AmpliTaq Gold® Fast PCR Master Mix, UP (2×)

Protocol

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Preface

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Safety

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.

Definitions

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.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

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 vii.)
- 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.

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 **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field of the MSDS Search page:
   a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.
3. To view, download, or print the document of interest:
   a. Right-click the document title.
   b. Select:
      - **Open** – To view the document
      - **Save Target As** – To download a PDF version of the document to a destination that you choose
      - **Print Target** – To print the document

4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
   a. Select **Fax** or **Email** below the document title.
   b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
   c. Enter the required information.
   d. Click **View/Deliver Selected Documents Now**.

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 the 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

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; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Blood borne Pathogens: **http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

How to Obtain Support

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

At the Support page, you can:

- 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

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Protocol

About This Protocol

This protocol provides:

- Background information about DNA amplification on the Veriti™ 96-Well Thermal Cycler systems
- A list of equipment and materials for using the AmpliTaq Gold® Fast PCR Master Mix, UP (2✕)
- Procedures for using the AmpliTaq Gold® Fast PCR Master Mix, UP (2✕).

Chemistry Overview

The AmpliTaq Gold® Fast PCR Master Mix, UP (2✕) contains a hot-start polymerase chain reaction that has been optimized to increase the overall PCR amplification speed. The master mix is a premix of all components except the primers and DNA template necessary to amplify your DNA target.

Use the AmpliTaq Gold® Fast PCR Master Mix, UP (2✕) (PN 4390937, PN 4390939, or PN 4390941) for fast DNA amplification on the Applied Biosystems Veriti™ 96-Well Fast Thermal Cycler and Veriti™ 96-Well Thermal Cycler.

Materials and Equipment

Master Mix Contents

The AmpliTaq Gold® Fast PCR Master Mix, UP (2X) (PN 4390937, PN 4390939, and PN 4390941) contains:

Table 1 Master mix contents

Part Number	Contents	Number of Reactions
4390937	1 X 250 µL tube	25
4390939	2 X 1.25 mL tubes	250
4390941	2 X 12.5 mL bottles	2500

Each bottle of AmpliTaq Gold® Fast PCR Master Mix, UP (2X) is 2X the recommended usage concentration. Each bottle contains the following:

- AmpliTaq Gold® DNA Polymerase, UP
- PCR Buffer
- dNTP
- $MgCl_2$
- Stabilizers

Storage and Stability

The AmpliTaq Gold® Fast PCR Master Mix, UP (2X) should be stored at 2 °C to 8 °C until expiration date as indicated on the label.

User-Supplied Materials and Equipment

The tables below include user-supplied equipment, materials, and documentation for performing the PCR with AmpliTaq Gold® Fast PCR Master Mix, UP (2X). These items are not supplied in the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) kits.

Unless otherwise noted, many items listed in Table 2 are available from major laboratory suppliers (MLS).

Table 2 User-supplied materials and equipment

Materials and Equipment	Source and Part Number
MicroAmp® Adhesive Seal Applicator	Applied Biosystems: PN 4333183
Applied Biosystems Veriti™ 96-Well Thermal Cycler or Veriti™ 96-Well Fast Thermal Cycler	Applied Biosystems: PN 4375786 or PN 4375305
Applied Biosystems AmpliTaq Gold® Fast PCR Master Mix, UP (2X)	Applied Biosystems: 4390937 (25 Rxns), 4390939 (250 Rxns), or 4390941 (2500 Rxns)
MicroAmp® Clear Adhesive Film	Applied Biosystems: PN 4306311
MicroAmp® Optical 96-Well Fast Thermal Cycling Plates with Barcode (code 128), 20 plates	Applied Biosystems: PN 4346906
MicroAmp® Optical 96-Well Reaction Plate, 10 plates	Applied Biosystems: PN N8010560
Reagent Tubes With Caps, 10 mL	Applied Biosystems: PN 4305932
Centrifuge with plate holders	MLS (Major Lab Suppliers)
Disposable gloves	MLS
Gel electrophoresis and UV equipment	MLS
Microcentrifuge	MLS
Pipette tips, aerosol resistant, nuclease-free: 1 µL to 20 µL range, 20 µL to 200 µL range, 100 µL to 1000 µL range	MLS
Pipettors (positive-displacement, air-displacement, or multichannel): 1 µL to 20 µL range, 20 µL to 200 µL range, 100 µL to 1000 µL range	MLS
Polypropylene tubes	MLS
RNase-free, sterile-filtered water	MLS

