

MicroSEQ® Microbial Identification System

Lab Setup Guide

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

TRADEMARKS

The trademarks mentioned herein are the property of Life Technologies Corporation unless otherwise noted.

ExoSAP-IT® is a trademark of USB Corporation. Performa® is a trademark of Edge Biosystems. FlashGel® is a trademark of Cambrex Bio Science.

© 2010 Life Technologies Corporation. All rights reserved.

Part Number 4453843 Rev. A
05/2010

Contents

	About This Guide	5
	Safety information	5
	Safety alert words	5
	SDSs	5
SETUP GUIDE	Laboratory Setup	7
	Product overview	7
	Area 1: DNA preparation	8
	Activities	8
	Equipment	8
	Area 2: PCR setup	9
	Activities	9
	Equipment	9
	Area 3: Post-amplification	10
	Activities	10
	Equipment	11
APPENDIX A	Recommended Materials	13
	Area 1: equipment, consumables, and reagents	13
	DNA preparation equipment	13
	DNA preparation consumables	13
	DNA preparation reagents	14
	Area 2: equipment, consumables, and reagents	15
	PCR setup equipment	15
	PCR setup consumables	15
	PCR setup reagents	16
	Area 3: equipment, consumables, and reagents	17
	Post-amplification equipment	17
	Post-amplification consumables	17
	Post-amplification reagents	18
	Area 3: equipment, consumables, and reagents for capillary electrophoresis	19
	Capillary electrophoresis equipment	19
	Capillary electrophoresis consumables	20
	Capillary electrophoresis reagents	21

APPENDIX B	MicroSEQ® Reagents Ordering Guide	23
APPENDIX C	Good PCR Practices	25
	Prevent contamination and nonspecific amplification	25
	PCR good laboratory practices	25
APPENDIX D	Safety	27
	Instrumentation safety	28
	Symbols on instruments	28
	General instrument safety	29
	Electrical safety	30
	Workstation safety	31
	Safety and electromagnetic compatibility (EMC) standards	31
	Chemical safety	33
	General chemical safety	33
	SDSs	33
	Chemical waste safety	34
	Biological hazard safety	35
	Safety alerts	36
	General alerts for all chemicals	36
	General alerts for instrumentation	36

About This Guide

Safety information



Note: For general safety information, see this section and [Appendix D, “Safety” on page 27](#). When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety alert words

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



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



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



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



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

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments (see “Safety symbols” on page 28).*

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see [“SDSs” on page 33](#).



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

Laboratory Setup

Product overview

The MicroSEQ[®] Microbial Identification System combines all of the instrumentation, reagents, sequence libraries, software, and validation support required for automated microbial identification using DNA sequencing.

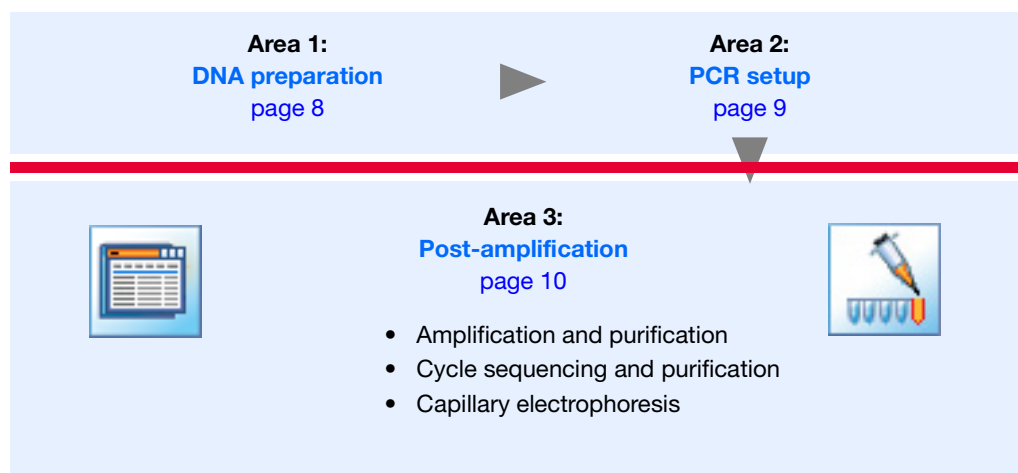
The MicroSEQ[®] system is a simple, rapid, and optimized system for the routine identification of all bacterial and fungal isolates, including organisms that are difficult to grow, non-viable, or unidentifiable using phenotypic methods. The MicroSEQ[®] system identifies bacterial and fungal isolates from a small sample of pure culture without preliminary testing or growth on selective media.

This chapter provides information on how to properly setup and equip a DNA amplification and sequencing laboratory for use with the MicroSEQ[®] Microbial Identification System.

