USER GUIDE

applied biosystems® by life technologies™

Platinum® Multiplex PCR Master Mix

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life technologies™

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About This Guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix of this document.

Purpose

The *Platinum® Multiplex PCR Master Mix User Guide* provides detailed information for performing multiplex end-point PCR over a wide range of DNA templates, including challenging GC-rich sequences, using the Platinum® Multiplex PCR Master Mix.

Prerequisites

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

User attention words

Three user attention words appear in this document. 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 or accurate chemistry kit use.

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.

Platinum® Multiplex PCR Master Mix User Guide

Product information

Introduction

A variant of PCR, end-point multiplex PCR enables simultaneous amplification of many targets in a single tube using multiple pairs of primers. Multiplex PCR has been applied in research, forensic, and diagnostic laboratories where simultaneous analysis of multiple markers is required.

Examples of experiments performed using multiplex PCR:

- Genotyping: Deletions, mutations, and high-throughput SNP profiling
- Detection of pathogens or genetically modified organisms
- Microsatellites analysis
- Forensic analysis: Human identification and paternity testing
- Quantitative and reverse transcription PCR assays for gene expression

Purpose of the product

The Platinum® Multiplex PCR Master Mix contains all of the components (except for primers and templates) for multiplex end-point PCR in a single tube, including Platinum® DNA Polymerase. The kit also includes the GC Enhancer, which is used for difficult-to-amplify templates, especially for templates with high GC content.

This master mix is optimized for simultaneous amplification of multiple targets ranging in size from 50–2500 bp from a DNA template with a wide range of GC content.

The PCR products are then analyzed by using:

- Agarose gel electrophoresis
- Capillary electrophoresis
- Agilent® Bioanalyzer™ Instrument
- Agilent® Lab901

Kit contents and storage conditions

Part Number	Description	Storage Conditions
4464268	Platinum® Multiplex PCR Master Mix **50 reaction kit**	Store unopened at –15°C to –25°C until the expiration date on the label. After opening, the master mix may be stored at –15°C to –25°C until the expiration date on the label, or at 4°C for up to 30 days. The GC Enhancer must be kept at –15°C to –25°C.
	Platinum® Multiplex PCR Master Mix, 2× (1 tube, 1.25 mL)	
	GC Enhancer (1 tube, 0.3 mL)	
4464269	Platinum® Multiplex PCR Master Mix **250 reaction kit**	
	Platinum® Multiplex PCR Master Mix, 2× (5 tubes, 1.25 mL each)	
	GC Enhancer (1 tube, 1.25 mL)	
4464270	Platinum® Multiplex PCR Master Mix **2000 reaction kit**	
	Platinum® Multiplex PCR Master Mix, 2× (5 bottles, 10 mL each)	
	GC Enhancer (1 bottle, 10 mL)	

Workflow

Prepare for PCR

Select an instrument and reaction plate (page 10)

▼

Calculate the number of required reactions (page 10)

Design, verify and synthesize the primers

Select your amplicon site and design the primers (page 10)

▼

Verify primers for multiplex PCR (page 11)

▼

Synthesize the primers (page 12)

▼

Experimentally verify primers for multiplex PCR (page 12)

Prepare and run the PCR reaction

Prepare primer mix (page 12)

▼

Prepare the PCR reaction mix (page 12)

▼

Add template DNA to the PCR reaction mix (page 13)

▼

Set up and run PCR (page 14)

Analyze the PCR products (page 15)

Prepare for PCR

Select an instrument and reaction plate

Before you begin PCR, review "PCR good laboratory practices" on page 23 and select an instrument and a reaction plate. You can perform the PCR amplification with any of the thermal cyclers, equipment or materials listed on page 18.

Calculate the number of required reactions

Calculate the number of reactions needed to perform each assay, including no-template control (NTC) reactions. In your calculations, account for volume loss from pipetting by adding at least one extra reaction for every 10 required reactions. For example, for a 96-well plate, prepare enough volume for approximately 110 reactions.