Table 3 Documentation

Document	Part Number
Applied Biosystems Veriti™ Thermal Cycler User Guide	4375799
ABI PRISM® 6100 Nucleic Acid PrepStation User Guide	4326242
DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol	4343586

Table 3 Documentation *(continued)*

Document	Part Number
ABI PRISM® 310 Genetic Analyzer User Guide	4317588
Applied Biosystems 3130/3130xl Genetic Analyzers: Getting Started Guide	4352715
Applied Biosystems 3730/3730xl DNA Analyzers: Getting Started Guide	4331468
Variant Reporter™ Software Version 1.0 Getting Started Guide	4376590

Preventing Contamination

Overview

The repetitive nature of PCR assays requires special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The PCR process allows for the amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

General PCR Practices

General PCR practices to prevent contamination:

- Maintain separate areas, dedicated equipment, and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Do not bring amplified PCR products into the PCR setup area.
- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use positive-displacement or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.

Protocol Procedure Overview

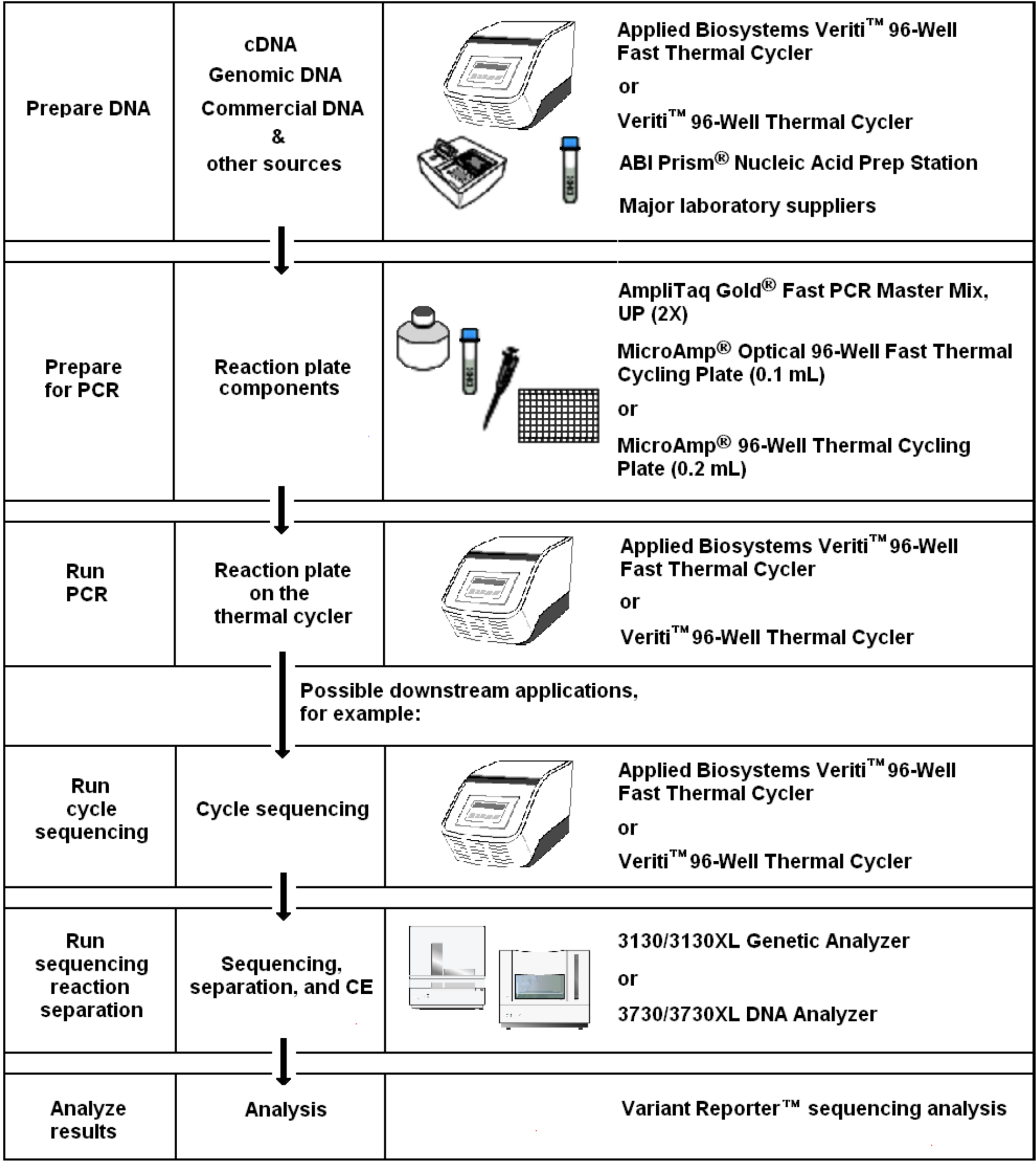


Figure 1 Protocol procedure overview

Preparing DNA Samples

You can use either complementary DNA (cDNA) or genomic DNA (gDNA) samples with the AmpliTaq Gold® Fast PCR Master Mix, UP (2✕) for fast DNA amplification.