To avoid cross-contamination and false amplifications when using the MicroSEQ[®] Microbial Identification System, consider setting up three separate laboratory areas, as shown in the figure below.

Areas 1 and 2 can be combined in one room. If this is the case, perform Area 1 activities in a Biological Safety Cabinet (BSC) and Area 2 activities in a PCR workstation or another BSC. Ideally, set up Areas 1 and 2 in a separate room from Area 3.

! **IMPORTANT!** If Areas 1, 2, and 3 are located in a single room, keep the three areas as separate as possible.



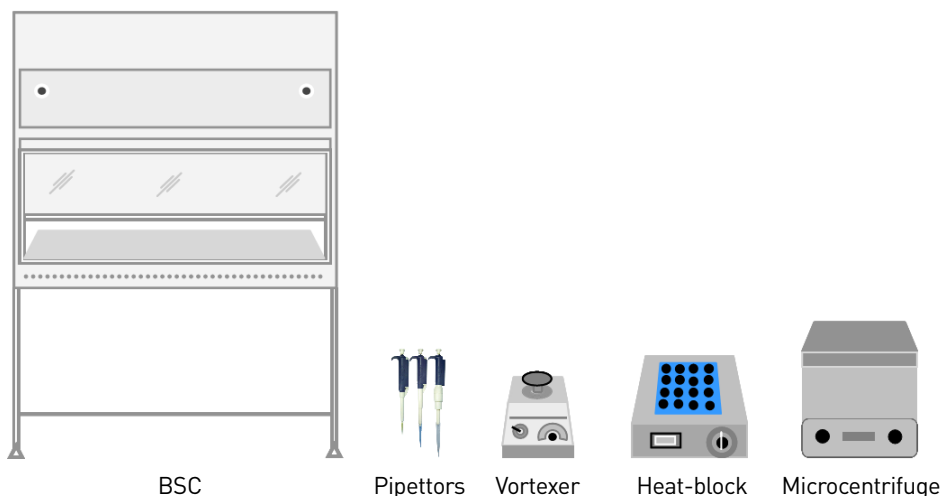
This guide contains instructions for setting up Areas 1, 2, and 3 for use with the MicroSEQ[®] Microbial Identification System.

Area 1: DNA preparation

Activities

1. Store PrepMan® Ultra Sample Preparation Reagent
2. Prepare for PCR
 - a. Dilute extracted DNA in nuclease-free water (not DEPC treated)
 - b. Add diluted DNA to the PCR master mix prepared in Area 2

Equipment



For information on the equipment listed below, refer to [“DNA preparation equipment”](#) on page 13.

1. Biological Safety Cabinet (BSC)
2. Pipettors
 - a. L200 (reagents only) — for pipetting PrepMan® Ultra Reagent
 - b. L200 — for transferring extracted DNA supernatant
 - c. L20 — for diluting extracted DNA and pipetting samples and positive controls into the PCR reaction
 - d. L1000 (reagents only) — for pipetting nuclease-free water

IMPORTANT! Do not share pipettors between the different areas. Do not use Area 1 pipettors in Areas 2 or 3.

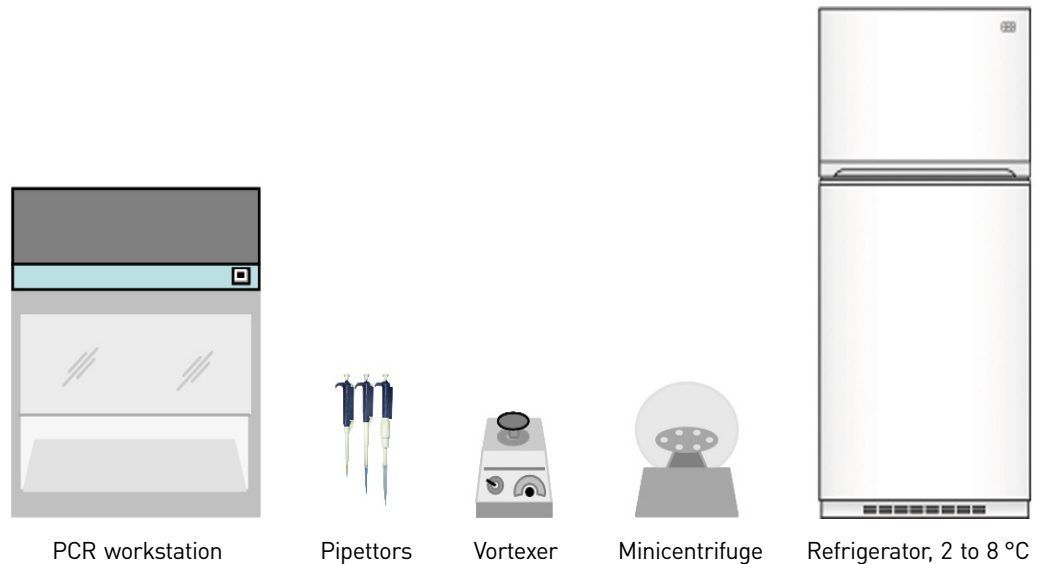
3. Vortexer
4. Heat-block
5. Microcentrifuge for 2 mL tubes; for recommended models refer to Appendix A on [page 13](#).

