Fast MicroSeq® 500
16S rDNA Bacterial Identification Kits

Protocol
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Part Number 4370453 Rev. B
06/2010
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Preface

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Purpose

This protocol is written for users of the Applied Biosystems MicroSeq® Microbial Identification System for bacterial identification.

Audience

This guide is intended for novice and experienced users who are isolating DNA, performing PCR, cycle sequencing, and electrophoresis, and then analyzing data.

This guide assumes some experience with PCR techniques.

Safety

Safety Alert Words

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

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

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

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

Chemical Hazard Warning

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

Chemical Safety Guidelines

To minimize the hazards of chemicals:

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

About MSDSs

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

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

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to https://docs.appliedbiosystems.com/msdssearch.html.

2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click Search.

3. Find the document of interest, right-click the document title, then select any of the following:
   - Open – To view the document
   - Print Target – To print the document
   - Save Target As – To download a PDF version of the document to a destination that you choose

4. To have a copy of a document sent by fax or e-mail, select Fax or Email to the left of the document title in the Search Results page, then click RETRIEVE DOCUMENTS at the end of the document list.

5. After you enter the required information, click View/Deliver Selected Documents Now.

Chemical Waste Hazard

⚠️ WARNING CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
• Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

• Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

• Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

• Handle chemical wastes in a fume hood.

• After emptying the waste container, seal it with the cap provided.

• Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

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

• Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

• Ensure the health and safety of all personnel in your laboratory.

• Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

**WARNING** BIOHAZARD. Biological samples such as tissues, body fluids, 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 eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; [http://bmbiod.nih.gov](http://bmbiod.nih.gov))
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; [http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)).

Additional information about biohazard guidelines is available at: [http://www.cdc.gov](http://www.cdc.gov)

How to Obtain Services and Support

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

At the Services and Support page, you can:

- Obtain worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches
Information about the MicroSeq ID Analysis Software is available in the:

- MicroSeq® ID Analysis Software Version 2.0 Online Help
- Applied Biosystems MicroSeq web site at www.microseq.com
Introduction

Product Description

The Fast MicroSeq® 500 16S rDNA Bacterial Identification Kits are part of the MicroSeq Microbial Identification System, which combines the advantages of MicroSeq bacterial and fungal application kits, MicroSeq ID Analysis Software, easy PrepMan® Ultra sample preparation reagents and protocols, and industry-leading thermal cyclers and sequencing systems. The Applied Biosystems Fast technology is the most rapid method available for the identification of microorganisms, and allows identification in five hours.

The Fast MicroSeq 500 16S rDNA Bacterial Identification PCR Kit and the MicroSeq 500 16S rDNA Bacterial Identification Sequencing Kit provide an accurate, easy-to-use method for routine bacterial identifications. Using the power of DNA sequencing technology, the MicroSeq system can identify bacteria that are difficult to grow, biochemically inert, or difficult to identify with conventional phenotypic methods and non-viable bacteria.

Note: To identify a full 16S rDNA sequence bacterial species, use the MicroSeq Full Gene Kit. See the Applied Biosystems Web site (http://www.appliedbiosystems.com) for more information.

The PCR and sequencing kits contain reagents for amplifying and sequencing the first 500 base pairs of the 16S ribosomal RNA bacterial gene (16S rDNA). The resulting DNA sequence is analyzed using MicroSeq® ID Analysis Software and compared to a bacterial 16S rDNA gene library containing SSU rDNA sequence entries from ~1,700 validated species. Variation within the first 500 base pairs of the 16S ribosomal RNA region is sufficient to identify most organisms.

Identifying bacteria through comparative sequence analysis yields accurate and reproducible results, especially for biochemically inert species or “fall through” samples.

Unlike other bacterial identification systems, the Fast MicroSeq kits do not require gram stains, biochemical information, or special growth conditions to identify bacteria. DNA extraction is greatly simplified using the Applied Biosystems PrepMan® Ultra Sample Preparation Reagent (PN 4322547), which can be used for all types of bacteria.

With dye terminator labeling, each of the four dideoxy terminators (ddNTPs) is tagged with a different fluorescent dye. When dye-labeled terminators are present in the reaction mix, extension products are simultaneously terminated and labeled with the dye that corresponds to a given base, as shown in the following figure.

For more information about dye terminator and other sequencing chemistries, refer to the ABI PRISM® Automated DNA Sequencing Chemistry Guide (PN 4305080).

For optimum performance of the MicroSeq Microbial Identification System, use the following instruments:

- Applied Biosystems 9800 Fast Thermal Cycler
- Applied Biosystems 3130 and 3130xl Genetic Analyzers

**Note:** You can use a GeneAmp® PCR System 9700 instead of a 9800 Fast Thermal Cycler, but the 9700 does not provide the reduced amplification and sequencing times allowed by the new fast PCR chemistry and the 9800 Fast Thermal Cycler.

For information on older instruments that can also be used, see “Appendix B Additional Applied Biosystems Instrument Platforms Supported” on page 30.
Protocol Overview

About This Protocol

This protocol provides:

- A list of materials and equipment required to determine the sequence of the first 500 base pairs of the bacterial rRNA gene
- Instructions for performing PCR and cycle sequencing, and purifying PCR and extension products
- Information about interpreting results

Procedure Workflow

The following workflow provides a simplified overview of the procedure for using the MicroSeq 500 bacterial identification kits.
PCR Good Laboratory Practices

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these assays can lead to amplification of a single DNA molecule (Saiki et al., 1985; Mullis and Faloona, 1987).