cDNA Samples

On an Applied Biosystems thermal cycler, synthesize cDNA from RNA using one of the following Applied Biosystems reverse transcription (RT) kits:

- HIV Reverse Transcriptase (PN AM2045)
- M-MLV Reverse Transcriptase (PN 2043)
- RETROscript Kit (AM1710 from Ambion): Use the 2-step protocol. After cDNA has been synthesized with RT enzyme and RT-buffer, proceed with PCR reaction with AmpliTaq Gold® Fast PCR Master Mix, UP (2✕).

Note: Ambion, an Applied Biosystems company, has a wide variety of products and specialized kits designed for isolation of RNA from numerous sources. To view product offerings and a kit selection guide, go to: **http://www.ambion.com**.

For a complete list of all RT enzymes and kits available for reverse transcription, go to **http://www.ambion.com** or **http://www.appliedbiosystems. com**.

The general procedure is as follows:

1. Obtain an RNase-free RNA sample
2. Prepare RT Master Mix and set up the reaction according to the kit instructions.

gDNA Samples

Use the Applied Biosystems 6100 Nucleic Acid PrepStation and one of the following Applied Biosystems chemistry sets to purify gDNA.

- NucPrep® Chemistry System
- BloodPrep® Chemistry System
- ABI PRISM® TransPrep System
- MagMax Total Nucleic Acid Isolation Kit (AM1840)

Refer to the *ABI PRISM® 6100 Nucleic Acid PrepStation User Guide*, PN 4326242, and the *DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol*, PN 4343586, for procedures to purify gDNA from various sample types.

General Procedure

To prepare cDNA or gDNA samples for amplification:

1. Digest sample with digestion buffer
2. Incubate
3. Add purification solution
4. Set up the ABI PRISM® 6100 Nucleic Acid PrepStation and software
5. Pass lysates across a 96-well purification tray
6. Wash lysates
7. Elute purified DNA
8. Purify cDNA or gDNA

Performing PCR Amplification

Overview

To perform PCR amplification using the AmpliTaq Gold® Fast PCR Master Mix, UP (2X), you can run one of the protocols listed in the table below.

Table 4 Protocols for PCR amplification

Protocol	Page Number
Fast PCR Protocol	page 10
Optimizing the AmpliTaq Gold® Fast PCR Protocol	page 16

About Fast PCR

During PCR amplification, the polymerase in the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) amplifies target DNA using sequence-specific primers. Customize the protocol for your specific DNA sample and primers.

AmpliTaq Gold® Fast PCR Master Mix, UP (2X) is provided at a convenient 2X concentration. Only the addition of DNA template and primers is required.

Reagent Preparation Guidelines

Follow these guidelines to ensure optimal PCR performance:

- Prior to use:
  - Mix the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) thoroughly by swirling the bottle.
  - Mix the primers, then centrifuge the tube briefly.
  - Place frozen DNA samples on ice to thaw. After the samples are thawed, mix them, then briefly centrifuge the tubes.
- Prepare the PCR reaction mix.

Note: Assembled reactions can be stable at room temperature for up to 18 hours.

Fast PCR Protocol

Overview

Applied Biosystems recommends performing four replicates of each reaction in the following protocol for fast PCR amplification. For one reaction, follow the steps listed in "Preparing the Reaction Mix" on page 11. The recommended reaction volume is 20 µL for the MicroAmp® Optical 96-Well Fast Thermal Cycling Plate or the MicroAmp® Optical 96-Well Thermal Cycling Plate, when using the Veriti 96-Well Thermal Cyclers.

Reaction Components

The table below lists the components, volumes, and concentrations that comprise the reaction mix.

Table 5 Reaction components

Component	Volume per Reaction‡ (µL)	Final Conc.
Deionized water	see below§	—
User-provided Primer 1	_	0.2 to 1.0 µM
User-provided Primer 2	_	0.2 to 1.0 µM
User-provided experimental template	see below§	<1 µg/reaction#
AmpliTaq Gold® Fast PCR Master Mix, UP (2X)	10	1X
Total volume	20	—

‡ Reaction volume can be adjusted to your experimental design, keeping the concentrations of reactants constant.

