AmpliTaq Gold® PCR Master Mix

Protocol

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**Introduction**

**Product Features**

AmpliTaq Gold® PCR Master Mix (P/Ns 4318739, 4327058, 4327059) is a convenient reagent premix used to perform polymerase chain reaction (PCR) amplification of single or multiple DNA targets. PCR Master Mix includes all of the chemical components, except primers and template, necessary for PCR.

PCR Master Mix has the following features:

♦ Incorporates the Hot Start technique by using AmpliTaq Gold® DNA Polymerase. This chemical Hot Start technique increases sensitivity, specificity, and yield of PCR products.

♦ Because it is a premixed formulation, it provides a quality controlled, reproducible reaction solution that limits contamination in PCR reactions.

♦ Saves labor and costs by minimizing pipetting steps.

♦ Can be conveniently stored at 4 °C.

**About This Protocol**

This protocol describes how to use PCR Master Mix to amplify samples. It also provides guidelines for optimizing thermal cycling, preventing contamination, and troubleshooting problems.

**About AmpliTaq Gold DNA Polymerase**

AmpliTaq Gold is a chemically modified form of AmpliTaq DNA Polymerase. When the chemical moiety is attached to the enzyme, the enzyme is inactive. This allows for flexibility in reaction setup, including pre-mixing of PCR reagents at room temperature. Because the enzyme is inactive during set-up and during the first ramp of PCR, when the reaction goes through sub-optimal primer annealing temperatures, mis-primed primers will not be extended. AmpliTaq Gold DNA Polymerase requires a heat activation step, well above optimal annealing, to activate the enzyme, which provides an automated chemical hot start. AmpliTaq Gold DNA Polymerase can be completely or partially activated in a pre-PCR heat step, or it can be allowed to activate slowly during thermal cycling. Slow activation can provide a hot start and a “time release” of active enzyme, where polymerase activity builds as PCR product accumulates.
Benefits of Hot Start and “Time Release” PCR

The Hot Start and “Time Release” techniques improve amplification of most templates by lowering non-specific background and increasing amplification of required specific products.

High background (non-specificity) and low specific product yield can occur in a PCR system when reaction components are mixed at low or permissive temperatures (4 °C to 25 °C), (Chou et al., 1992). This can also occur during the first ramp of cycling when the reaction goes through suboptimal annealing temperatures. At these temperatures, non-specific primer annealing occurs, and since active enzyme is present at these temperatures, the mis-primed primers will be extended. These non-specific constructs are amplified throughout the remaining PCR cycles, resulting in mis-primed products and primer oligomers.

Mis-primed PCR products can obscure detection of specific target bands in gel analysis and impair quantitative PCR and sequencing of PCR products. Furthermore, amplification of mis-primed products competes for reactants (dNTPs, primers) resulting in poor yields of specific product. For further information, see “Adjusting the Hold Period for AmpliTaq Gold Activation” on page 13.

GeneAmp PCR Gold Buffer

AmpliTaq Gold PCR Master Mix is formulated with GeneAmp® PCR Gold Buffer. This provides flexible, efficient activation of AmpliTaq Gold DNA Polymerase, resulting in highly specific and robust PCR amplification. The ionic strength and pH of GeneAmp PCR Gold Buffer has been optimized for use with AmpliTaq Gold DNA Polymerase.
Materials

AmpliTaq Gold PCR Master Mix

AmpliTaq Gold PCR Master Mix (see table below for available sizes) contains all the reagents (except primers and template) needed for PCR amplifications. It is provided at a convenient 2X concentration for easier dilution when adding template and primers.

Tube Contents

Each tube of AmpliTaq Gold PCR Master Mix is at 2X the recommended usage concentration, and contains the following:

- AmpliTaq Gold DNA Polymerase, 250 U (0.05 U/µL)
- GeneAmp PCR Gold Buffer, 30 mM Tris/HCl, pH 8.05, 100 mM KCl
- dNTP, 400 µM each
- MgCl₂, 5 mM
- Stabilizers

Available Sizes

<table>
<thead>
<tr>
<th>Volume per Tube (mL)</th>
<th>Number of Tubes</th>
<th>Units Per Tube</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>250</td>
<td>4318739</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>250</td>
<td>4327058</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>2500</td>
<td>4327059</td>
</tr>
</tbody>
</table>