Area 2: PCR setup

Activities

1. Store the MicroSEQ[®] PCR kits.
2. Prepare the PCR reactions.

Equipment



For information on the equipment listed below, refer to [“DNA preparation equipment” on page 13](#).

1. PCR workstation; if Areas 1 and 2 are in the same room
2. Pipettors
 - a. L200 (reagents only) – for pipetting PCR reagents
 - b. L20 – for pipetting PCR reagents and negative control

IMPORTANT! Do not share pipettors between the different areas. Pipette positive controls and samples in **Area 1** using the L20 pipettor. Do not use Area 2 pipettors in Areas 1 or 3.

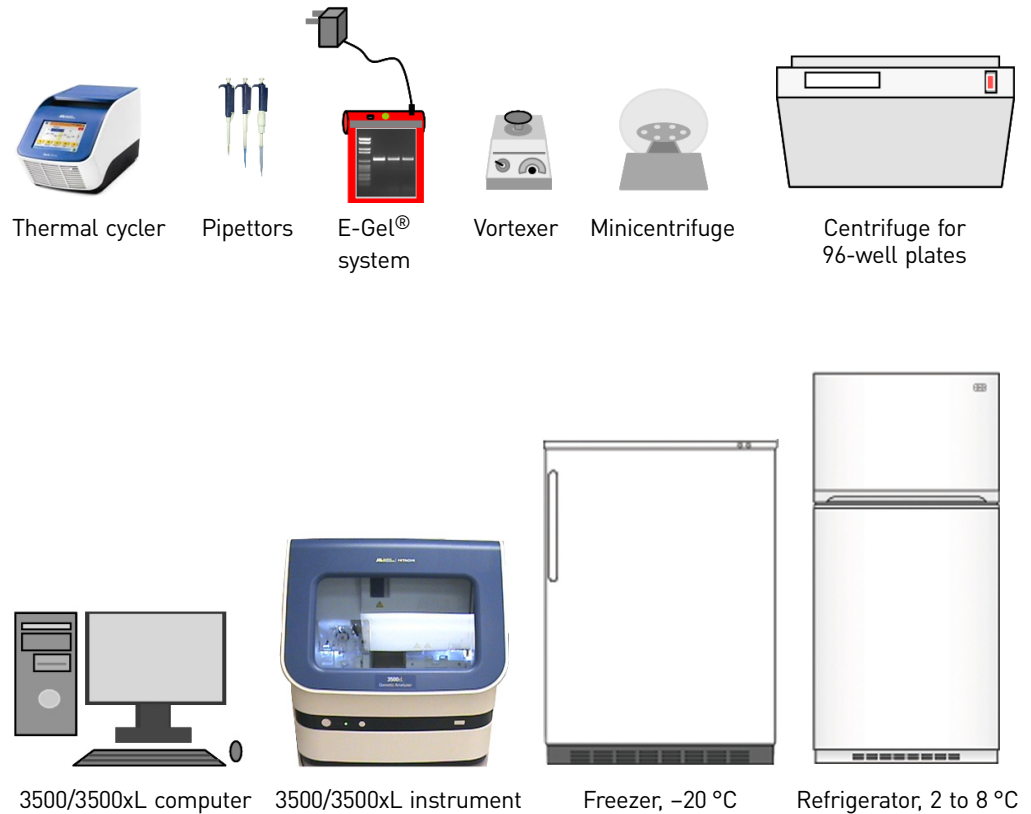
3. Vortexer
4. Benchtop minicentrifuge
5. 2 to 8 °C refrigerator; for storage of MicroSEQ[®] PCR Kits

Area 3: Post-amplification

Activities

1. Store the following items:
 - a. MicroSEQ[®] Identification Kits
 - b. PCR purification reagents
 - c. Genetic Analyzer consumables
 - d. Sequencing purification kits
 - e. Hi-Di[™] Formamide
2. Perform the following:
 - a. PCR thermal cycling
 - b. PCR check using agarose gel electrophoresis (OPTIONAL)
 - c. PCR purification
 - d. Cycle sequencing
 - e. Sequencing purification
 - f. Capillary electrophoresis

Equipment



For information on the equipment listed below, refer to [“DNA preparation equipment”](#) on page 13.