§ Use any combination of water and template as long as the total volume of the PCR Master Mix, sample, and primers equals 20 µL.

Preferably >10^2 copies of template but <0.2 µg gDNA per reaction.

Preparing the Reaction Mix

To prepare the reaction mix:

Step	Action
1.	Thaw the reagents, frozen DNA templates, and primers (if necessary) on ice. See "Reaction Components" on page 16. **Note:** AmpliTaq Gold® Fast PCR Master Mix, UP (2X) and PCR reactions do not need to be placed on ice during setup.
2.	Calculate the amount of template, primers, and deionized water needed to make up a total volume of 20 µL when added to the master mix. **Note:** The amount of each component depends on your specific primer and template concentrations. See "Reaction Components" on page 16.
3.	• If using the Veriti™ 96-Well Fast Thermal Cycler: Pipette the calculated amount of template, primers, and deionized water into replicate wells onto a MicroAmp® optical 96-Well Fast thermal cycling plate. • If using the Veriti 96-Well Thermal Cycler: Pipette the calculated amount of template, primers, and deionized water into replicate wells onto a MicroAmp® optical 96-Well reaction plate.
4.	Thoroughly mix the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) by gently swirling the container. Minimize bubbles in the master-mix solution.
5.	Pipette 10 µL of the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) into each reaction well.
6.	Seal the plate with MicroAmp® Clear Adhesive Film. **IMPORTANT!** Ensure good contact between the film and the plate by using the MicroAmp® Adhesive Seal Applicator.
7.	Place the plate in a plate centrifuge and spin the plate briefly to ensure that the liquid collects at the bottom of each well.
8.	Perform thermal cycling using the parameters described in "Thermal Cycling" on page 12.

Thermal Cycling

The Veriti™ instruments come with a default PCR thermal cycling method. The **Create** screen displays this default method (Figure 2 on page 12). The default PCR thermal profile uses three-step PCR. You can run the default method, or use it as a template to create a customized method.

If the firmware is not version 1.2.0 or later, you must create a new method and adjust the parameters to match Table 6 on page 13. For creating or adjusting your protocol, please refer to the documentation for your thermal cycler.

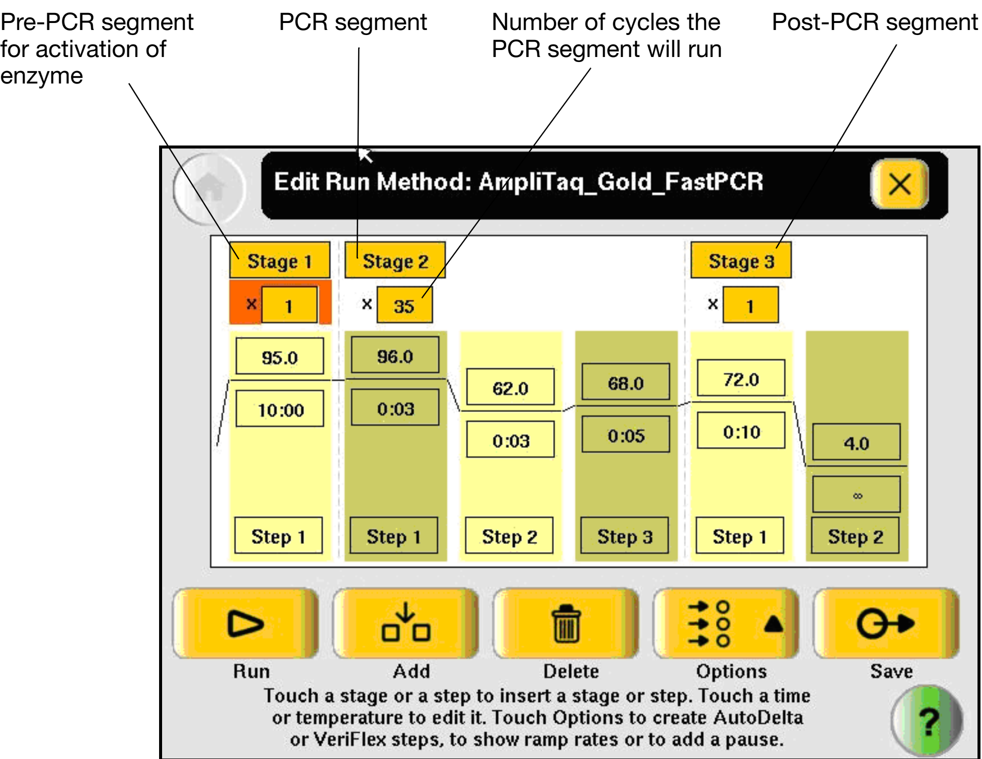


Figure 2 AmpliTaq Gold® Fast PCR method

IMPORTANT! Be sure to set the activation time at 10:00 (10 minutes).