Materials required but not supplied:

In addition to AmpliTaq Gold PCR Master Mix, the items listed in the following table may be required:

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneAmp® PCR System 9700, 9600, or 2400 Thermal Cycler</td>
<td>Applied Biosystems</td>
</tr>
<tr>
<td>MicroAmp® disposables</td>
<td>Applied Biosystems</td>
</tr>
<tr>
<td>Agarose</td>
<td>Major laboratory supplier (MLS)</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>Electrophoresis apparatus</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
</tbody>
</table>
Storage and Stability

Upon receipt, store the AmpliTaq Gold PCR Master Mix at 2-8 °C. For long-term storage, keep the PCR Master Mix at –15 °C to –25 °C. It can be freeze-thawed for up to 10 cycles; however, repeated freeze-thaw cycles are not recommended.

Performance Characteristics

Each lot of AmpliTaq Gold PCR Master Mix has been shown to yield a specifically expressed visible band on an ethidium bromide-stained agarose gel, which corresponds to approximately 142 bp product when starting with 10 copies of HIV-1 Positive Control DNA per reaction.

Materials required but not supplied: (continued)

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipets, positive-displacement or air-displacement</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipet tips with filter plugs</td>
<td>MLS</td>
</tr>
<tr>
<td>Polypropylene tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>Tris-EDTA (TE) buffer</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortex</td>
<td>MLS</td>
</tr>
</tbody>
</table>
Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note  Calls attention to useful information.

IMPORTANT  Indicates information that is necessary for proper instrument operation.

⚠️ CAUTION  Indicates a potentially hazardous situation which, 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 which, if not avoided, could result in death or serious injury.

⚠️ DANGER  Indicates an imminently hazardous situation which, 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

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

♦ 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.

♦ Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

♦ Do not leave chemical containers open. Use only with adequate ventilation.

♦ Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended on the MSDS.

♦ Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.
Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<thead>
<tr>
<th>To order MSDSs...</th>
<th>Then...</th>
</tr>
</thead>
</table>
| Over the Internet | a. Go to our Web site at www.appliedbiosystems.com/techsupp  
b. Click MSDSs |

<table>
<thead>
<tr>
<th>If you have...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>The MSDS document number or the Document on Demand index number</td>
<td>Enter one of these numbers in the appropriate field on this page.</td>
</tr>
<tr>
<td>The product part number</td>
<td>Select Click Here, then enter the part number or keyword(s) in the field on this page.</td>
</tr>
<tr>
<td>Keyword(s)</td>
<td></td>
</tr>
</tbody>
</table>

c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or email.

By automated telephone service

Use “To Obtain Documents on Demand” under “Technical Support.”

By telephone in the United States

Dial 1-800-327-3002, then press 1.

By telephone from Canada

<table>
<thead>
<tr>
<th>To order in...</th>
<th>Dial 1-800-668-6913 and...</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Press 1, then 2, then 1 again</td>
</tr>
<tr>
<td>French</td>
<td>Press 2, then 2, then 1</td>
</tr>
</tbody>
</table>

By telephone from any other country

See the specific region under “To Contact Technical Support by Telephone or Fax” under “Technical Support.”

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.
Preventing Contamination

Overview
Due to the enormous amplification that occurs during PCR, small levels of DNA contamination can result in product formation even in the absence of purposefully added template DNA (Kwok and Higuchi, 1989).

DNA contamination can come from:
♦ Previous PCR amplifications
♦ Samples with high DNA concentration
♦ Cross contamination
♦ Positive control templates

General PCR Practices
Follow these recommendations:
♦ Maintain separate areas and dedicated equipment and supplies for:
  − Sample preparation
  − PCR setup
  − PCR amplification
  − Analysis of PCR products
♦ 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.
♦ 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 a 10% bleach solution, and dry thoroughly before use.
♦ Use dedicated or disposable sterile vessels, solutions, and pipets (preferably positive-displacement pipets or tips with hydrophobic filters) to minimize cross-contamination during DNA preparation, reaction mixing, and sample analysis (Kwok, 1990).
Protocol for Amplification of Samples

**Overview**
AmpliTaq Gold PCR Master Mix is provided at a convenient 2X concentration. Only the addition of template and primer is required.