- 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.
- Maintain separate areas, dedicated equipment, and supplies for:
  - Sample preparation and PCR setup
  - PCR amplification and post-PCR analysis
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Do not splash or spray PCR samples.
- Quick-spin PCR samples whenever residual sample is present on the inside lid (such as after dropping a tube or when there is condensation on the tube from heating or thawing)
- Keep reactions and components capped as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.

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.

Maintain separate areas, dedicated equipment, and supplies for:
- Sample preparation and PCR setup
- PCR amplification and post-PCR analysis

Never bring amplified PCR products into the PCR setup area.

Open and close all sample tubes carefully. Do not splash or spray PCR samples.

Quick-spin PCR samples whenever residual sample is present on the inside lid (such as after dropping a tube or when there is condensation on the tube from heating or thawing)

Keep reactions and components capped as much as possible.

Use positive-displacement pipettes or aerosol-resistant pipette tips.

Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.
Materials and Equipment

Kit Contents

The table below describes the contents of the two kits:

- The Fast MicroSeq 500 16S rDNA Bacterial Identification PCR Kit
- The MicroSeq 500 16S rDNA Bacterial Identification Sequencing Kit

PCR Kit (PN 4370489)

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast PCR Master Mix</td>
<td>One tube containing 2X PCR Master Mix, which is designed to amplify 16S rRNA gene sequences from bacterial genomic DNA. This tube contains enough PCR Master Mix to perform 50 PCR amplifications, five negative control assays, and five positive control assays.</td>
</tr>
<tr>
<td>Positive Control DNA</td>
<td>One tube of Positive Control DNA (E. coli) at 1 ng/µL, sufficient to perform 10 positive control assays.</td>
</tr>
<tr>
<td>Negative Control (Water)</td>
<td>One tube of Negative Control</td>
</tr>
</tbody>
</table>

Sequencing Kit (PN 4346480)

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Sequencing Mix</td>
<td>Two tubes, containing enough mix to perform a total of 55 reactions (50 sequencing and 5 control reactions)</td>
</tr>
<tr>
<td>Reverse Sequencing Mix</td>
<td>Two tubes, containing enough mix to perform a total of 55 reactions (50 sequencing and 5 control reactions)</td>
</tr>
</tbody>
</table>
Storage Guidelines

<table>
<thead>
<tr>
<th>Kit</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upon Receipt</td>
</tr>
<tr>
<td>PCR</td>
<td>– 15 to – 25 °C</td>
</tr>
</tbody>
</table>

- Avoid excess freeze-thaw cycles. Aliquot reagents in smaller amounts, if necessary.
- Before each use of the kits, allow the frozen stocks to thaw at room temperature.

**IMPORTANT!** Do not heat the reagents.
- Whenever possible, keep thawed materials on ice during use.
- Mix the contents of each tube thoroughly, but do not vortex vigorously. Centrifuge the tubes briefly to collect the liquid at the bottom of the tube.

Equipment and Materials Not Included

The items in the following two tables are required in addition to the reagents supplied in the MicroSeq Fungal Identification kits.