Program the Veriti™ using a combination of the parameters in the table below for your specific target sequence.

Table 6 Program parameters

Step	Activation of Enzyme	PCR			PCR (Final Step)	
	HOLD	Cycle (35 Cycles)			HOLD	HOLD
		Denature	Annealing	Extension‡		
Temp	95 °C	96 °C	Primer T_m	68 °C	72 °C	4 °C
Time	10 min	3 sec	3 sec	See Table 7 below	10 sec§	∞

‡ Use the Primer T_m calculator on an Applied Biosystems Thermal Cycler, or go to **http://www.appliedbiosystems.com/support/techtools/calc/**
§ For cloning applications, increase final extension time to 5 min.

Table 7 Recommended extension times

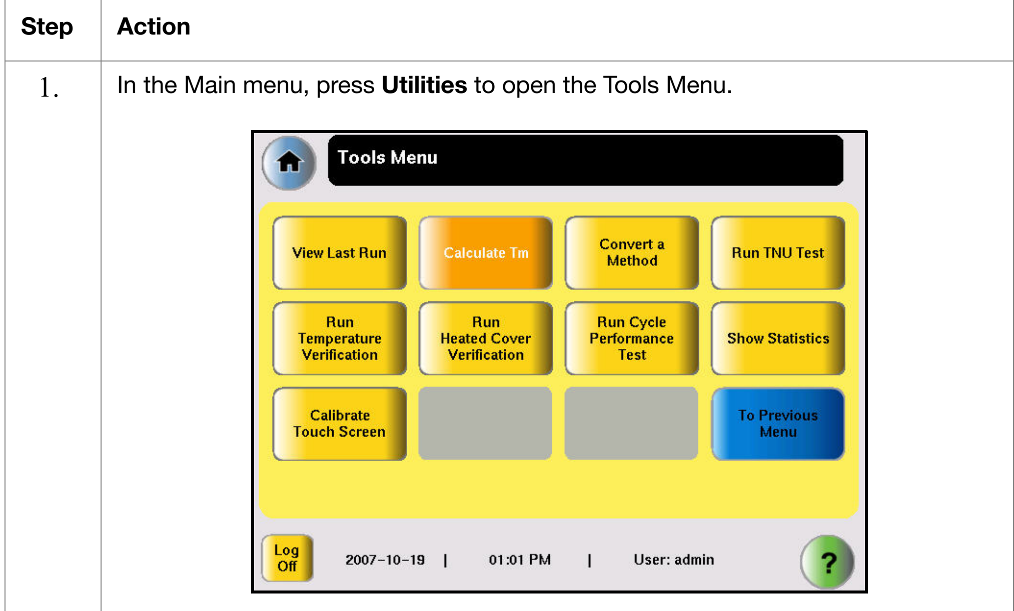
Length (kb)	Extension Time (sec)
0.5	5
1.0	15
1.5	30

Using the T_m Calculator

The Applied Biosystems Web site, www.appliedbiosystems.com, provides a T_m calculator for your use. Point your browser to:

http://www.appliedbiosystems.com/support/techtools/calc/

To use the T_m calculator to determine the annealing/extension temperature of a primer set of known sequence.

Step	Action
1.	In the Main menu, press **Utilities** to open the Tools Menu. 

2.	Press **Calculate Tm** to open the Tm Calculator.
3.	Enter the salt concentration using values of 5 to 1000. **Note:** The default, 50mM, should be used for the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) reaction.
4.	Enter the primer concentration using values of 0.01 to 10.00. **Note:** The default is 0.20.
5.	Enter the primer sequence in **Primer 1 Sequence**. Maximum length for each primer is 30.
6.	Enter the primer sequence in **Primer 2 Sequence**, then press **Calculate** to calculate the T_ms. **Note:** The melting points are displayed. Use this information to program a run. For more information about creating methods and runs, refer to the *Veriti™ Thermal Cycler User Guide* (PN 4375799).
7.	Press the Home icon to go back to the Utilities screen.