Note: Multiple PCRs can be run on one reaction plate, but include controls for each run on the plate.

Design, verify and synthesize the primers

Select your amplicon site and design the primers

IMPORTANT! If GC-rich targets are unavoidable when selecting your amplicon site and primers, keep the amplicon sizes shorter than 300 bp. Avoid simultaneous amplification in a single reaction of targets with over 70% GC and those with less than 25% GC when mixing primers. GC Enhancer is included as an optional PCR additive for targets with greater than 65% GC content.

Choose one of the following methods for selecting your amplicon site and designing the primers:

- **Primer Express® Software**: Refer to the *Primer Express® Version 3.0 Getting Started Guide* and *Software Help* for using this software.
- **Primer3Plus**: Freeware provided at **www.bioinformatics.nl** under **Useful Links**. Follow the instructions beginning on this page.

  Note: Primer3Plus provides a Basic Local Alignment Search Tool (BLAST) algorithm, which is useful for comparing sequence information. Other software options do not provide this algorithm.

Primer3Plus Freeware

1. In the Main tab, paste your sequence ID and the source sequence.
2. In the General Settings tab, use the following parameters:

Variable	Definition
Primer size	21-30 nt
Primer T_m	59-65°C
Primer GC%	40-60%
Concentration of monovalent cations	50 mM
Annealing oligo concentration	50 nM
Mispriming/Repeat Library	Based on your assay
Remaining variables	Default or User-defined

3. In the Advanced Settings tab, define the product size:
   - Amplicon size differentiation between adjacent amplicons for sufficient separation:
     – For GC-rich sites, amplicon size should be <300 bp.
     – The table below defines the guidelines for differentiating the sizes if the PCR products are resolved on a 4% High-Resolution E-gel.

Size of the adjacent amplicons	Minimum difference
50–100 bp	10 bp
100–200 bp	20 bp
200–400 bp	40 bp
400–700 bp	50 bp
700–900 bp	100 bp
900–2500 bp	500 bp

     – If other E-gels are to be used, the following table gives a rough idea of the resolutions:

Gel type	Agarose	Run length	Resolution
E-gel® single comb	0.8%	5.8 cm	800 bp–3 kb
	1.2%		100 bp–3 kb
	2%		100 bp–2 kb

Verify primers for multiplex PCR

Verify the designed primers for their multiplex PCR compatibility using the in-silico PCR modeling feature at the UCSC Genome Bioinformatics website:

1. Go to **http://genome.ucsc.edu** and select **PCR** on the top menu bar.
2. Enter the sequences of forward and reverse primers.
3. Click **Submit**.
4. Verify the following:
   - The PCR product is unique.
   - The size of the product differentiates from adjacent amplicons.
   - The T_m for each primer is between 59–65°C.
5. Copy the sequence of the product, then click **BLAT**.
6. Paste the in-silico amplicon sequence into the text box in the Blat window and click **Submit** to conduct Blat.
7. Click **Details** for each retrieved fragment to ensure there is no homology in the primer targeting sites.

Synthesize the primers

Synthesize primers with a reliable oligo vendor and dissolve them in 0.1× TE buffer (10 mM Tris–HCl, 0.1 mM EDTA: pH 8 for regular oligos, and pH 7 for fluorescently labeled oligos). De-salted primers are generally pure enough for multiplex PCR.

We recommend measuring the concentrations of each primer with a UV spectrophotometer before use.

Experimentally verify primers for multiplex PCR

Verify the primers in singleplex PCR and by gel electrophoresis before use in multiplex PCR. Use only primers that produce single bands with the correct size.

Note: A 4% agarose E-gel is recommended.

If the template DNA is homozygous for the amplified target, use only the primers that generate a single band with the correct size for multiplex PCR. If a primer pair produces multiple bands when the template DNA is homozygous, redesign the primers by changing the location of the targeting site on the genome until a single band is produced.

Store the primers

Store primers in small aliquots at –20°C and avoid repeated freezing and thawing.