1. Thermal cycler
 2. Pipettors
 - a. L20 (reagents only) —for pipetting ExoSAP-IT reagent and MicroSEQ[®] sequencing reagents
 - b. L20 — for pipetting PCR product
- IMPORTANT!** Do not share pipettors between the different areas. Do not use Area 3 pipettors in Areas 1 or 2.
3. Agarose gel electrophoresis system (for example, E-Gel[®] system) (OPTIONAL)
 4. Vortexer
 5. Benchtop minicentrifuge
 6. Centrifuge for spinning 96-well plates or spin column format
 7. Genetic Analyzer

8. 20 °C freezer; for storage of sequencing kits, ExoSAP-IT[®] Kit, Hi-Di[™] Formamide, and PCR product
 9. 2 to 8 °C refrigerator; for storage of MicroSEQ[®] Identification Kits
- Note:** Use the 2 to 8 °C refrigerator used for storage of reagents in Areas 2 and 3.

A

Recommended Materials

Area 1: equipment, consumables, and reagents

DNA preparation equipment

Equipment	Supplier	Part number
Biological Safety Cabinet (BSC)	MLS [†]	—
Microcentrifuge for 1.5- to 2.0-mL tubes	Hettich, Eppendorf, or equivalent	Hettich Micro 22, or Eppendorf 5415 D, or Eppendorf 5430
Vortexer	MLS	Scientific Industries Vortex Genie
Heat block for 1.5- to 2.0-mL tubes	MLS	VWR Heatblock digital 2-blk 115V (13259-052)
Pipettors L20 (n=1), L200 (n=2), L1000 (n=1)	MLS	Rainin LTS, or Gilson Pipetman P

[†] MLS: Major Laboratory Supplier

DNA preparation consumables

Consumables	Supplier	Part number
Pipette tips with Barrier	Applied Biosystems or MLS [†]	<ul style="list-style-type: none"> • AM12665 (1000 µL) • AM12655 (200 µL) • AM12645 (20 µL) or, for Rainin LTS pipettors: <ul style="list-style-type: none"> • Rainin RT-L10F (20 µL) • RT-L200F (200 µL) • RT-L1000F (1000 µL)
Bag/Stand Note: For discarding used tips	MLS	—
Non-stick RNase-Free 1.5-mL microcentrifuge tubes	Applied Biosystems	AM12450
2-mL screw cap tubes or 2-mL safe-lock tubes	MLS	Eppendorf 2-mL Safe-Lock tubes (022363352)
Tube rack for 1.5- to 2.0-mL tubes	MLS	—

1- μ L loops Note: for use in picking up colonies	MLS	—
Powder-free gloves	MLS	—

† MLS: Major Laboratory Supplier

DNA preparation reagents

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

Reagents	Supplier	Part number
PrepMan [®] Ultra Sample Preparation Reagent, 200 reactions	Applied Biosystems	4322547 (with protocol) or 4318930 (without protocol)
Nuclease-free water (not DEPC treated)	Applied Biosystems	AM9937 (10 x 50-mL) or AM9938 (1 x 100-mL)
DNAZap [™] Solution	Applied Biosystems	AM9890

Area 2: equipment, consumables, and reagents

PCR setup equipment

Equipment	Supplier	Part number
Pipettors, L20 and L200	MLS‡	Rainin LTS, or Gilson Pipetman P
Refrigerator; 2 to 8 °C	MLS	—
Minicentrifuge	MLS	—
Vortexer	MLS	Scientific Industries Vortex Genie
PCR workstation	MLS	—
MicroAmp® Cap Installing Tool† Note: If using caps to cover reactions in 96-well plates	Applied Biosystems	4330015
MicroAmp® Adhesive Film Applicator Kit† Note: If using adhesive file to seal plates	Applied Biosystems	4333183
MicroAmp® Splash Free 96-Well Base†	Applied Biosystems	4312063
MicroAmp® 96-Well Tray/Retainer Set for Veriti® systems† Note: For use with Veriti® 0.2 mL Thermal Cycler, if using MicroAmp® 8-Tubes Strip, 0.2-mL; or MicroAmp® Reaction Tubes without Caps, 0.2 mL	Applied Biosystems	4381850
MicroAmp® Fast 96-Well Tray	Applied Biosystems	4358305

† These items are part of the Thermal Cycling Starter Kit that ships with the Veriti® Thermal Cycler.