Optimizing the AmpliTaq Gold® Fast PCR Protocol

Overview

Follow the guidelines described below to optimize the Fast PCR protocol for your target sequence. For creating a new method, refer to the *Veriti™ Thermal Cycler User Guide* (PN 4375799).

Reaction Components

The table below lists the components, volumes, and concentrations that comprise the reaction mix.

Table 8 Reaction components

Component	Volume per Reaction‡ (µL)	Final Conc.
Deionized water	see below§	—
User-provided Primer 1	_	0.2 to 1.0 µM
User-provided Primer 2	_	0.2to 1.0 µM
User-provided template	see below§	<1 µg/reaction#
AmpliTaq Gold® Fast PCR Master Mix, UP (2×)	10	1×
Total volume	20	—

‡ Reaction volume can be adjusted to your experimental design, keeping the concentrations of reactants constant.
§ Use any combination of water and template as long as the total volume of the PCR Master Mix, sample, and primers equals 20 µL.
# Preferably >10^2 copies of template, but <0.2 µg gDNA per reaction.

Optimizing the Template Concentration

Use the following guidelines to optimize the template concentration:

- Start with enough copies of the template to obtain a signal after 30 to 40 cycles; preferably more than 10^2 copies, but less than 0.2 µg of DNA per 20 µL reaction.

- If the target DNA concentration is low, more than 35 cycles may be required to produce sufficient product for analysis. You can amplify as few as 1 to 10 target copies (Saiki *et al*., 1988; Chou *et al*., 1992). To avoid statistically arising dropouts (false negatives), validate for low copy number amplifications for an average of 5 to 10 target molecules per sample.

Note: Proteases may be present in the sample DNA. Proteases may degrade the AmpliTaq Gold® Fast PCR polymerase, resulting in little or no yield of DNA product. You can inactivate proteases by heating the DNA samples to 95 °C for 5 minutes before adding PCR Master Mix. You can automate this step with any of the Applied Biosystems PCR instrument systems.

Designing the Primers

Use the following guidelines to design your primers:

- The single-stranded DNA primers should be 15 to 30 bases in length.
- The %G+C of primers should be near 50%, to maximize specificity.
- Calculate the melting temperature of your primer(s) with the T_m calculator (see "Using the Tm Calculator" on page 14). Use this number as a starting point to determine the annealing temperature.
- To avoid potential problems, purify primers 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 restriction avoids template-independent amplification of primer sequences (or "primer dimer"), which can lead to other, larger primer artifacts. Primer-dimer may occur to some extent even without an apparent overlap.
- Use primer design software to assist in primer selection.

Optimizing the Primer Concentration

Use the following guidelines to optimize the primer concentration:

- Determine optimal primer concentrations empirically by testing concentrations in the range of 0.1 µM to 1.0 µM.
  - Primer concentrations that are too low 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 µM to 0.5 µM work for most PCR amplifications.
- Reducing each primer concentration (for example, to 0.2 µM) helps reduce amplification of non-specific products.

Preparing the Reaction Mix

To prepare the reaction mix:

Step	Action
1.	Thaw reagents, frozen DNA templates, and primers (if necessary) on ice. See "Reaction Components" on page 16. **Note:** AmpliTaq Gold® Fast PCR Master Mix, UP (2X) and PCR reactions do not require placement on ice during setup.
2.	Calculate the amount of template, primers, and deionized water needed to make up a total volume of 20 µL when added to the master mix. **Note:** The amount of each component depends on your specific primer and template concentrations. See "Reaction Components" on page 16.
3.	• If using the Veriti™ 96-Well Fast Thermal Cycler: Pipette the calculated amount of template, primers, and deionized water into replicate wells onto a MicroAmp® optical 96-Well Fast thermal cycling plate • If using the Veriti 96-Well Thermal Cycler: Pipette the calculated amount of template, primers, and deionized water into replicate wells onto a MicroAmp® optical 96-Well reaction plate.
4.	Thoroughly mix the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) by gently swirling the bottle. **Note:** Minimize bubbles in the master mix solution.
5.	Pipette 10 µL of the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) into each reaction well.
6.	Seal the plate with MicroAmp® Clear Adhesive Film. **IMPORTANT!** Ensure good contact between the film and the plate by using the MicroAmp® Adhesive Seal Applicator.
7.	Place the plate in a plate centrifuge and spin briefly to ensure the liquid collects at the bottom of each well.
8.	Perform thermal cycling using the parameters described in "Thermal Cycling" on page 19.