**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 25–30 cycles; preferably more than $10^4$ copies, but 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 (Saiki *et al*., 1988; Chou *et al*., 1992). Validation for low copy number amplifications is best done for an average of 5–10 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 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 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:

- Optimal primer concentrations can be determined empirically by testing concentrations in the range of 0.1–1.0 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 0.2–0.5 µM will work for most PCR amplifications.
- Reducing each primer concentration (e.g., to 0.2 µM) will help reduce amplification of non-specific products.

About Proteases

Proteases (e.g., impure genomic DNA), may be present in the sample DNA. Proteases may degrade AmpliTaq Gold DNA Polymerase and result in little or no yield. Proteases can be inactivated by heating samples to 95 °C for 5 minutes before adding PCR Master Mix. This step can be carried out automatically with any of the Applied Biosystems GeneAmp PCR Instrument Systems.

Preparing the Reaction Mix

To prepare the reaction mix:

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thaw reagents (see Reaction Components on page 10) on ice if necessary.</td>
</tr>
<tr>
<td>2</td>
<td>Gently vortex to mix the reagent.</td>
</tr>
<tr>
<td>3</td>
<td>Pipette template, primers, and deionized water (if necessary) into your reaction tubes.</td>
</tr>
</tbody>
</table>
| 4    | **If...**
|      | proteases are present in the sample DNA, |
|      | **Then...**
|      | inactivate the proteases by heating samples to 95 °C for 5 minutes before adding PCR Master Mix. |
|      | proteases are not present, |
|      | continue with the next step. |
| 5    | Pipette the PCR Master Mix into your reaction tubes. |
Perform thermal cycling using the parameters supplied in “Thermal Cycling Optimization Guidelines for Custom Applications” on page 11.

Reaction Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Reaction(a)</th>
<th>Final Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>see below(b)</td>
<td>—</td>
</tr>
<tr>
<td>User-provided Primer 1</td>
<td>1–5 µL</td>
<td>0.2–1.0 µM</td>
</tr>
<tr>
<td>User-provided Primer 2</td>
<td>1–5 µL</td>
<td>0.2–1.0 µM</td>
</tr>
<tr>
<td>User-provided experimental</td>
<td>see below(b)</td>
<td>&lt;1 µg/reaction(c)</td>
</tr>
<tr>
<td>template</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR Master Mix (2X)</td>
<td>25 µL</td>
<td>1X</td>
</tr>
<tr>
<td>Total volume</td>
<td>50 µL</td>
<td>—</td>
</tr>
</tbody>
</table>

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

b. Any combination of water and template can be used as long as the total volume of the PCR Master Mix, sample, and primers equals 50 µL.

c. Preferably >10⁶ copies of template but <1 µg DNA per reaction.
Thermal Cycling Optimization Guidelines for Custom Applications

**About Thermal Cycling Optimization**

Thermal cycling conditions can be optimized for each DNA template by using the following PCR temperature conditions:

- Two-temperature PCR
- Three-temperature PCR

**Two-Temperature vs. Three-Temperature PCR**

**Which One to Choose?**

- Use the two-temperature PCR when primer annealing temperatures are above 60 °C.
- Use the three-temperature PCR when the templates have high G+C content and/or secondary structure, or desired primer annealing temperatures are below 60 °C.
Two-Temperature Thermal Cycling

Two-temperature PCR consolidates the annealing and extension steps into one. The extension is completed at the annealing temperature. Two-temperature PCR consists of the following two steps:

♦ Denaturation of DNA template
♦ Annealing and extension of primers

Note Fifteen seconds for denaturation and annealing is adequate when using GeneAmp PCR System thermal cyclers which display a calculated sample temperature. Some models of thermal cyclers may require longer times.