Instruments from Applied Biosystems

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems 3130/3130-xl Genetic Analyzer</td>
<td>Contact your local Applied Biosystems sales office. For information on older instruments that can also be used, see &quot;Appendix B Additional Applied Biosystems Instrument Platforms Supported&quot; on page 30.</td>
</tr>
<tr>
<td>Applied Biosystems 9800 Fast Thermal Cycler</td>
<td>Note: You can use a GeneAmp PCR System 9700 instead of a 9800 Fast Thermal Cycler, but the 9700 does not provide the reduced amplification and sequencing times allowed by the new fast PCR chemistry and the 9800 Fast Thermal Cycler.</td>
</tr>
</tbody>
</table>
User-supplied materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>3100/3130 BigDye® Terminator v1.1 Sequencing Standard</td>
<td>Applied Biosystems (PN 4336791)</td>
</tr>
<tr>
<td>(310, 3100, 3100-Avant, 3130, and 3130xl instruments)</td>
<td></td>
</tr>
<tr>
<td>Plates, covers, tubes, and caps as needed if using the</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems 9800 Fast Thermal Cycler:</td>
<td></td>
</tr>
<tr>
<td>• Optical 96-Well Fast Thermal Cycling Plate with Barcode</td>
<td>Applied Biosystems (PN 4346906)</td>
</tr>
<tr>
<td>• Fast Reaction Tubes, 0.1 mL</td>
<td>Applied Biosystems (PN 4358293)</td>
</tr>
<tr>
<td>• Fast 96-Well Retainer Tray</td>
<td>Applied Biosystems (PN 4346905)</td>
</tr>
<tr>
<td>• MicroAmp™ Splash Free Support Base</td>
<td>Applied Biosystems (PN 4312063)</td>
</tr>
<tr>
<td>• MicroAmp™ Caps, 8 Caps/Strip or 12 Caps/Strip</td>
<td>Applied Biosystems (PN N8010535 or N8010534)</td>
</tr>
<tr>
<td>Plates, covers, tubes, and caps as needed if using the</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems GeneAmp® PCR System 9700:</td>
<td></td>
</tr>
<tr>
<td>• MicroAmp™ 96-Well Support Base</td>
<td>Applied Biosystems (PN N8010531)</td>
</tr>
<tr>
<td>• MicroAmp™ 96-Well Tray</td>
<td>Applied Biosystems (PN N8010541)</td>
</tr>
<tr>
<td>• MicroAmp™ 96-Well Optical Reaction Plate with Barcode</td>
<td>Applied Biosystems (4306737)</td>
</tr>
<tr>
<td>• MicroAmp™ 96-Well Full Plate Covers</td>
<td>Applied Biosystems (PN N8010550)</td>
</tr>
<tr>
<td>• MicroAmp® Reaction Tubes, 0.2 mL</td>
<td>Applied Biosystems (PN N8010533)</td>
</tr>
<tr>
<td>• MicroAmp™ Caps, 8 Caps/Strip or 12 Caps/Strip</td>
<td>Applied Biosystems (PN N8010535 or N8010534)</td>
</tr>
<tr>
<td>PrepMan® Ultra Sample Preparation Reagent</td>
<td>Applied Biosystems (PN 4322547)</td>
</tr>
<tr>
<td>Nuclease-Free Water (not DEPC treated)</td>
<td>Ambion (PN 9937)</td>
</tr>
<tr>
<td>No-Stick RNase-Free 1.5-mL microfuge tubes</td>
<td>Ambion (PN 12450)</td>
</tr>
<tr>
<td>Table-top centrifuge, with 96-tube tray adaptor</td>
<td>Eppendorf (5804) or equivalent</td>
</tr>
<tr>
<td>Fixed-angle microcentrifuge</td>
<td>Eppendorf (5415D) or equivalent</td>
</tr>
<tr>
<td>FlashGel™ System as needed:</td>
<td>Cambrex</td>
</tr>
<tr>
<td>• FlashGel Starter Pack</td>
<td>• 57026</td>
</tr>
<tr>
<td>• FlashGel Dock</td>
<td>• 57025</td>
</tr>
<tr>
<td>• FlashGel Casette</td>
<td>• 57023</td>
</tr>
<tr>
<td>Montage® PCR Filter Unit</td>
<td>Millipore (UFC7 PCR 50)</td>
</tr>
</tbody>
</table>
### User-supplied materials (continued)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension Product Purification product as needed:</td>
<td></td>
</tr>
<tr>
<td>- CentriSep Spin Column</td>
<td>Applied Biosystems (PN 401763)</td>
</tr>
<tr>
<td>- DyeEx® 96 Kit</td>
<td>Qiagen (PN 63181)</td>
</tr>
<tr>
<td>- DyeEx® 2.0 Spin Kit</td>
<td>Qiagen (PN 63204)</td>
</tr>
<tr>
<td>Hi-Di™ Formamide (optional, for greater stability of extension products)</td>
<td>Applied Biosystems (PN 4311320)</td>
</tr>
<tr>
<td>ExoSAP-IT® Reagent</td>
<td>USB® (78200)</td>
</tr>
<tr>
<td>2-mL screw-cap microcentrifuge tubes</td>
<td>Major Laboratory Supplier (MLS)</td>
</tr>
<tr>
<td>Vortexer</td>
<td>MLS</td>
</tr>
</tbody>
</table>

Isolating DNA

**PrepMan® Ultra Sample Preparation Reagent**

Isolate bacterial genomic DNA using Applied Biosystems PrepMan® Ultra Sample Preparation Reagent (PN 4322547). Follow the instructions in the PrepMan® Ultra Sample Preparation Reagent Protocol (PN 4318925).

**IMPORTANT!** The ideal colony size is 2 to 3 mm. For smaller colonies, decrease the amount of PrepMan Ultra Sample Preparation Reagent to 50 µL from the suggested 100 µL in the PrepMan® Ultra Sample Preparation Reagent Protocol.

**WARNING** CHEMICAL HAZARD. PrepMan Ultra contains a material that may cause eye, skin, and respiratory tract irritation, and adverse effects on the kidneys and blood and central nervous system. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**Storing the DNA Sample**

The isolated DNA (supernatant) can be stored at –20 °C indefinitely or at 4 °C for up to one month.

**Preparing the Working Stock of Bacterial Genomic DNA**

At the end of the PrepMan Ultra protocol, you obtain a supernatant that contains bacterial genomic DNA.

**To make the working stock of bacterial genomic DNA:**

1. Pipette 495 µL of nuclease-free water (Ambion #9937) into a 1.5-mL microcentrifuge tube (Ambion #12450).
   
   For samples with low biomass, you can make a smaller dilution (for example, use 195 µL of nuclease-free water to make a 1:40 dilution).

2. Add 5 µL of the supernatant to get a 1:100 dilution.

3. Vortex the tube to mix the solution.

4. Store the remaining supernatant at –20 °C.
Performing PCR – Amplifying the 16S rDNA Region

Minimizing the Effects of PCR Inhibition

PCR inhibition can interfere with amplification of the desired region of the rRNA gene.

One way to avoid PCR inhibition is to further dilute your working stock of bacterial genomic DNA before proceeding to PCR. For example, add 45 µL of nuclease-free water to 5 µL of your working stock to make a 1:1000 dilution of your original PrepMan Ultra supernatant. Making several dilutions of each sample and performing PCR for each of them increases your chances of obtaining a PCR product of the correct size.

