ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit

For –21 M13 and M13 REV Primers

Protocol

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1 Introduction

Chapter Summary

In This Chapter The following topics are covered in this chapter:

About the Kit

Reagent Requirements

- The ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS, requires **new** instrument (matrix) files for the ABI PRISM® 310 Genetic Analyzer, ABI PRISM® 377 DNA Sequencers, and ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel installed and **new** spectral calibrations for the ABI PRISM® 3700 DNA Analyzer and the ABI PRISM® 3100 Genetic Analyzer:
  - The 310, 377, and 373 instruments require the ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421) for instrument (matrix) file generation.
  - The 3700 instrument requires ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309) for spectral calibration.
  - The 3100 instrument requires ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303) for spectral calibration.
- The dRhodamine Matrix Standards and Matrix Standard Set DS-01 are not compatible with BigDye™ primers v3.0, BigDye™ terminators v3.0, or dGTP BigDye™ terminators v3.0.
- The alcohol precipitation methods are different from those recommended for the original BigDye primers.
- There are new v3.0 mobility files.
- The basecallers are the same.

BigDye Primer v3.0 Ready Reaction Kit

The ABI PRISM BigDye Primer v3.0 Ready Reaction Cycle Sequencing Kit combines AmpliTaq® DNA Polymerase, FS, the BigDye™ primers v3.0, and all the required components for the sequencing reaction.

The kit contains nucleotide mixes that have been specifically optimized for AmpliTaq DNA Polymerase, FS. The deoxy- and dideoxynucleotide ratios in the nucleotide mixes have been formulated to give a good signal balance above 700 bases. These formulations also contain 7-deaza-dGTP in place of dGTP to minimize band compressions.

In the Ready Reaction format, the dye-labeled primers, deoxynucleoside triphosphates, dideoxynucleoside triphosphates, AmpliTaq DNA Polymerase, FS, and buffer are premixed into A, C, G,

and T Ready Reaction cocktails to eliminate time-consuming reagent preparation. These reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, on polymerase chain reaction (PCR) fragments, and on large templates, *e.g.*, the ends of BAC clones.

Cycle Sequencing with AmpliTaq DNA Polymerase, FS

The kit formulation contains the sequencing enzyme AmpliTaq DNA Polymerase, FS. This enzyme is a variant of *Thermus aquaticus* DNA polymerase that contains a point mutation in the active site. This results in less discrimination against dideoxynucleotides and a much more even peak intensity pattern.

This enzyme also has a second mutation in the amino terminal domain that virtually eliminates the 5´→3´ nuclease activity of AmpliTaq DNA Polymerase. The enzyme has been formulated with a thermally stable inorganic pyrophosphatase to eliminate problems associated with pyrophosphorolysis.

Cycle sequencing protocols that rely on the use of AmpliTaq DNA Polymerase, FS offer the following advantages over traditional sequencing methods:

- Less hands-on operation
- No alkaline denaturation step required for double-stranded DNA
- Same protocol for both single- and double-stranded templates
- Less starting template needed
- More reproducible results

BigDye Primer v3.0 Appearance on the 377 or 373 Instrument Gel Image

The dye/base relationships and colors of the BigDye primers v3.0 as they appear on the gel image are shown below for the 377 and 373 instruments.

Base	Primer	Color of Bands on ABI PRISM 377 or 373 Instrument Gel Image
A	V3 Dye 2	Green
C	V3 Dye 1	Blue
G	V3 Dye 3	Yellow
T	V3 Dye 4	Red

Instruments

Instrument Platforms

The ABI PRISM BigDye Primer v3.0 Ready Reaction Cycle Sequencing Kit is for use with the following instruments:

- ABI PRISM® 3700 DNA Analyzer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 377 DNA Sequencer (all models[1])

This kit can also be used with ABI PRISM® 373 DNA Sequencers with the new ABI PRISM® BigDye Filter Wheel installed.[2] Refer to the *ABI PRISM BigDye Filter Wheel User Bulletin* (P/N 4304367) for more information.

General instructions are given for using the kit reagents to generate samples for these instruments. For more detailed instructions, refer to the appropriate instrument user's manual.

IMPORTANT This kit is not designed for use with ABI PRISM® 373 DNA Sequencers and ABI PRISM® 373 DNA Sequencers with XL Upgrade that do not have the ABI PRISM BigDye Filter Wheel.

Thermal Cyclers

The protocols provided in this document were optimized using Applied Biosystems thermal cyclers, including:

- GeneAmp® PCR Instrument Systems 9700, 9600, and 2400
- DNA Thermal Cycler 480
- DNA Thermal Cycler (TC1)

If you use a thermal cycler not manufactured by Applied Biosystems, you may need to optimize thermal cycling conditions. Ramping time is very important. If the thermal ramping time is too fast (>1°/sec), poor (noisy) data may result.

1. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.
2. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Required Software

Dye/Filter Sets and Matrix Standards for the 310, 377, and 373 Instruments

The dye/filter sets and matrix standards required for the 310, 377, and 373 instruments are listed in the table below.