Prepare and run the PCR reaction

Prepare primer mix

1. Combine all primers for the multiplex PCR reaction in one tube, and adjust the primer mix to a final concentration of 0.5 µM per primer using 0.1× TE (10 mM Tris–HCl, 0.1 mM EDTA) buffer.
2. Dispense the primer mix into smaller aliquots that can be stored at –20°C for one year or 4°C for up to two months.

IMPORTANT! Avoid repeated freezing and thawing.

Prepare the PCR reaction mix

1. Thaw the primer mixes, templates, GC Enhancer (optional), and Platinum® Multiplex PCR Master Mix.
2. Mix the Platinum® Multiplex PCR Master Mix by inverting the tube or bottle about 10 times.

IMPORTANT! Avoid generating bubbles when mixing.

3. Place the Platinum® Multiplex PCR Master Mix tube or bottle on ice.
4. Mix the contents of the remaining tubes well by inverting each tube a few times and spinning briefly. Place the tubes on ice.

5. Prepare the PCR reaction mix as follows, using your number of calculated reactions as described on page 10:

Component	Volume per 50–µL reaction (µL)	Final concentration
Platinum® Multiplex PCR Master Mix, 2×	25 µL	1×
Primer mix (0.5 µM each primer)	5–10 µL†	50–100 nM each primer
GC Enhancer (Optional)	0–10 µL‡	0–20%
PCR-grade water	Variable: Use to bring final volume to 50 µL minus the volume of template DNA.	—

† Use 10 µl of primer mix (final concentration: 100 nM of each primer) when less than 0.1 µg of DNA is used.

‡ For targets with 65 to 75% GC, use 6 µL of the GC Enhancer in a 50–µL reaction. For targets with >75% GC, start with 10 µL in a 50–µL reaction. Refer to Optimize enhancer concentration (page 25) for more information.

6. Combine the components in an appropriate tube.
7. Close the tube and mix the solution well by inverting the tube a few times.
8. Centrifuge the tube briefly to spin down the contents.
9. Dispense appropriate volumes (50 µL minus the volume of template DNA) of the PCR reaction mix to the bottom of the wells of a reaction plate or PCR tubes.

Add template DNA to the PCR reaction mix

1. Add 0.1–0.2 µg of DNA template to the PCR reaction mix to bring the final volume to 50 µL.
2. For the no-template control reactions, adjust the final reaction volume of each well or tube to 50 µL with PCR-grade water.
3. Seal the plate with MicroAmp Clear Adhesive Film or cap the tubes with MicroAmp® 8-Cap Strips.
4. Mix the contents well by inverting the plates or tubes a few times then briefly centrifuging.

Set up and run PCR

1. Choose an amplification protocol based on your analysis method.
2. Configure the run method as outlined in your instrument user manual.
3. If the products will be analyzed by **agarose gel electrophoresis, Lab901** or **Agilent® 2100 Bioanalyzer™** instruments, use the following parameters:

Stage	Step	Temperature	Time
Holding	Activation of Platinum® Multiplex PCR Master Mix	95°C	2 min
Cycling (30 to 40 cycles)	Denature	95°C	30 sec
	Anneal	60°C	90 sec
	Extend	72°C	60 sec/kb of the largest amplicon
Holding	Final Extension	72°C	10 min
Holding	Final Hold	4°C	∞

If the products will be analyzed by **capillary electrophoresis**, use the following parameters:

Stage	Step	Temperature	Time
Holding	Activation of Platinum® Multiplex PCR Master Mix	95°C	2 min
Cycling (25 to 40 cycles)	Denature	95°C	30 sec
	Anneal	60°C	90 sec
	Extend	72°C	60 sec/kb of the largest amplicon
Holding	Final Extension	60°C	30 min
Holding	Final Hold	4°C	∞

4. Load the reaction plate or tubes into the instrument, and start the run. See your instrument user manual for detailed instruction on how to load and run the reactions.

Analyze the PCR products

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

You may analyze results using gel electrophoresis, capillary electrophoresis, Lab901 or the Agilent® 2100 Bioanalyzer™ instrument. Refer to instrument or PCR system documentation for analysis details.