‡ MLS: Major Laboratory Supplier

PCR setup consumables

Consumables	Supplier	Part number
MicroAmp® Optical 96-Well Reaction Plate with Barcode, 20 plates or MicroAmp® Optical 96-Well Reaction Plate with Barcode, 10 plates Note: For use with Veriti® 0.2 mL Thermal Cycler	Applied Biosystems	4306737 or N8010560

MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL Note: For use with 9800 Fast Thermal Cycler	Applied Biosystems	4346906
MicroAmp® Clear Adhesive Film Note: If using adhesive film to seal plates	Applied Biosystems	4306311
MicroAmp® Caps Note: If using 8-tube strips or single tubes without cap, or if using caps instead of adhesive film to cover reactions in 96-well plates	Applied Biosystems	8 Caps/Strip N8010535 or 12 Caps/Strip N8010534
MicroAmp® Reaction Tubes with cap, 0.2-mL; or MicroAmp® Reaction Tubes without Cap, 0.2-mL; or MicroAmp® 8-Tube Strip, 0.2-mL Note: For use with Veriti® 0.2 mL Thermal Cycler, if using tubes instead of plates	Applied Biosystems	N8010612, N8010533, or N8010580
MicroAmp® Fast 8-Tube Strip, 0.1-mL Note: For use with 9800 Fast Thermal Cycler, if using 8-tube strips instead of plates	Applied Biosystems	4358293
Pipette tips with Barrier	Applied Biosystems or MLS†	AM12645 (20 µL) or Rainin RT-L10F (20 µL), for Rainin LTS pipettors
Bag/Stand Note: For discarding used tips	MLS	—
Powder-free gloves	MLS	—

† MLS: Major Laboratory Supplier

PCR setup reagents

Reagents	Supplier	Part number
Fast MicroSEQ® 500 16S rDNA Bacterial ID PCR Kit	Applied Biosystems	4370653 (with Protocol) or 4370489 (without Protocol)
MicroSEQ® Full Gene 16S rDNA Bacterial ID PCR Kit	Applied Biosystems	4349161 (with Protocol) or 4349155 (without Protocol)
Fast MicroSEQ® D2 rDNA Fungal ID PCR Kit	Applied Biosystems	4396787 (with Protocol) or 4382397 (without Protocol)

Area 3: equipment, consumables, and reagents

Post-amplification equipment

Equipment	Supplier	Part number
Veriti® 96-Well Thermal Cycler, 0.2 mL	Applied Biosystems	4375786
Table-top centrifuge with 96-deep-well adapter, if using Performa® 96; or microcentrifuge, if using Performa® single columns	Eppendorf or equivalent	Eppendorf 5804, Eppendorf 5430, or Eppendorf 5415 D
Pipettors; 2 × L20	MLS†	Rainin LTS, or Gilson Pipetman P
Freezer, -20 °C	MLS	—
Agarose gel system (OPTIONAL): <ul style="list-style-type: none"> E-Gel® System <ul style="list-style-type: none"> E-Gel® PowerBase™ v.4 E-Gel® EX Gel, 2%, 10-Pak <i>or</i> <ul style="list-style-type: none"> FlashGel® System <ul style="list-style-type: none"> FlashGel® Dock FlashGel® Cassettes (1.2% agarose, 12 + 1 well format) 	Invitrogen Lonza	Invitrogen: <ul style="list-style-type: none"> G6200-4 G4010-02 Lonza: <ul style="list-style-type: none"> 57025 57023
Gel documentation system (OPTIONAL)	MLS	—
Minifuge	MLS	—
Vortexer	MLS	Scientific Industries Vortex Genie

† MLS: Major Laboratory Supplier

Post-amplification consumables

Consumables	Supplier	Part number
MicroAmp® Optical 96-Well Reaction Plate with Barcode, 20 plates or MicroAmp® Optical 96-Well Reaction Plate, 10 plates Note: For use with Veriti® 0.2 mL Thermal Cycler	Applied Biosystems	4306737 or N8010560

MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL Note: For use with 9800 Fast Thermal Cycler	Applied Biosystems	4346906
MicroAmp® Clear Adhesive Film Note: If using adhesive film to seal plates	Applied Biosystems	4306311
MicroAmp® Caps Note: If using 8-tube strips or single tubes without cap, or if using caps instead of adhesive film to cover reactions in 96-well plates	Applied Biosystems	8 Caps/Strip N8010535 or 12 Caps/Strip N8010534
MicroAmp® Reaction Tubes with cap, 0.2-mL; or MicroAmp® Reaction Tubes without Cap, 0.2-mL; or MicroAmp® 8-Tube Strip, 0.2-mL Note: For use with Veriti® 0.2 mL Thermal Cycler, if using tubes instead of plates	Applied Biosystems	N8010612, N8010533, or N8010580
MicroAmp® Fast 8-Tube Strip, 0.1-mL Note: For use with 9800 Fast Thermal Cycler, if using 8-tubes strip instead of plates	Applied Biosystems	4358293
Pipette tips with Barrier	Applied Biosystems or MLS†	AM12645 (20 µL) or Rainin RT-L10F (20 µL), for Rainin LTS pipettors
Bag/Stand Note: For discarding used tips	MLS	—
Powder-free gloves	MLS	—