If the PrepMan Ultra supernatant is colored (typically blackish or greenish), PCR inhibition is likely to occur. If you do not obtain a PCR product from any of the diluted samples, use a DNA extraction kit to isolate pure DNA. Alternatively, you can try the bead-beating method as explained on the Biospec Web site (http://www.biospec.com).
Performing PCR  PCR amplifies the first 500 base pairs of the 16S ribosomal RNA gene (16S rDNA) in the Applied Biosystems 9800 Fast Thermal Cycler as explained in the following procedure.

**Note:** You can use a GeneAmp® PCR System 9700 (in 9600 emulation mode) instead of a 9800 Fast Thermal Cycler, but the 9700 does not provide the reduced amplification and sequencing times allowed by the new fast PCR chemistry and the 9800 Fast Thermal Cycler.

To amplify the 16S rDNA region:

1. Prepare samples and controls in tubes or 96-well plates as follows. Use the plate or tubes appropriate for your thermal cycler (see page 7).

<table>
<thead>
<tr>
<th>If preparing...</th>
<th>Then combine...</th>
</tr>
</thead>
</table>
| Negative Controls | • 15 µL 2X Fast PCR Master Mix  
                   | • 15 µL nuclease-free water                                                   |
| Positive Controls | • 15 µL 2X Fast PCR Master Mix  
                   | • 15 µL of the positive-control DNA                                           |
| Samples           | • 15 µL PCR 2X Fast PCR Master Mix  
                   | • 15 µL working stock (1:100 dilution of PrepMan® Ultra supernatant)         |

**Note:** The 15 µL of working stock solution is intended to provide an amount of DNA close to the 25 ng that is optimal for the PCR kit.

2. Cap the tubes or seal the 96-well plate with the 96-well heat seal, then place them in the thermal cycler.
To amplify the 16S rDNA region: (continued)

3. Use the following conditions for the 9800 Fast Thermal Cycler

**Note:** You can use the same conditions for a 9700 thermal cycler, but the run will take ~40 minutes (20 minutes longer than the 9800 run).

<table>
<thead>
<tr>
<th>Initial Step</th>
<th>Each of 30 Cycles</th>
<th>Final Extension</th>
<th>Final Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOLD</td>
<td>Melt 95 °C 10 sec</td>
<td>Anneal 95 °C 0 sec</td>
<td>CYCLE 64 °C 15 sec</td>
</tr>
</tbody>
</table>

a. You can increase the number of cycles to increase the PCR yield, but doing so can cause additional background signal from the negative control.

4. Set the reaction volume for thermal cycling to 30 µL.

5. Start the run.

6. Store the PCR products at −15 to −25 °C until you are ready to use them.

PCR products are stable for at least six months or longer at −20 °C without detectable degradation.

**Analyzing PCR Products**

Determine if a PCR product is present in your samples by running a 1.2% agarose (Cambrex FlashGel cassette 57023). Loading 5 µL of the PCR product per lane is sufficient to detect amplified DNA.

**Note:** Running a FlashGel is a time-efficient way (2 to 7 minutes) to determine if PCR products are present. Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
Performing PCR – Amplifying the 16S rDNA Region

The positive control and samples should display a PCR product between 460 to 560 bp, depending on the bacterial species. No product should be visible for the negative control.

If your samples show no PCR product, PCR inhibition is the most likely cause. “Troubleshooting” on page 22 provides more information about potential PCR problems and solutions.

Preparing PCR Products for Cycle Sequencing

After gel analysis, you have 25 µL of PCR product mixture. Remove unused dNTPs and primers from the PCR product mixture using one of the following products:

Clean-up the PCR product using one of the following methods:

- ExoSAP-IT® (USB PN 78200; http://www.usbweb.com)
  Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Montage® PCR Filter Unit (Millipore PN UFC7 PCR50)

Be sure you follow the guidelines for the starting sample volume for clean-up as directed in the product literature.
Performing Cycle Sequencing

Cycle sequencing occurs when successive rounds of denaturation, annealing, and extension in a 9800 Fast Thermal Cycler result in linear amplification of extension products. The products are then loaded into a genetic analyzer to determine the sequence.

Note: You can use a GeneAmp® PCR System 9700 (in 9600 emulation mode) instead of a 9800 Fast Thermal Cycler, but the 9700 does not provide the reduced amplification and sequencing times allowed by the new fast PCR chemistry and the 9800 Fast Thermal Cycler.

For additional information about cycle sequencing chemistries, refer to the Automated DNA Sequencing Chemistry Guide (PN 4305080).

Preparing Cycle Sequencing Reactions

Prepare one forward and one reverse sequencing reaction for each PCR product.