For successful analysis using gel electrophoresis, follow these guidelines:

1. Unload the reaction plate or tubes after the run is complete.
2. (*Optional*) Store the plate or tubes at 4°C for up to 72 hours, or at –15 to –25°C for longer-term storage.
3. Choose the appropriate agarose concentration according to the total number of amplicons, the size difference between adjacent amplicons and the amplicon size range in each reaction.
4. Load and run gels according to the manufacturer's instructions to achieve the maximum separation of adjacent amplicons.
5. Take pictures of the gels on a UV transilluminator.

Troubleshooting

Observation	Possible cause	Recommended action
Excessive amount of primer dimer	Primer design is not optimal	Review primer design and composition, and ensure each primer pair gives a single band and no primer dimer in singleplex PCR.
	Cycle number is too high	Reduce the cycle number in increments of three cycles.
	Primer manufacturing error	Order primers from a reliable vendor and ensure that a purification process like de-salting and HPLC is used.
	Pipetting errors	Calibrate pipettes regularly to ensure the accuracy of pipetting.
	Quality of template DNA is too low	Always use high-quality, purified DNA templates.
Low levels of PCR product or no product band visible	Template concentration is too low	Increase the sample input to 0.1–1 µg/ 50 µL reaction.
	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™ Blocks 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™ Blocks 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.
	Preincubation/activation time is not sufficient	Increase the pre-PCR heat step in increments of 1 minute, or use the "Time Release" protocol (see "Adjust the hold period for activation" on page 24).

Observation	Possible cause	Recommended action
Product bands are smeared	Potential secondary products	Reduce final concentrations of the primers for the select amplicons to 50 nM while maintaining all other at 100 nM.
	Carryover contamination	• Use the GeneAmp® PCR Carry-Over Prevention Kit (Part no. 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.
	Sample amount is too low	Increase the sample input to 0.1–1 µg/50 µL reaction

A

Ordering Information

Kit Contents

Part Number	Description	Storage Conditions
4464268	Platinum® Multiplex PCR Master Mix **50 reaction kit**	Store unopened at –15°C to –25°C until the expiration date on the label. After opening, the master mix may be stored at –15°C to –25°C until the expiration date on the label, or at 4°C for up to 30 days. The GC Enhancer must be kept at –15°C to –25°C.
	Platinum® Multiplex PCR Master Mix, 2× (1 tube, 1.25 mL)	
	GC Enhancer (1 tube, 0.3 mL)	
4464269	Platinum® Multiplex PCR Master Mix **250 reaction kit**	
	Platinum® Multiplex PCR Master Mix, 2× (5 tubes, 1.25 mL each)	
	GC Enhancer (1 tube, 1.25 mL)	
4464270	Platinum® Multiplex PCR Master Mix **2000 reaction kit**	
	Platinum® Multiplex PCR Master Mix, 2× (5 bottles, 10 mL each)	
	GC Enhancer (1 bottle, 10 mL)	

Materials and equipment not included

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

Thermal cyclers

Item†	Applied Biosystems Part No.
Veriti® 60-Well Thermal Cycler	Part No. 4384638
Veriti® 96-Well Fast Thermal Cycler	Part No. 4375305
Veriti® 96-Well Thermal Cycler	Part No. 4375786
2720 Thermal Cycler	Part No. 4359659
Aluminum 96-Well GeneAmp® PCR System 9700	Part No. 4314879
Gold-plated 96-Well GeneAmp® PCR System 9700	Part No. 4314878
Silver 96-Well GeneAmp® PCR System 9700	Part No. N8050001

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

Reagents

Item	Source
Nuclease-free water (not DEPC-treated), 500 mL	Life Technologies (Part No. AM9930)
Tris-EDTA (TE) buffer, pH 8.0	Life Technologies (Part No. AM9849) or MLS
10× BlueJuice™ Gel Loading Buffer	Life Technologies (Part No. 10816015)
E-Gel® 50 bp DNA Ladder	Life Technologies (Part No. 10488099)
E-Gel® Low Range Quantitative DNA Ladder	Life Technologies (Part No. 12373031)