† MLS: Major Laboratory Supplier

Post-amplification reagents

Reagents	Supplier	Part number
MicroSEQ® 500 16S rDNA Bacterial ID Sequencing Kit	Applied Biosystems	4346479 (with Protocol) or 4346480 (without Protocol)
MicroSEQ® Full Gene 16S rDNA Bacterial ID Sequencing Kit	Applied Biosystems	4347483 (with Protocol) or 4347484 (without Protocol)
MicroSEQ® D2 rDNA Fungal ID Sequencing Kit	Applied Biosystems	4347482 (with Protocol) or 4347481 (without Protocol)
MicroSEQ® ID Cleanup Kit; ExoSAP-IT® Reagent bundled with 2X Performa® DTR Ultra 96-Well Kit†	Applied Biosystems	4415506

MicroSEQ [®] ID Purification Combo Kit 2; ExoSAP-IT [®] Reagent bundled with 36 Performa [®] DTR Gel Filtration Cartridges [†]	Applied Biosystems	4427807
MicroSEQ [®] ID Sequencing Cleanup Plates Note: For use with Performa [®] DTR Ultra 96-Well Kit [†]	Applied Biosystems	4408227
MicroSEQ [®] ID Sequencing Cleanup Cartridges Note: For use with Performa [®] DTR Gel Filtration Cartridges [†]	Applied Biosystems	4408228
MicroSEQ [®] ID Microbial Identification Software bundle (MSID analysis software with bacterial and fungal libraries)	Applied Biosystems	4371298
Hi-Di [™] Formamide or Hi-Di [™] Formamide, 5-mL	Applied Biosystems	4311320 or 4440753

[†] Applied Biosystems offers different configurations of PCR and sequencing cleanup kits. You can choose the most appropriate combinations for your purpose (see "MicroSEQ[®] Reagents Ordering Guide" on page 23 for reference).

Area 3: equipment, consumables, and reagents for capillary electrophoresis

Capillary electrophoresis equipment

Equipment	Supplier	Part number
3500/3500xL Genetic Analyzer	Applied Biosystems	<ul style="list-style-type: none"> 3500 (8 caps): 4440464 3500xL (24 caps): 4440465 3500: 4406019 3500xL: 4406018
3130/3130xL Genetic Analyzer	Applied Biosystems	<ul style="list-style-type: none"> 3130 (4 caps): 4363198, 4427003, or 4427004 3130xL (16 caps): 4363199, 4427005, or 4427006
Refrigerator; 2 to 8 °C [†]	MLS [‡]	—

[†] Use the same 2 to 8 °C refrigerator as that used for storage of reagents in Areas 2 and 3.

[‡] MLS: Major Laboratory Supplier; For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Capillary electrophoresis consumables

Consumables	Supplier	Part number
Tubes, 50-mL	Applied Biosystems	AM12501
Powder-free gloves	MLS [†]	—
Lint-free tissues	MLS	for example Kimwipes [®]
3500/3500xL Genetic Analyzer consumables		
96-Well retainer & base set (Standard) 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] Optical 96-Well Reaction Plate for capillary electrophoresis	Applied Biosystems	4410228
96-Well retainer & base set (Fast) for 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] Fast Optical 96-Well Reaction Plate for capillary electrophoresis	Applied Biosystems	4409530
96-Well Septa for 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] Optical 96-Well Reaction Plate or MicroAmp [®] Fast Optical 96-Well Reaction Plate for capillary electrophoresis	Applied Biosystems	4412614
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] 8-Tube Strip, 0.2 mL for capillary electrophoresis	Applied Biosystems	4410231
8-Tube retainer & base set (Fast) for 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] Fast 8-Tube Strip, 0.1 mL for capillary electrophoresis	Applied Biosystems	4410233
8-Strip Septa for 3500/3500xL Genetic Analyzers Note: If using MicroAmp [®] 8-Tube Strip, 0.2 mL or MicroAmp [®] 8-Tube Strip, 0.1 mL for capillary electrophoresis	Applied Biosystems	4410701
Septa Cathode Buffer Container, 3500 Series	Applied Biosystems	4410715