Two-temperature thermal cycling on the GeneAmp PCR System 9700, 9600, or 2400:

<table>
<thead>
<tr>
<th>Step</th>
<th>AmpliTaq Gold Enzyme Activation</th>
<th>PCR</th>
<th>PCR (Final Step)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOLD (30 cycles)</td>
<td>CYCLE (30 cycles)</td>
<td>HOLD</td>
</tr>
<tr>
<td>Denature</td>
<td></td>
<td>Denature</td>
<td></td>
</tr>
<tr>
<td>Anneal/Extend</td>
<td></td>
<td>Anneal/Extend</td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>95 °C^a</td>
<td>95 °C</td>
<td>60–70 °C^b</td>
</tr>
<tr>
<td>Time</td>
<td>5 min^a</td>
<td>15 sec</td>
<td>60 sec/kb</td>
</tr>
</tbody>
</table>

a. Adjust the time according to the desired initial enzyme activation (refer to "Thermal Activation Profile" on page 15). Start with an initial activation of 95 °C for 5 minutes and adjust as required. Refer to "Adjusting the Hold Period for AmpliTaq Gold Activation" on page 13 for more details.

b. Adjust the temperature according to the primer melting temperature.
Three-temperature PCR consists of the following three steps:

- Denaturation of DNA template
- Annealing of the primers to the template
- Extension of the primers

**Note**  Fifteen seconds for denaturation and annealing is adequate when using GeneAmp PCR System thermal cyclers which display a calculated sample temperature. Some models of thermal cyclers may require longer times.

Three-temperature thermal cycling on the GeneAmp PCR System 9700, 9600, or 2400:

<table>
<thead>
<tr>
<th>Step</th>
<th>AmpliTaq Gold Enzyme Activation</th>
<th>PCR (Final Step)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOLD CYCLE (30 cycles)</td>
<td>HOLD</td>
</tr>
<tr>
<td>Temp</td>
<td>95 °C</td>
<td>95 °C</td>
</tr>
<tr>
<td>Time</td>
<td>5 minα</td>
<td>15 sec</td>
</tr>
</tbody>
</table>

a. Adjust the time according to the desired initial enzyme activation (refer to “Thermal Activation Profile” on page 15). Start with an initial activation of 95 °C for 5 minutes and adjust as required. Refer to “Adjusting the Hold Period for AmpliTaq Gold Activation” below for more details.

b. Adjust the temperature according to the primer melting temperature.

---

For general PCR runs, we recommend a pre-PCR activation setup of 95 °C for 5 minutes. A titration of pre-PCR activation times (2–10 minutes in 1 minute intervals) should be done to find the best upfront enzyme activity for your reaction. Activation of AmpliTaq Gold DNA Polymerase can also be modulated to release active enzyme slowly over time (time release), allowing enzyme activity to increase with cycle number as the amount of template increases. This type of PCR can be accomplished as follows:

- With no activation during the pre-PCR hold period
- With limited activation during the pre-PCR hold period

In a “Time Release” protocol, the enzyme is released slowly to match the template concentration which further increases the specificity. When a no or limited (1–2 minute) pre-PCR activation is used, the enzyme is
released gradually during the denaturation step (95 °C for 15 seconds) of each cycle. Because the enzyme is released slowly, up to 5 additional cycles may be required.

Limiting the amount of active enzyme at the beginning of the amplification reaction when low amounts of substrate molecules are present enhances high specificity in the early PCR cycles.

See page 15 for the thermal activation profile for AmpliTaq Gold DNA Polymerase.

---

**Adjusting the Denaturation Conditions**

- 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 95–96 °C (Gelfand *et al*., 1990).
- 15 seconds is adequate when using GeneAmp PCR System thermal cyclers which display a calculated sample temperature. Some models of thermal cyclers may require longer denaturation times.

---

**Adjusting the Annealing Conditions**

- For increased product specificity, use annealing temperatures greater than 45 °C (Saiki *et al*., 1988; Rychlik *et al*., 1990).
- Use two-temperature PCR for annealing temperatures >60 °C.
- The optimum annealing temperature can be determined empirically by testing at 1–2 °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. A T_m calculator can be found on the Applied Biosystems web site at http://www.appliedbiosystems.com/techsupport/tmcalc.html
  In addition, the GeneAmp PCR System 9700 Thermal Cycler also contains a T_m calculator.
- 15 seconds is adequate when using GeneAmp PCR System thermal cyclers which display a calculated sample temperature. Some models of thermal cyclers may require longer annealing times.
Adjusting the Extension Conditions

The following are guidelines for adjusting the extension conditions:

- The length of the target sequence will affect the required extension time. Longer targets require increased extension times. As a general rule, 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. This can be compensated for, by increasing the extension time in later cycles.

- For two-temperature PCR, extension temperatures are typically lower than 72 °C and thus extension times may need to be lengthened accordingly.