WARNING CHEMICAL HAZARD. MicroSeq 500 Sequencing Mixes (Forward and Reverse) cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare cycle sequencing reactions:

1. In a reaction tube or 96-well plate, combine in parallel for each PCR product:
   - 7 µL of the purified PCR product + 13 µL of the forward sequencing mix
   - 7 µL of the purified PCR product + 13 µL of the reverse sequencing mix

2. Cap the tubes or seal the 96-well plate with the 96-well heat seal, then place them in the thermal cycler.
Performing Cycle Sequencing

To perform cycle sequencing:

1. Program the 9800 Fast Thermal Cycler or the GeneAmp® PCR System 9700 (in 9600 emulation mode) using the following thermal-cycling conditions:

<table>
<thead>
<tr>
<th>Initial Step</th>
<th>Each of 25 Cycles</th>
<th>Final Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOLD</td>
<td>Melt</td>
<td>CYCLE HOLD</td>
</tr>
<tr>
<td>96 °C 1 min</td>
<td>96 °C 10 sec</td>
<td>4 °C ∞</td>
</tr>
<tr>
<td></td>
<td>50 °C 5 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 °C 1 min 15 sec</td>
<td></td>
</tr>
</tbody>
</table>

2. Set the reaction volume for thermal cycling to 20 µL.

3. Start the run.

4. If you are using a CentriSep Spin column to purify extension products (see the section below), hydrate the column.

5. If necessary, store the extension products overnight at 4 °C before purifying them.
   You can store extension products at –20 °C for up to 1 week.

Purifying Extension Products

After cycle sequencing, you must remove excess dye terminators and primers from the cycle sequencing reactions using one of the following:

<table>
<thead>
<tr>
<th>If you performed PCR in ...</th>
<th>Then use...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcentrifuge tubes</td>
<td>CentriSep Column (Applied Biosystems PN 401763) or DyeEx® 2.0 Spin Kit (Qiagen PN 63204)</td>
</tr>
<tr>
<td>96-well plates</td>
<td>DyeEx 96 Kit (Qiagen PN 63181)</td>
</tr>
</tbody>
</table>
Follow the guidelines and procedures that accompany the kits.
For greater stability of extension products, you can mix the eluate with an equal volume of Hi-Di™ Formamide.

⚠️ WARNING CHEMICAL HAZARD. Formamide. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Performing Electrophoresis of Extension Products

**IMPORTANT!** You must use the 50-cm capillary array length regardless of the instrument that you are using. Refer to your instrument user guide for more information.

**Instrument Configuration for Electrophoresis**


Select the Run Module, Basecaller, and Dye/Set Primer specified below.

**Note:** If the resulting signal strength is too high, you might have to decrease injection times in the run module.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Filter Set</th>
<th>Run Module</th>
<th>Basecaller</th>
<th>DyeSet/Primer (Mobility File)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems 3130 or 3130xl</td>
<td>E</td>
<td>StdSeq50POP6</td>
<td>KB.bcp</td>
<td>KB_3130_POP6_BDT v1.mob file</td>
</tr>
</tbody>
</table>

**Note:** You can also use the StdSeq50_POP7 Run Module and the KB_3130_POP7_BDT v1.mob file for shorter sequencing times. However, quality of data may be reduced within the first 40 bases on the 5’ end.

Refer to the MicroSeq® ID Analysis Software Online Help for more information about naming conventions for Basecaller and DyeSet/Primer files.
Performing the Run

<table>
<thead>
<tr>
<th></th>
<th>To perform the run:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Load 15 µL of the purified extension products into a 96-well plate (PN 4306737).</td>
</tr>
<tr>
<td>2</td>
<td>Load the plate into your instrument.</td>
</tr>
<tr>
<td>3</td>
<td>Start the run.</td>
</tr>
<tr>
<td>4</td>
<td>When the run is complete, analyze the data using the MicroSeq® ID Analysis Software. For information on using the software, refer to the MicroSeq ID Analysis Software Online Help, the <em>MicroSeq® ID Analysis Software Version 2.0 Quick Reference Card</em> and the <em>MicroSeq® ID Analysis Software Version 2.0 Getting Started Guide</em>.</td>
</tr>
</tbody>
</table>
Analyzing Data

**MicroSeq® ID Analysis Software**

MicroSeq® ID Analysis Software analyzes sequences obtained with any of the MicroSeq® Microbial Identification Kits.

When analyzing data generated using the MicroSeq® 500 16S rDNA Bacterial Identification Kits, the software assembles the 16S rDNA sequence for the unknown, then compares the sequence with 16S rDNA sequences in the MicroSeq® ID 16S rDNA 500 Library (v2.0). Based on the comparison, the software provides a potential ID for the unknown bacterial species.

With the software you can perform:

- Basecalling with assignment of quality values.
- Clear-range determination, which lets you exclude data near sequence ends (typically poor-quality data) from analysis.
- Assembly and alignment of sequences to generate a high-quality consensus sequence.
- Comparison of the consensus sequence to the MicroSeq® ID proprietary libraries to generate a list of the closest matches, including percentage match scores.
- Exports of projects and consensus sequences to facilitate data-sharing between collaborators.

The software also has features that assist with 21 CFR Part 11 compliance requirements.

For more information, refer to the *MicroSeq® ID Analysis Software Version 2.0 Quick Reference Card* and the *MicroSeq® ID Analysis Software Version 2.0 Getting Started Guide* provided with the software.

**MicroSeq® Proprietary Libraries**

The MicroSeq® ID 16S rDNA 500 Library (v2.0) includes over 1,700 validated 16S rDNA sequences. All sequences and strains are carefully checked and quality controlled to achieve maximum reliability. Polymorphic positions are taken into account to ensure the highest degree of accuracy.
Custom Libraries

In addition to accessing the proprietary libraries, MicroSeq® ID Analysis Software allows you to create custom libraries. Create custom libraries using data generated by the MicroSeq® ID software, or using downloaded sequences from public databases. Custom libraries are easy to import and export, making information sharing convenient.