Thermal Cycling

The Veriti™ instruments come with a default PCR thermal cycling method. The Create screen displays this default method. The default PCR thermal profile uses three-step PCR. You can run the default method, or use it as a template to create a customized method.

If optimization is required, adjust the method below. For creating or adjusting your protocol, please refer to the documentation for your thermal cycler.

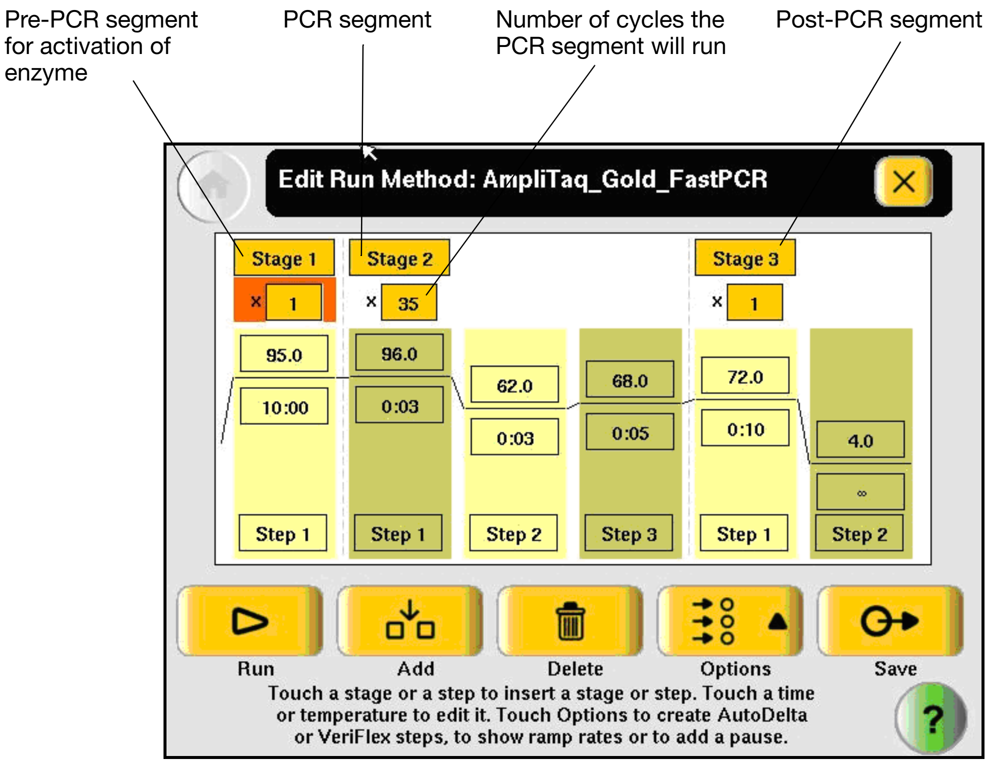


Figure 3 Veriti™, AmpliTaq Gold® Fast PCR method

IMPORTANT! Be sure to set the activation time at 10:00 (10 minutes).

Fast Thermal Cycling Profile

Program the Fast Thermal Cycler using a combination of the following parameters for your specific target sequence. For creating a new method on the Fast Thermal Cycler, refer to the *Fast Thermal Cycler User Guide*.

Table 9 Program parameters

Step	Activation of Enzyme	PCR			PCR (Final Step)	
	HOLD	Cycle (35 Cycles)			HOLD	HOLD
		Denature	Annealing	Extension‡		
Temp	95 °C	96 °C	Primer T_m	68 °C	72 °C	4 °C
Time	10 min	3 sec	3 sec	See Table 10 below	10 sec§	∞

‡ Use the Primer Tm calculator on an Applied Biosystems Thermal Cycler, or go to **http://www.appliedbiosystems.com/support/techtools/calc/**

§ For cloning applications, increase final extension time to 5 min.

Using the T_m Calculator

The Applied Biosystems Web site, www.appliedbiosystems.com, provides a T_m calculator for your use. Point your browser to:

http://www.appliedbiosystems.com/support/techtools/calc/

See "Using the Tm Calculator" described on page 14 for a procedure on how to use the T_m calculator to determine the annealing temperature.

Adjusting the Denaturation Conditions

The following are guidelines for adjusting the denaturation conditions:

- Ten minutes (10:00) is required when using the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) for the initial activation step.
- It is very important in the early cycles to make sure that your DNA template is completely denatured.
- The maximum denaturation temperature should not exceed 97 °C.