Thermal Activation Profile

The activation profile of AmpliTaq Gold DNA Polymerase in Gold Buffer at different temperatures is shown in the graph below.
# Appendix A. Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Cause</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced or no product band visible</td>
<td>Template concentration too low</td>
<td>Increase sample concentration.</td>
</tr>
<tr>
<td></td>
<td>Experimental sample DNA damaged or degraded</td>
<td>Use sample that has been processed to minimize shearing and nicking.</td>
</tr>
<tr>
<td></td>
<td>Denaturation time too short or too long</td>
<td>Adjust time in increments of 5 seconds.</td>
</tr>
<tr>
<td></td>
<td>Denaturation temperature too low or too high</td>
<td>Adjust temperature in increments of 1 °C.</td>
</tr>
<tr>
<td></td>
<td>Annealing/extension temperature too high</td>
<td>Lower temperature in increments of 2 °C.</td>
</tr>
<tr>
<td></td>
<td>Annealing/extension time too short</td>
<td>Lengthen time in increments of 15 seconds.</td>
</tr>
<tr>
<td></td>
<td>Cycle number too low</td>
<td>Increase cycle number in increments of three cycles.</td>
</tr>
<tr>
<td></td>
<td>Primer design not optimal</td>
<td>Review primer design and composition.</td>
</tr>
<tr>
<td></td>
<td>Preincubation/activation time not sufficient</td>
<td>Increase pre-PCR heat step in increments of 1 minute, or use “Time Release” protocol.</td>
</tr>
<tr>
<td>Product band is smeared</td>
<td>Carryover contamination</td>
<td>See “Preventing Contamination” on page 7.</td>
</tr>
<tr>
<td></td>
<td>Denaturation time too short or too long</td>
<td>Adjust time in increments of 5 seconds.</td>
</tr>
<tr>
<td></td>
<td>Denaturation temperature too low</td>
<td>Increase temperature in increments of 1 °C.</td>
</tr>
<tr>
<td></td>
<td>Annealing/extension time too long</td>
<td>Shorten time in increments of 15 seconds.</td>
</tr>
<tr>
<td></td>
<td>Cycle number too high</td>
<td>Shorten cycle number in increments of three cycles.</td>
</tr>
<tr>
<td></td>
<td>Experimental sample DNA degraded</td>
<td>Test a new aliquot of sample.</td>
</tr>
<tr>
<td>Non-specific amplification with or without a product band</td>
<td>Carryover contamination</td>
<td>See “Preventing Contamination” on page 7.</td>
</tr>
<tr>
<td></td>
<td>Non-specific priming</td>
<td>Increase the anneal temperature in 1–2 °C increments.</td>
</tr>
<tr>
<td></td>
<td>Too much initial enzyme activity</td>
<td>Reduce pre-PCR activation time or use a “Time Release” protocol.</td>
</tr>
<tr>
<td></td>
<td>Primer design not optimal</td>
<td>Review primer design and composition.</td>
</tr>
</tbody>
</table>
Appendix B. References


Appendix C. Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section “To Obtain Documents on Demand” following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

<table>
<thead>
<tr>
<th>Product Area</th>
<th>E-mail address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Analysis (DNA Sequencing)</td>
<td><a href="mailto:galab@appliedbiosystems.com">galab@appliedbiosystems.com</a></td>
</tr>
<tr>
<td>Sequence Detection Systems and PCR</td>
<td><a href="mailto:pcrlab@appliedbiosystems.com">pcrlab@appliedbiosystems.com</a></td>
</tr>
<tr>
<td>Protein Sequencing, Peptide and DNA Synthesis</td>
<td><a href="mailto:corelab@appliedbiosystems.com">corelab@appliedbiosystems.com</a></td>
</tr>
<tr>
<td>Biochromatography</td>
<td><a href="mailto:tsupport@appliedbiosystems.com">tsupport@appliedbiosystems.com</a></td>
</tr>
<tr>
<td>PerSeptive DNA, PNA and Peptide Synthesis systems</td>
<td></td>
</tr>
<tr>
<td>FMAT™ 8100 HTS System</td>
<td></td>
</tr>
<tr>
<td>CytoFluor® 4000 Fluorescence Plate Reader</td>
<td></td>
</tr>
<tr>
<td>Voyager™ Mass Spectrometers</td>
<td></td>
</tr>
<tr>
<td>Mariner™ Mass Spectrometers</td>
<td></td>
</tr>
<tr>
<td>LC/MS (Applied Biosystems/MDS Sciex)</td>
<td><a href="mailto:support@sciex.com">support@sciex.com</a></td>
</tr>
<tr>
<td>Chemiluminescence (Tropix)</td>
<td><a href="mailto:tropix@appliedbiosystems.com">tropix@appliedbiosystems.com</a></td>
</tr>
</tbody>
</table>