During the analysis process, you can search both proprietary and custom libraries simultaneously to determine the 20 closest matches to the sequence of your unknown.

Generated Reports

MicroSeq® ID Analysis Software generates four detailed reports:

- **Analysis QC Report** – Allows you to quickly scan the unknowns in a project to gather information about the samples, including the top percent identity match and specimen score to measure data quality. Figure 1 on page 20 shows a sample Analysis QC Report.

- **Library Search Report** – Provides more detailed information about the libraries searched, including a list of all the top matches and the total number of bases searched. Figure 2 on page 21 shows a sample Library Search Report.

- **Audit Trail Report** – Tracks changes made to projects after analysis.

- **Electronic Signature History Report** – Reports a summary of the electronic signatures performed by user in a project.

All reports can be generated on a project level and on a per specimen level. In addition, the software allows you to create custom reports. For information, refer to the MicroSeq® ID Analysis Software Version 2.0 Online Help.
Figure 1  Example Analysis QC Report
Figure 2  Sample Library Search Report
Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR product</td>
<td>No biomass</td>
<td>Use more bacterial cells</td>
</tr>
<tr>
<td>No PCR product</td>
<td>PCR inhibition</td>
<td>Make additional dilutions (1:10, 1:100, 1:1000) of the working stock (see page 9) before proceeding to PCR.</td>
</tr>
<tr>
<td>No PCR product</td>
<td>Fungal sample</td>
<td>Use the MicroSeq® D2 LSU rDNA Fungal Identification Kits.</td>
</tr>
<tr>
<td>Cells were not disrupted by the PrepMan® Ultra method</td>
<td>Fungal sample</td>
<td>Use the MicroSeq® D2 LSU rDNA Fungal Identification Kits.</td>
</tr>
<tr>
<td>No PCR product</td>
<td>PCR inhibition</td>
<td>Make additional dilutions (1:10, 1:100, 1:1000) of the PrepMan Ultra supernatant before proceeding to PCR.</td>
</tr>
<tr>
<td>No PCR product</td>
<td>Bacterial sample</td>
<td>Use the MicroSeq® 500 or the MicroSeq® Full Gene 16S rDNA Bacterial Identification Kit.</td>
</tr>
<tr>
<td>Cells were not disrupted by the PrepMan Ultra method</td>
<td>Bacterial sample</td>
<td>Use the MicroSeq® 500 or the MicroSeq® Full Gene 16S rDNA Bacterial Identification Kit.</td>
</tr>
<tr>
<td>Short sequence, the first part of which is very bright and off-scale and the remainder of which has very low intensity</td>
<td>High starting amount of DNA or too much DNA template in the sequencing reaction</td>
<td>Decrease the amount of bacterial cell material (smaller colony or pellet).</td>
</tr>
<tr>
<td>Both my results and raw data show occasional high spikes for all four dye colors</td>
<td>Bubbles in the capillary</td>
<td>Check the instrument manual.</td>
</tr>
</tbody>
</table>
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large regions of overlapping sequence</td>
<td>DNA sample is contaminated (that is, the DNA is derived from more than one species of bacteria)</td>
<td>Clone the PCR product (using a kit such as the Invitrogen™ Topo® PCR Cloning Kit) before performing sequencing.</td>
</tr>
</tbody>
</table>
| Small regions of overlapping sequence    | • In bacterial species with multiple copies of the rRNA gene, insertions or deletions in a subset of the genes can result in a shift by 1 to 3 bp.  
• Similarly, in bacteria with multiple copies of the rRNA gene, the gene can be polymorphic, resulting in overlap of up to 1% of the sequence. | No action needed.                                                          |
| Cannot call bases for large regions of sequence | DNA sample is contaminated (that is, the DNA is derived from more than one species of bacteria) | Clone the PCR product (using a kit such as the Invitrogen™ Topo® PCR Cloning Kit) before performing sequencing. |
Frequently Asked Questions

**Sensitivity and Quantification**

What is the sensitivity of the MicroSeq® 500 kits?

As long as you start from a visible colony or cell pellet, MicroSeq kits will work.

Can I use the MicroSeq 500 Kits to quantify bacteria?

No. The PCR is an endpoint assay.

**Sample Preparation and Storage**

What is the best way to prepare yeast samples?

Prepare yeast samples using the Prepman® Ultra Sample Preparation Reagent or bead-beating method, just as you prepare bacterial samples. Extra dilutions of the working fungal stock are sometimes necessary.

Which kits should I use to identify yeast samples?

Use the MicroSeq® D2 LSU rDNA Fungal Identification Kits to sequence and identify yeast samples.

Are there alternative methods for preparing genomic DNA?

If the PrepMan Ultra kit method does not successfully disrupt cells, you can use the bead-beating method to isolate bacterial genomic DNA from bacterial colonies or cultures. Use 100 µM zirconia/silica beads (Biospec Products PN 11079101Z). Refer to the Biospec Web site (**http://www.biospec.com**) for the bead-beating protocol.

Alternatively, you can use a DNA extraction kit (available from vendors like Qiagen) to isolate pure DNA.

Can I use less PrepMan Ultra Sample Preparation Reagent if I start with a smaller colony?