Adjusting the Annealing Conditions

The following are guidelines for adjusting annealing conditions:

- A T_m calculator can be found at:

http://www.appliedbiosystems.com/support/techtools/calc/

- The optimum annealing temperature can be determined empirically by testing at 1 °C to 2 °C increments, until the maximum specificity is reached.
- Computer programs designed to calculate primer melting temperatures (T_m) can assist you in narrowing the range of annealing temperatures for empirical determination.

  In addition, the Applied Biosystems Thermal Cycler also contains a T_m calculator. See "Using the Tm Calculator" described on page 14 for a procedure to determine the starting point for optimizing the annealing temperature.

Adjusting the Extension Conditions

The following are guidelines for adjusting extension conditions:

- The length of the target sequence affects the required extension time. As a general rule, allow an extension time of approximately 1 to 3 seconds per 100 base pairs. Longer targets require more time.

Table 10 Recommended extension times

Length (kb)	Extension time
0.5	5 sec
1.0	15 sec
1.5	30 sec

- As the amount of DNA increases, the number of DNA polymerase molecules may become limiting. This limitation can be compensated for by increasing the extension time in later cycles using the Veriti™ 96-Well Fast Thermal Cycler's Auto Delta function.

For creating a new method on the Veriti™ 96-Well Fast Thermal Cycler, refer to the *Applied Biosystems Veriti*™ 96-Well Fast Thermal Cycler *Block Base Module User Guide*.

Analyzing Results

Data analysis depends on the type of analysis you have selected. You can use gel electrophoresis, cloning, and cycle sequencing to analyze amplified samples.

For information on how to analyze the data, refer to the documentation for your specific sample analysis.

Troubleshooting

Table 11 comprises troubleshooting tips for using AmpliTaq Gold® Fast PCR Master Mix, UP (2X).

Table 11 Troubleshooting the AmpliTaq Gold® Fast PCR Master Mix, UP (2X)

Observation	Possible Cause	Recommended Action
Reduced or no product band visible.	Template concentration too low.	Increase sample concentration.
	Experimental sample DNA damaged or degraded.	Use sample that has been processed and stored properly to minimize shearing and nicking.
	Denaturation time too short or too long.	Adjust time in increments of 2 seconds.
	Denaturation temperature too low or too high.	Adjust temperature in increments of 1 °C.
	Annealing temperature too low or too high.	Adjust temperature in increments of 2 °C.
	Extension time too short.	Adjust extension time in increments of 5 to 10 seconds (see Table 7 on page 13).
	Cycle number too low.	Increase cycle number in increments of three cycles.
	Primer design not optimal.	Redesign primers with T_m of 62 °C or higher (see "Designing the Primers" on page 17).
	Difficult templates (for example, for AT).	1. Increase annealing time by increments of 5 seconds. 2. Adjust annealing temperature by increments of 5 °C.
	Difficult templates (for example, GC rich).	1. Adjust by increasing denaturing time and temperature as described above. 2. Adjust annealing time and temperature as described above.
	Assembled reaction at room temperature for more than 18 hours.	Maintain assembled reaction at room temperature for less than 18 hours or keep on ice.

Table 11 Troubleshooting the AmpliTaq Gold® Fast PCR Master Mix, UP (2X) *(continued)*

Observation	Possible Cause	Recommended Action
Product band is smeared.	Carryover contamination.	See “Preventing Contamination” on page 5.
	Annealing temperature too low.	Increase temperature in increments of 2 °C.
	Cycle number too high.	Decrease cycle number in increments of three cycles.
	Experimental sample DNA degraded.	Test a new aliquot of sample.
	Assembled reaction at room temperature for more than 18 hours.	Maintain assembled reaction at room temperature for less than 18 hours or keep on ice.
Non-specific amplification with or without a product band.	Carryover contamination.	See “Preventing Contamination” on page 5.
	Non-specific priming.	Increase the anneal temperature in 1 °C to 2 °C increments.
	Primer design not optimal	Review primer design and composition.
	Incomplete denaturation of amplicon (region).	Increase the denaturation temperature in 1 °C increments.

Customer Issues

Some issues encountered by customers are listed below. The responses are based on preliminary tests.

Table 12 Customer issues and test results

Issue	Response
Master Mix is frozen.	Supportive information has seen no effect on PCR performance for up to 10 freeze-thaw cycles.
Master Mix did not freeze.	Master Mix contains glycerol, so it may not freeze.
Ice is not available for reaction setup.	Room temperature setup is standard for AmpliTaq Gold® Fast PCR Master Mix, UP (2✕).

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