Times for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

<table>
<thead>
<tr>
<th>Product</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemiluminescence</td>
<td>8:30 a.m. to 5:30 p.m. Eastern Time</td>
</tr>
<tr>
<td>Framingham support</td>
<td>8:00 a.m. to 6:00 p.m. Eastern Time</td>
</tr>
<tr>
<td>All Other Products</td>
<td>5:30 a.m. to 5:00 p.m. Pacific Time</td>
</tr>
</tbody>
</table>
To Contact Technical Support by Telephone or Fax

In North America
To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial 1-800-831-6844 and press 1.)

<table>
<thead>
<tr>
<th>Product or Product Area</th>
<th>Telephone Dial...</th>
<th>Fax Dial...</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI PRISM® 3700 DNA Analyzer</td>
<td>1-800-831-6844, then press 8</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>DNA Synthesis</td>
<td>1-800-831-6844, then press 2, then 1</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>Fluorescent DNA Sequencing</td>
<td>1-800-831-6844, then press 2, then 2</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>Fluorescent Fragment Analysis (includes GeneScan® applications)</td>
<td>1-800-831-6844, then press 2, then 3</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)</td>
<td>1-800-831-6844, then press 2, then 4</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>ABI PRISM® 3100 Genetic Analyzer</td>
<td>1-800-831-6844, then press 2, then 6</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>Peptide Synthesis (433 and 43X Systems)</td>
<td>1-800-831-6844, then press 3, then 1</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>Protein Sequencing (Precise® Protein Sequencing Systems)</td>
<td>1-800-831-6844, then press 3, then 2</td>
<td>1-650-638-5981</td>
</tr>
<tr>
<td>PCR and Sequence Detection</td>
<td>1-800-762-4001, then press 1 for PCR, 2 for the 7700, 7900 or 5700, 6 for the 6700 or dial 1-800-831-6844, then press 5</td>
<td>1-240-453-4613</td>
</tr>
<tr>
<td>♦ Voyager™ MALDI-TOF Biospectrometry</td>
<td>1-800-899-5858, then press 1, then 3</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>♦ Mariner™ ESI-TOF Mass Spectrometry Workstations</td>
<td></td>
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### Outside North America