Yes. The ideal colony size is 2 to 3 mm. For smaller colonies, you can decrease the amount of PrepMan Ultra Sample Preparation Reagent to 50 µL from the suggested 200 µL in the *PrepMan® Ultra Sample Preparation Reagent Protocol* (PN 4318925).
Can I enrich my genomic DNA by using less PrepMan Ultra Sample Preparation Reagent?

Yes. However, be careful not to overload the PCR mix. Enriched samples tend to have more cellular and other debris, which can interfere with PCR.

At what temperature should I store my PrepMan Ultra kit-isolated DNA?

Applied Biosystems recommends storing DNA at –20 °C, although you may safely keep it at room temperature overnight.

Contamination

How can I tell if my sequence is representative of a single species?

The DNA sequence from a single species should be distinct (easy to call base pairs), without significant regions of overlapping sequence.

If my initial DNA sample is contaminated (that is, it comes from multiple species), how can I sequence my PCR product?

Clone the PCR product using a kit such as the Topo® PCR Cloning Kit from Invitrogen™.

Overlapping Sequences

My sequence has large regions of overlap (>5% mixed bases). What does this mean?

The presence of large regions of overlapping sequence indicates that the DNA is derived from more than one species of bacteria. You can still derive the sequence of each of the bacterial species by cloning the PCR products (using a kit such as the Invitrogen Topo® PCR Cloning Kit).

My sequence has small regions (up to 1%) of overlap. What does this mean?

No action is needed. Some bacterial species can have multiple copies of the rRNA gene. Small regions of overlapping sequence can be caused by insertions or deletions in a subset of these genes, resulting in a shift of 1 to 3 bp. Similarly, the gene can be polymorphic, leading to overlap of up to 1% of the sequence.
PCR

Can I always expect the same size PCR product for all species?

PCR products can vary depending on the species. Expected product sizes for the:

- **MicroSeq 500 Kit** – 1 band at 460 to 560 bp
- **MicroSeq Full Gene Kit** – 1 band at 460 to 560 bp and 2 bands at 700 to 780 bp
- **Fungal Kit** – 1 band at 300 to 500 bp

Can I increase the number of cycles to increase the PCR yield?

You can increase the number of cycles to increase the PCR yield, but doing so can cause additional background signal from the negative control.

BigDye® Terminator Chemistry

What is the difference between the BigDye® Terminator chemistry currently used in the kit and the dRhodamine chemistry used in previous versions of the kit?

BigDye Terminator chemistry allows you to read clear sequences close to the primer regions. Additionally, overall signal strength is increased. Refer to the *ABI PRISM® Automated DNA Sequencing Chemistry Guide* (PN 4305080) for more information about BigDye Terminator chemistry.

Species Libraries

How are species in the Applied Biosystems libraries validated?


Where are the species in the Applied Biosystems libraries derived from?

The species are derived from the American Type Culture Collection (ATCC) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures).

Are there pathological species included in the libraries?

Yes.
What is the difference between the libraries for the MicroSeq Full Gene Kits and the MicroSeq 500 Kits?

The sequences in the library for the MicroSeq 500 kits are \( \approx 500 \) bp, which is the expected size of the PCR products for these kits. The sequences in the library for the MicroSeq Full Gene kits, on the other hand, are \( \approx 1440 \) bp—the maximum sequence length that the kits allow you to determine.

How many entries are included in the MicroSeq ID Analysis Software libraries?

<table>
<thead>
<tr>
<th>Kit</th>
<th>Library</th>
<th>Number of Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroSeq 500</td>
<td>MicroSeq® ID 16S rDNA 500 Library v2.0</td>
<td>1716</td>
</tr>
<tr>
<td>MicroSeq Full Gene</td>
<td>MicroSeq® ID 16S rDNA Full Gene Library v2.0</td>
<td>1261</td>
</tr>
<tr>
<td>MicroSeq Fungal</td>
<td>MicroSeq® ID Fungal Gene Library v2.0</td>
<td>1113</td>
</tr>
</tbody>
</table>

The three libraries are available as part of the MicroSeq® ID Analysis Software v2.0, Application and Libraries (PN 4371298), but can also be purchased individually. For more information, see the Applied Biosystems Web site (http://www.appliedbiosystems.com).

Where can I find additional information about MicroSeq ID Analysis Software?

Additional information about MicroSeq ID Analysis Software is available in the:

- MicroSeq® ID Analysis Software Version 2.0 Online Help
- Applied Biosystems MicroSeq web site at www.microseq.com
## Appendix A MicroSeq® Microbial Identification System Products