Other equipment and consumables

Item	Source
1.5-mL microcentrifuge tubes	MLS
Agarose	MLS
Pre-cast agarose gels, 1% to 4% with ethidium bromide stain	MLS
Centrifuge with plate adapter	MLS†
Disposable gloves	MLS
Electrophoresis apparatus	MLS
Invitrogen E-Gel® 4% High-Resolution Agarose Gel	Invitrogen (Part No. G5018-04)
Invitrogen E-Gel® 48 4% Agarose Gel	Invitrogen (Part No. G8008-04)
MicroAmp® Optical 96-Well Reaction Plates	Applied Biosystems (Part No. N8010560)
MicroAmp® Splash-Free 96-Well Base	Applied Biosystems (Part No. 4312063)
MicroAmp® 8-Tube Strip, 0.2 mL	Applied Biosystems (Part No. N8010580)

Item	Source
MicroAmp® 8-Cap Strip	Applied Biosystems (Part No. N8010535)
MicroAmp® 96-well Base	Applied Biosystems (Part No. N8010531)
MicroAmp® 96-Well Tray/Retainer Set, 10 sets	Life Technologies (Part No. 403081)
MicroAmp® Clear Adhesive Film	Applied Biosystems (Part No. 4306311)
MicroAmp® Optical Film Compression Pad‡.	Applied Biosystems (Part No. 4312639
Microcentrifuge	MLS
Pipettes, positive-displacement or air-displacement	MLS
Pipette tips with filter plugs	MLS
Polypropylene tubes	MLS
Vortexer	MLS

† 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.

‡ See instrument manual for compatibility.

For more product recommendations, visit the PCR technology page at:
www.appliedbiosystems.com

B Guidelines for Designing PCR Assays

This appendix covers:

- PCR good laboratory practices 23
- Adjust thermal cycling 24
- Optimize the PCR conditions 24

PCR good laboratory practices

When preparing samples for PCR amplification:

- 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 (Part no. AM9890).

Adjust thermal cycling

Adjust the hold period for activation

For general PCR runs, Applied Biosystems recommends a pre-PCR activation setup of 95°C for 2 minutes.

Adjust 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.
- 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.

Adjust annealing conditions

- For increased product specificity, use annealing temperatures higher than 45°C.
- Determine the optimum annealing temperature by testing at increments of 5 or fewer degrees Celsius until the maximum specificity is reached.
- Use computer programs that calculate primer melting temperatures (T_m) to help you narrow the range of annealing temperatures to test. For such a T_m calculator, go to **http://www.appliedbiosystems.com**, then select **Support ▸ Tools ▸ Research Tools ▸ T_m Calculator for PCR Primers**. The GeneAmp® PCR System 9700 Thermal Cycler also contains a T_m calculator.
- Thirty (30) 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.

Adjust 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

Optimize template concentration

- The DNA segment to be amplified from the template can be up to 2.5 kb long, although 100 to 1500 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 30 ng (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, however, this may increase the likelihood of primer dimers.

Optimize enhancer concentration

The GC Enhancer helps amplify challenging amplicons, including amplicons that are GC-rich, have GC-repeats, or generate nonspecific products. GC enhancer is generally not needed if the GC contents of the targets are between 25 and 70%.

In a 50-µL reaction, for targets with:

- 65 to 75% GC, start with 6 µL.
- >75% GC, start with 10 µL.

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

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

C

Safety

This appendix covers:

- General safety 27
- Chemical safety 28
- Biological hazard safety 29

General safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device. Always determine what chemicals have been used in the instrument before changing reagents or instrument components.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the 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! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **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**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

You can download the following documents and other product-support documents from **www.appliedbiosystems.com**:

Document	Part number
Platinum® Multiplex PCR Master Mix, 2X Product Insert	4463721
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
Getting Started Guide: Primer Express® Software Version 3.0	4362460

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.appliedbiosystems.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

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