<table>
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<tr>
<td>Biochromatography (BioCAD® Workstations and POROS® Perfusion Chromatography Products)</td>
<td>1-800-899-5858, then press 1, then 4</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>Expedite™ Nucleic acid Synthesis Systems</td>
<td>1-800-899-5858, then press 1, then 5</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)</td>
<td>1-800-899-5858, then press 1, then 5</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>PNA Custom and Synthesis</td>
<td>1-800-899-5858, then press 1, then 5</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>FMAT™ 8100 HTS System</td>
<td>1-800-899-5858, then press 1, then 6</td>
<td>1-508-383-7855</td>
</tr>
<tr>
<td>Cytofluor® 4000 Fluorescence Plate Reader</td>
<td></td>
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</tr>
<tr>
<td>Chemiluminescence (Tropix)</td>
<td>1-800-542-2369 (U.S. only), or 1-781-271-0045</td>
<td>1-781-275-8581</td>
</tr>
<tr>
<td>LC/MS (Applied Biosystems/MDS Sciex)</td>
<td>1-800-952-4716</td>
<td>1-508-393-7899</td>
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<table>
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<tbody>
<tr>
<td><strong>Africa and the Middle East</strong></td>
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<tr>
<td>Africa (English Speaking) and West Asia</td>
<td>27 11 478 0411</td>
<td>27 11 478 0349</td>
</tr>
<tr>
<td>(Fairlands, South Africa)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa (French Speaking; Courtaboeuf Cedex,</td>
<td>33 1 69 59 85 11</td>
<td>33 1 69 59 85 00</td>
</tr>
<tr>
<td>France)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa (Johannesburg)</td>
<td>27 11 478 0411</td>
<td>27 11 478 0349</td>
</tr>
<tr>
<td>Middle Eastern Countries and North Africa</td>
<td>39 (0)39 8389 481</td>
<td>39 (0)39 8389 493</td>
</tr>
<tr>
<td>(Monza, Italia)</td>
<td></td>
<td></td>
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<tr>
<td>Region</td>
<td>Telephone Dial...</td>
<td>Fax Dial...</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-------------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Eastern Asia, China, Oceania</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia (Scoresby, Victoria)</td>
<td>61 3 9730 8600</td>
<td>61 3 9730 8799</td>
</tr>
<tr>
<td>China (Beijing)</td>
<td>86 10 64106608 or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>86 800 8100497</td>
<td></td>
</tr>
<tr>
<td>Hong Kong</td>
<td>852 2756 6928</td>
<td>852 2756 6968</td>
</tr>
<tr>
<td>India (New Delhi)</td>
<td>91 11 653 3743/3744</td>
<td>91 11 653 3138</td>
</tr>
<tr>
<td>Korea (Seoul)</td>
<td>82 2 593 6470/6471</td>
<td>82 2 593 6472</td>
</tr>
<tr>
<td>Malaysia (Petaling Jaya)</td>
<td>60 3 79588268</td>
<td>603 79549043</td>
</tr>
<tr>
<td>Singapore</td>
<td>65 896 2168</td>
<td>65 896 2147</td>
</tr>
<tr>
<td>Taiwan (Taipei Hsien)</td>
<td>886 2 2358 2838</td>
<td>886 2 2358 2839</td>
</tr>
<tr>
<td>Thailand (Bangkok)</td>
<td>66 2 719 6405</td>
<td>66 2 319 9788</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria (Wien)</td>
<td>43 (0)1 867 35 75 0</td>
<td>43 (0)1 867 35 75 11</td>
</tr>
<tr>
<td>Belgium</td>
<td>32 (0)2 532 4484</td>
<td>32 (0)2 582 1886</td>
</tr>
<tr>
<td>Czech Republic and Slovakia (Praha)</td>
<td>420 2 35365189</td>
<td>420 2 35364314</td>
</tr>
<tr>
<td>Denmark (Naerum)</td>
<td>45 45 58 60 00</td>
<td>45 45 58 60 01</td>
</tr>
<tr>
<td>Finland (Espoo)</td>
<td>358 (0)9 251 24 250</td>
<td>358 (0)9 251 24 243</td>
</tr>
<tr>
<td>France (Paris)</td>
<td>33 (0)1 69 59 85 85</td>
<td>33 (0)1 69 59 85 00</td>
</tr>
<tr>
<td>Germany (Weiterstadt)</td>
<td>49 (0) 6150 101 0</td>
<td>49 (0) 6150 101 101</td>
</tr>
<tr>
<td>Hungary (Budapest)</td>
<td>36 (0)1 270 8398</td>
<td>36 (0)1 270 8288</td>
</tr>
<tr>
<td>Italy (Milano)</td>
<td>39 (0)39 83891</td>
<td>39 (0)39 838 9492</td>
</tr>
<tr>
<td>Norway (Oslo)</td>
<td>47 23 12 06 05</td>
<td>47 23 12 05 75</td>
</tr>
<tr>
<td>Poland, Lithuania, Latvia, and Estonia (Warszawa)</td>
<td>48 (22) 866 40 10</td>
<td>48 (22) 866 40 20</td>
</tr>
<tr>
<td>Portugal (Lisboa)</td>
<td>351 (0)22 605 33 14</td>
<td>351 (0)22 605 33 15</td>
</tr>
<tr>
<td>Russia (Moskva)</td>
<td>7 502 935 8888</td>
<td>7 502 564 8787</td>
</tr>
<tr>
<td>South East Europe (Zagreb, Croatia)</td>
<td>385 1 34 91 927/838</td>
<td>385 1 34 91 840</td>
</tr>
<tr>
<td>Spain (Tres Cantos)</td>
<td>34 (0)91 806 1210</td>
<td>34 (0)91 806 1206</td>
</tr>
<tr>
<td>Sweden (Stockholm)</td>
<td>46 (0)8 619 4400</td>
<td>46 (0)8 619 4401</td>
</tr>
<tr>
<td>Switzerland (Rotkreuz)</td>
<td>41 (0)41 799 7777</td>
<td>41 (0)41 790 0676</td>
</tr>
</tbody>
</table>
To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Access the Applied Biosystems Technical Support Web site.</td>
</tr>
<tr>
<td>2</td>
<td>Under the Troubleshooting heading, click Support Request Forms, then select the relevant support region for the product area of interest.</td>
</tr>
<tr>
<td>3</td>
<td>In the Personal Assistance form, enter the requested information and your question, then click Ask Us RIGHT NOW.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Telephone Dial...</th>
<th>Fax Dial...</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Netherlands (Nieuwerkerk a/d IJssel)</td>
<td>31 (0)180 392400</td>
<td>31 (0)180 392409 or 31 (0)180 392499</td>
</tr>
<tr>
<td>United Kingdom (Warrington, Cheshire)</td>
<td>44 (0)1925 825650</td>
<td>44 (0)1925 282502</td>
</tr>
<tr>
<td>All other countries not listed (Warrington, UK)</td>
<td>44 (0)1925 282481</td>
<td>44 (0)1925 282509</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Telephone Dial...</th>
<th>Fax Dial...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan (Hacchobori, Chuo-Ku, Tokyo)</td>
<td>8120 477392 (Toll free within Japan) or 81 3 5566 6230</td>
<td>8120 477120 (Toll free within Japan) or 81 3 5566 6507</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Telephone Dial...</th>
<th>Fax Dial...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caribbean countries, Mexico, and Central America</td>
<td>52 55 35 3610</td>
<td>52 55 66 2308</td>
</tr>
<tr>
<td>Brazil</td>
<td>0 800 704 9004 or 55 11 5070 9654</td>
<td>55 11 5070 9694/95</td>
</tr>
<tr>
<td>Argentina</td>
<td>800 666 0096</td>
<td>55 11 5070 9694/95</td>
</tr>
<tr>
<td>Chile</td>
<td>1230 020 9102</td>
<td>55 11 5070 9694/95</td>
</tr>
<tr>
<td>Uruguay</td>
<td>0004 055 654</td>
<td>55 11 5070 9694/95</td>
</tr>
</tbody>
</table>
To Obtain Documents on Demand

