ concentration for each primer set:

- Use the supplied 25 mM $MgCl_2$ to adjust the magnesium ion concentration.
- Vary the concentration of $MgCl_2$ around 1.8 mM. A typical range is 1.0 to 4.0 mM.
- Raise the $MgCl_2$ concentration in the reaction mix proportionately if the samples contain EDTA, citrate, or other chelators.
- Adjust the $MgCl_2$ concentration in parallel with significant changes in the concentration (higher or lower) of sample DNA or dNTPs to keep the free magnesium ion concentration constant. For example, reduce the concentration of dNTP from 200 µM each to 40 µM each, and reduce the $MgCl_2$ concentration from the higher concentration to 640 µM.

Optimizing dNTP concentration

The dNTP concentration that is recommended for PCR is 200 µM for each dNTP. If the blend of dNTPS is changed, and the concentration of any one dNTP is significantly different from the other dNTPs, then AmpliTaq® 360 DNA Polymerase tends to misincorporate, slow down, and/or terminate prematurely (Innis *et al.*, 1988). Lower concentrations of dNTPs (40 µM) tend to promote polymerase fidelity (Eckert and Kunkel, 1992).

Optimizing enzyme concentration

For difficult-to-amplify targets, increasing the AmpliTaq® 360 DNA Polymerase up to 5 U per 50 µL reaction may improve the yield.

Appendix C Safety

This appendix covers:

Chemical hazard warnings

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page 26.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard warning

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Chemical alerts

General alerts for all chemicals

May cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

⚠ **CHEMICAL HAZARD. AmpliTaq® 360 DNA Polymerase**.

⚠ **CHEMICAL HAZARD. AmpliTaq® 360 Buffer, 10×**.

⚠ **WARNING! CHEMICAL HAZARD. Ethidium bromide** causes eye, skin, and respiratory tract irritation and is a known mutagen (that is, it can change genetic material in a living cell and has the potential to cause cancer).

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Glossary

amplicon — A segment of DNA amplified during PCR.

biological replicates — Reactions that contain identical components and volumes, but evaluate separate samples of the same biological source (for example, multiple samples of the same liver tissue). *See also* technical replicates.

cycling stage — In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage.

diluent — A reagent used to dilute a sample or standard before it is added to the PCR reaction. The diluent can be buffer or water.

forward primer — Oligonucleotide that flanks the 5′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

holding stage — In the thermal profile, a stage that can include one or more steps. You can add a holding stage to the thermal profile to activate enzymes, to inactivate enzymes, or to incubate a reaction.

melting temperature (T_m) — In melt-curve experiments, the temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. The T_m is displayed in the melt curve.

mode — *See* ramp speed.

negative control (NC) — The task for targets in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Also called no template control (NTC).

no-template control (NTC) — *See* negative control (NC).

quantity — In quantitation experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.

ramp — The rate at which the temperature changes during the instrument run. The ramp is defined as a percentage. The ramp for the melt curve step is defined as a temperature increment. In the graphical view of the thermal profile, the ramp is indicated by a diagonal line.

ramp speed Speed at which the temperature ramp occurs during the instrument run. For optimal results using the AmpliTaq® 360 DNA Polymerase, Applied Biosystems recommends using the standard ramp speed.

reaction mix A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control).

replicate group A set of identical reactions in an experiment.

replicates *See* biological replicates or technical replicates.

reverse primer An oligonucleotide that flanks the 3′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

reverse transcriptase An enzyme that converts RNA to cDNA. Reverse transcriptase is added to the PCR reaction to perform 1-step RT-PCR.

run method Definition of the reaction volume and the thermal profile for the instrument run.

sample The template that you are testing.

stage In the thermal profile, a group of one or more steps. In core PCR, there are two types of stages: holding stage (including pre-PCR read and post-PCR read) and cycling stage (also called amplification stage).

step A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt-curve steps), hold temperature, and hold time (duration).

target The nucleic acid sequence that you want to amplify and detect.

technical replicates Identical reactions that contain identical components and volumes and that evaluate the same sample.

See also biological replicates.

thermal profile Part of the run method that specifies the temperature, time, and ramp for all steps and stages of the PCR instrument run.

T_m *See* melting temperature (T_m).

Documentation and Support

Related documentation

You can download the following documents and other product-support documents from:
http://docs.appliedbiosystems.com/search.taf

Document	Part number
AmpliTaq® 360 DNA Polymerase Product Insert	4398170
AmpliTaq® 360 DNA Polymerase Quick Reference Card	4398952
Applied Biosystems Veriti™ Thermal Cycler User Guide	4375799
GeneAmp® PCR System 9700 Base Module User's Manual	4303481
GeneAmp® PCR System 9700 96-Well Sample Block Module	4316011

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

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IMPORTANT! The e-mail address above is for submitting comments and suggestions relating only to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" above.

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