3500 Genetic Analyzer Capillary Array, 50 cm (8 caps) Note: If using 3500 Genetic Analyzer instrument (8 caps)	Applied Biosystems	4404685
3500xL Genetic Analyzer Capillary Array, 50 cm (24 caps) Note: If using 3500 Genetic Analyzer instrument (24 caps)	Applied Biosystems	4404689
3130/3130xL Genetic Analyzer consumables		
96-Well Plate Base for 3130 Series Note: If using MicroAmp® Optical 96-Well Reaction Plate for capillary electrophoresis	Applied Biosystems	4317237
96-Well Plate Retainer for 3130 Series Note: If using MicroAmp® Optical 96-Well Reaction Plate for capillary electrophoresis	Applied Biosystems	4317241
96-Well Plate Septa for 3130 Series	Applied Biosystems	4315933
3130/3100-Avant™ Genetic Analyzer Capillary Array, 50 cm (4 caps) Note: If using 3130 Genetic Analyzer instrument (4 caps)	Applied Biosystems	4333466
3130xL/3100 Genetic Analyzer Capillary Array, 50 cm (16 caps) Note: If using 3130 Genetic Analyzer instrument (16 caps)	Applied Biosystems	4315930

† MLS: Major Laboratory Supplier; For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Capillary electrophoresis reagents

Reagents	Supplier	Part number
Nuclease-free water (not DEPC treated)	Applied Biosystems	AM9937 (10 x 50-mL) or AM9938 (1 x 100-mL)
3500/3500xL Genetic Analyzer reagents		
POP-6™ Polymer for 3500/3500xL Genetic Analyzers Note: If using 3500 Genetic Analyzer Series with POP6	Applied Biosystems	4393717 (384 samples) or 4393712 (960 samples)

POP-7™ Polymer for 3500/3500xL Genetic Analyzers Note: If using 3500 Genetic Analyzer Series with POP7	Applied Biosystems	4393708 (384 samples) or 4393714 (960 samples)
Pouch Cap for Polymer Pouch, 3500 Series	Applied Biosystems	4412619
Anode Buffer Container (ABC), 3500 Series	Applied Biosystems	4393927
Cathode Buffer Container (CBC), 3500 Series	Applied Biosystems	4408256
Septa Cathode Buffer Container, 3500 Series	Applied Biosystems	4410715
Conditioning Reagent, 3500 Series	Applied Biosystems	4393718
Sequencing Install Standard, BigDye® Terminator v1.1, for 3500 Series	Applied Biosystems	4404314

3130/3130xL Genetic Analyzer reagents

POP-6™ Polymer for 3130/3130xL Genetic Analyzers Note: If using 3130 Genetic Analyzer Series with POP6	Applied Biosystems	4352757 (7-mL for 960 samples) or 4363783 (3.5-mL for 250 samples)
POP-7™ Polymer for 3130/3130xL Genetic Analyzers Note: If using 3130 Genetic Analyzer Series with POP7	Applied Biosystems	4352759 (7-mL for 960 samples) or 4363785 (3.5-mL for 250 samples)
Running Buffer, 10X for 3130 Series	Applied Biosystems	402824
3100/3130™ Genetic Analyzer Sequencing Standards, BigDye® Terminator v1.1	Applied Biosystems	4336791

B

MicroSEQ[®] Reagents Ordering Guide

The information in this ordering guide has been provided to allow you to determine the number of MicroSEQ[®] reagent kits required depending upon the number of identifications (IDs) to be evaluated, whether 50, 100, or 500 identifications.

Reagents	50 IDs [‡]	100 IDs	500 IDs
PrepMan [®] Ultra Sample Preparation Reagent; 20-mL bottle	5-mL	10-mL	50-mL (3 bottles)
Fast MicroSEQ [®] 500 16S rDNA Bacterial ID PCR Kit; for 50 IDs	1 kit	2 kits	10 kits
MicroSEQ [®] 500 16S rDNA Bacterial ID Sequencing Kit; for 50 IDs	1 kit	2 kits	10 kits
Fast MicroSEQ [®] D2 rDNA Fungal ID PCR Kit; for 50 IDs	1 kit	2 kits	10 kits
MicroSEQ [®] D2 rDNA Fungal ID Sequencing Kit; for 50 IDs	1 kit	2 kits	10 kits
MicroSEQ [®] Full Gene 16S rDNA Bacterial ID PCR Kit; for 10 IDs	5 kits	10 kits	50 kits
MicroSEQ [®] Full Gene 16S rDNA Bacterial ID Sequencing Kit; for 10 IDs	5 kits	10 kits	50 kits
MicroSEQ [®] Cleanup kits; ExoSAP-IT for PCR cleanup and Performa [®] DTR Ultra 96-Well plates for sequencing cleanup	1 kit 4415506 [§]	<ul style="list-style-type: none"> • 1 kit 4415506[§] • 1 kit 4408227^{‡‡} 	<ul style="list-style-type: none"> • 4 kits 4415506[§] • 2 kits 4408227^{‡‡}