Free, 24-hour access to Applied Biosystems technical documents, including MSDSs, is available by fax or e-mail or by download from our Web site.

<table>
<thead>
<tr>
<th>To order documents...</th>
<th>Then...</th>
</tr>
</thead>
</table>
|                       | b. Click the Index link for the document type you want, then find the document you want and record the index number.  
|                       | c. Use the index number when requesting documents following the procedures below. |
| by phone for fax delivery | a. From the U.S. or Canada, call 1-800-487-6809, or from outside the U.S. and Canada, call 1-858-712-0317.  
|                       | b. Follow the voice instructions to order the documents you want.  |
| Note                  | There is a limit of five documents per request. |

To submit technical questions from North America or Europe:

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
</table>
| 4    | In the Customer Information form, enter the requested information and your question, then click Ask Us RIGHT NOW.  
|      | Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert. |
To Obtain Customer Training Information

The Applied Biosystems Training web site at www.appliedbiosystems.com/techsupp/training.html provides course descriptions, schedules, and other training-related information.

<table>
<thead>
<tr>
<th>To order documents...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>through the Internet for fax or e-mail delivery</td>
<td>a. Access the Applied Biosystems Technical Support Web site at <a href="http://www.appliedbiosystems.com/techsupp">http://www.appliedbiosystems.com/techsupp</a></td>
</tr>
<tr>
<td></td>
<td>b. Under Resource Libraries, click the type of document you want.</td>
</tr>
<tr>
<td></td>
<td>c. Enter or select the requested information in the displayed form, then click Search.</td>
</tr>
<tr>
<td></td>
<td>d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click Deliver Selected Documents Now (or click the PDF icon for the document to download it immediately).</td>
</tr>
<tr>
<td></td>
<td>e. Fill in the information form (if you have not previously done so), then click Deliver Selected Documents Now to submit your order.</td>
</tr>
</tbody>
</table>

**Note** There is a limit of five documents per request for fax deliver but no limit on the number of documents you can order for e-mail delivery.