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MicroSeq® 500 16S rDNA Bacterial Identification kits</strong></td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® 500 16S rDNA Bacterial Identification PCR Kit</td>
<td>4370653</td>
</tr>
<tr>
<td>Includes reagents for 60 amplifications, Quick Reference Card, and this Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4370489.</td>
<td></td>
</tr>
<tr>
<td>MicroSeq® 500 16S rDNA Bacterial Identification Sequencing Kit</td>
<td>4370782</td>
</tr>
<tr>
<td>Includes reagents for 55 identifications (110 sequencing reactions), Quick Reference Card, and this Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4346480.</td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® 500 16S rDNA Bacterial Identification Kits Protocol</td>
<td>4370453</td>
</tr>
<tr>
<td>Fast MicroSeq® 500 16S rDNA Bacterial Identification Kits Quick Reference Card</td>
<td>4370455</td>
</tr>
<tr>
<td><strong>MicroSeq® Full Gene 16S rDNA Bacterial Identification kits</strong></td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® Full Gene 16S rDNA Bacterial Identification PCR Kit</td>
<td>4370654</td>
</tr>
<tr>
<td>Includes reagents for 20 amplifications, Quick Reference Card, and Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4370484.</td>
<td></td>
</tr>
<tr>
<td>MicroSeq® Full Gene 16S rDNA Bacterial Identification Sequencing Kit</td>
<td>4370783</td>
</tr>
<tr>
<td>Includes reagents for 15 identifications, Quick Reference Card, and Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4347484.</td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® Full Gene 16S rDNA Bacterial Identification Kits Protocol</td>
<td>4370457</td>
</tr>
<tr>
<td>Fast MicroSeq® Full Gene 16S rDNA Bacterial Identification Kits Quick Reference Card</td>
<td>4370459</td>
</tr>
<tr>
<td><strong>MicroSeq® D2 LSU rDNA Fungal Identification kits</strong></td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® D2 LSU rDNA Fungal Identification PCR Kit</td>
<td>4370652</td>
</tr>
<tr>
<td>Includes reagents for 60 amplifications, Quick Reference Card, and Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4370486.</td>
<td></td>
</tr>
<tr>
<td>MicroSeq® D2 LSU rDNA Fungal Identification Sequencing Kit</td>
<td>4370779</td>
</tr>
<tr>
<td>Includes reagents for 55 identifications (110 sequencing reactions), Quick Reference Card, and Protocol</td>
<td></td>
</tr>
<tr>
<td><strong>Note:</strong> For reagents only without documentation, order Part Number 4347481.</td>
<td></td>
</tr>
<tr>
<td>Fast MicroSeq® D2 LSU rDNA Fungal Identification Kits Protocol</td>
<td>4370456</td>
</tr>
<tr>
<td>Fast MicroSeq® D2 LSU rDNA Fungal Identifications Kits Quick Reference Card</td>
<td>4370458</td>
</tr>
</tbody>
</table>
### MicroSeq® ID Analysis Software v2.0, Application and Libraries

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Includes:</td>
<td></td>
</tr>
<tr>
<td>• MicroSeq® ID Fungal Gene Library v2.0</td>
<td></td>
</tr>
<tr>
<td>• MicroSeq® ID 16S rDNA Full Gene Library v2.0</td>
<td></td>
</tr>
<tr>
<td>• MicroSeq® ID 16S rDNA 500 Library v2.0</td>
<td></td>
</tr>
<tr>
<td>• MicroSeq® ID Analysis Software v2.0</td>
<td>4371298</td>
</tr>
</tbody>
</table>

**Note:** For information about separately purchasing software and libraries, see the Applied Biosystems Web site (http://www.appliedbiosystems.com)
Appendix B Additional Applied Biosystems Instrument Platforms Supported

To take advantage of the reduced amplification and sequencing times allowed by the new fast PCR chemistry and the 9800 Fast Thermal Cycler, Applied Biosystems recommends using the 9800 Fast Thermal Cycler and an Applied Biosystems 3130 or 3130xl Genetic Analyzer with the MicroSeq kits.

However, the MicroSeq kits can also be used with the following instruments:

- GeneAmp® PCR System 9700
- Applied Biosystems 3730 and 3730xl DNA Analyzers
- ABI PRISM® 3100 and 3100-Avant Genetic Analyzers
- ABI PRISM® 310 Genetic Analyzer

### Materials
The following matrix and sequencing standards are used with other instruments.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI PRISM® dRhodamine Matrix Standards Kit (310 instrument only)</td>
<td>Applied Biosystems (PN 4336805)</td>
</tr>
<tr>
<td>3700/3730 BigDye® Terminator v1.1 Sequencing Standard (3730 and 3730xl instruments)</td>
<td>Applied Biosystems (PN 4336799)</td>
</tr>
</tbody>
</table>
**Appendix B Additional Applied Biosystems Instrument Platforms Supported**

**Instrument Configuration for Electrophoresis**

Select the run module and mobility file appropriate for the instrument you use.

**Note:** If the resulting signal strength is too high, you might have to adjust injection times in the run module.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Filter Set</th>
<th>Run Module</th>
<th>Basecaller</th>
<th>DyeSet/Primer (Mobility File)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems 3730</td>
<td>E</td>
<td>LongSeq50_POP7</td>
<td>KB.bcp</td>
<td>KB_3730_POP7_BDT v1.mob</td>
</tr>
<tr>
<td>Applied Biosystems 3730x/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABI PRISM® 3100</td>
<td>E</td>
<td>StdSeq50_POP6</td>
<td>KB.bcp</td>
<td>KB_3100_POP6_BDT v1.mob</td>
</tr>
<tr>
<td>ABI PRISM® 3100-Avant™</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABI PRISM® 310</td>
<td>E</td>
<td>SeqPOP6(1 mL) E.md4</td>
<td>KB.bcp</td>
<td>KB_310_POP6_BDT v1_50Std.mob</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>SeqPOP6 Rapid (1 mL) E.md4</td>
<td></td>
<td>KB_310_POP6_BDTv1_36Rapid.mob</td>
</tr>
</tbody>
</table>
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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.636.5884

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