Reagents	50 IDs [‡]	100 IDs	500 IDs
MicroSEQ® Cleanup kits; ExoSAP-IT for PCR cleanup and Performa® DTR Gel Filtration Cartridges for sequencing cleanup	<ul style="list-style-type: none"> • 1 kit 4427807^{††} • 2 kits 4408228^{§§} 	<ul style="list-style-type: none"> • 1 kit 4427807^{††} • 5 kits 4408228^{§§} 	<ul style="list-style-type: none"> • 5 kits 4427807^{††} • 23 kits 4408228^{§§}
Hi-Di™ Formamide [†] ; 20-mL or 5-mL bottle	1-mL	2-mL	10-mL

[†] Hi-Di™ Formamide: 20-mL bottle, PN 4311320; 5-mL bottle, PN 4440753

[‡] IDs: Identifications (bacteria or fungi)

[§] 4415506: MicroSEQ® ID Cleanup Kit (ExoSAP-IT® Reagent for 150 ID's, Performa® DTR Ultra 96-Well for 96 IDs)

^{††} 4427807: MicroSEQ® ID Purification Combo Kit 2 (ExoSAP-IT® Reagent for 100 IDs, 36 Performa® DTR Gel Filtration Cartridges for 18 IDs)

^{‡‡} 4408227: MicroSEQ® ID Sequencing Cleanup Plates (Performa® DTR Ultra 96-Well kit for 96 IDs)

^{§§} 4408228: MicroSEQ® ID Sequencing Cleanup Cartridges (Performa® DTR Gel Filtration Cartridges for 18 IDs)



Good PCR Practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- 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.
 - Centrifuge PCR samples briefly whenever residual sample may be present on the inside lid (such as after dropping a tube or plate, or when there is condensation on the tube or plate from heating or thawing).
 - Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
 - Keep reactions and components capped or sealed as much as possible.
 - Use a positive-displacement pipette or aerosol-resistant pipette tips.
 - Use separate pipette tips for sample preparation, PCR setup, and amplification steps.
 - Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.
 - Clean the PCR set-up area with DNAZap™ reagent.
- ⓘ **IMPORTANT!** To avoid false positives due to cross-contamination:
- Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
 - Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.

C

Appendix C Good PCR Practices
Prevent contamination and nonspecific amplification

D

Safety

This appendix covers:

■ Instrumentation safety	28
Symbols on instruments	28
General instrument safety	29
Electrical safety	30
Workstation safety	31
Safety and electromagnetic compatibility (EMC) standards	31
■ Chemical safety	33
General chemical safety	33
SDSs	33
Chemical waste safety	34
Biological hazard safety	35
■ Safety alerts	36
General alerts for all chemicals	36
General alerts for instrumentation	36











Instrumentation safety

Symbols on instruments




Electrical symbols on instruments







The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the On position of the main power switch.
	Indicates the Off position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the Standby condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols


The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see [“Safety information” on page 5](#)). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.


Symbol	Description
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of a radiological hazard and to proceed with appropriate caution.
	Indicates the presence of a slipping hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.

Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). European Union customers: Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See www.appliedbiosystems.com for a list of customer service offices in the European Union.

General instrument safety

 **WARNING! PHYSICAL INJURY HAZARD.** Use these products only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

 **WARNING! PHYSICAL INJURY HAZARD.** Using these products in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.



Moving and lifting
the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting
stand-alone
computers and
monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the
instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See [“About SDSs” on page 33](#).

Cleaning or
decontaminating the
instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Electrical safety

Power



WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.



WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.



WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating The MicroSEQ[®] Microbial Identification System has an installation (overvoltage) category of II, and is classified as portable equipment.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.



CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.

These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC Standards

U.S. and Canadian safety standards



The MicroSEQ[®] Microbial Identification System has been tested to and complies with standard:

UL 3101-1/CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

FDA "Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11," as applicable.

The MicroSEQ[®] Microbial Identification System has been tested to and complies with the "Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11," as applicable.

The MicroSEQ[®] Microbial Identification System has been tested to and complies with standard EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide."



Canadian EMC
standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and
EMC
standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC
Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning



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

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See “About SDSs” on page 33.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Safety Data Sheets (SDSs) provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

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

Obtaining SDSs

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

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





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

Chemical waste safety

Chemical waste hazards



CAUTION! HAZARDOUS WASTE. Refer to 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 Safety Data Sheets (SDSs) 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 SDS.
- 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 SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

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

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

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

Biological hazard safety

General biohazard



WARNING! 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* (www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov



Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see [“Safety alert words” on page 5](#).

General alerts for all chemicals

Avoid contact with skin, eyes, and/or clothing. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

General alerts for instrumentation

Please refer to the instrument user manual provided with your instrumentation.

Part Number 4453843 Rev. A 05/2010



Applied Biosystems
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

Technical Resources and Support
For the latest technical resources and support information
for all locations, please refer to our Web site at
www.appliedbiosystems.com