IMPORTANT The instrument (matrix) file for the BigDye primers v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Instrument	Dye/Filter Set	Standards for Instrument (Matrix) File Generation
310 Genetic Analyzer	Filter Set E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)
377 DNA Sequencers[a]	Filter Set E	
373 DNA Sequencers with the BigDye Filter Wheel[b]	Filter Set A	

a. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

b. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Dye Sets and Spectral Standards for the 3700 and 3100 Instruments

The dye sets and spectral standards required for the 3700 and 3100 instruments are listed in the table below.

IMPORTANT Spectral calibrations for the BigDye primers v3.0 are not compatible with the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Instrument	Dye Set	Standards for Spectral Calibration
3700 DNA Analyzer	D	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309)
3100 Genetic Analyzer	Z	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303)

Instructions

For Generating Matrices

- For the 377 and 310 instruments, refer to the product insert for instructions on using the ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421) to generate matrices.
- For the 373 instruments, contact Technical Support for instructions on using the ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421) to generate matrices.

For Performing Spectral Calibrations

- For the 3700 instrument, refer to the product insert for instructions on using the ABI PRISM 3700 BigDye Terminator v3.0 Sequencing Standard (P/N 4390309) to perform spectral calibration.
- For the 3100 instrument, refer to the product insert for instructions on using the ABI PRISM BigDye Terminator v3.0 Sequencing Standard (P/N 4390303) to perform spectral calibration.

Dye Set/Primer (Mobility) Files

Available in Two Places

To analyze sequencing data generated with BigDye chemistries v3.0, you need dye set/primer (mobility) files that were created for v3.0 chemistries. The dye set/primer (mobility) files can be obtained from two places:

- The files can be installed from the two CD-ROMs or one floppy disk enclosed in the v3.0 matrix and sequencing standards listed below. See page 1-7 for instructions.
  - ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421)
  - ABI PRISM BigDye Terminator v3.0 Sequencing Standard (P/N 4390303)
  - ABI PRISM 3700 BigDye Terminator v3.0 Sequencing Standard (P/N 4390309)
- The files can be downloaded from the Internet. See page 1-7 for instructions.

Installing Files from the CD-ROMs or Floppy Disk Enclosed in the v3.0 Matrix and Sequencing Standards

If you wish to analyze your data using a ...	and your data was collected on a...	Refer to the CD-ROM or floppy disk labeled...
computer with the Windows NT® platform	3700, 3100, 310, or 377 instrument	PN 4326478, For Windows NT platform
Macintosh® computer with a CD-ROM drive	310, 377, 373 instrument	PN 4326479, For Macintosh platform
Macintosh computer with a floppy drive	310, 377, 373 instrument	PN 4326480, For Macintosh platform

Downloading Files from the Internet

Dye set/primer (mobility) files can be downloaded from our website:

http://www.appliedbiosystems.com/techsupp/swpps/SAsw.html

If you do not have access to the Internet, you can get the files from Applied Biosystems Technical Support, or from your local field applications specialist (call your local sales office for more information).

Reagents and Storage

Available Kits

The following kits are available:

Kit	Primer	Number of Reactions	Part Number
The ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS	-21 M13	100	4390161
	M13 reverse	100	4390163
	-21 M13	5000	4390157
	M13 reverse	5000	4390159

Storage and Use of the Kit

- Store the kit at –15 to –25 °C.
- Avoid excess (*i.e.,* no more than 5–10) freeze-thaw cycles. Aliquot reagents in smaller amounts if necessary.
- Before each use of the kit, allow the frozen stocks to thaw at room temperature (do not heat).

IMPORTANT Mix each stock thoroughly and then centrifuge briefly to collect all the liquid at the bottom of each tube.

- Whenever possible, thawed materials should be kept on ice during use. Do not leave reagents at room temperature for extended periods.

Materials Supplied by the User

Overview In addition to the reagents supplied in this kit, other items are required.

This section lists general materials needed for:

- Cycle sequencing
- Purifying extension products

Note Many of the items listed in this section are available from major laboratory suppliers (MLS) unless otherwise noted. Equivalent sources may be acceptable where noted.

Refer to the individual instrument protocols for the specific items needed for each instrument.

⚠ WARNING CHEMICAL HAZARD. Before handling the chemical reagents needed for cycle sequencing, read the safety warnings on the reagent bottles and in the manufacturers' Material Safety Data Sheets (MSDSs). Always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) when handling chemicals. Dispose of waste in accordance with all local, state/provincial, and national environmental and health regulations.

Materials for Cycle Sequencing

The table below lists the plates or tubes required for the recommended Applied Biosystems thermal cyclers (page 1-4).

Thermal Cycler	Plate or Tube	Applied Biosystems Part Number
GeneAmp® PCR System 9700	MicroAmp® 96-Well Reaction Plate	N801-0560
	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 or N801-0535
	ABI PRISM™ Optical Adhesive Cover Starter Pack or ABI PRISM® Optical Adhesive Covers	4313663 or 4311971
GeneAmp® PCR System 9600	MicroAmp® 96-Well Reaction Plate	N801-0560
	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 N801-0535
	ABI PRISM™ Optical Adhesive Cover Starter Pack or ABI PRISM® Optical Adhesive Covers	4313663 or 4311971
GeneAmp® PCR System 2400	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 N801-0535
DNA Thermal Cycler 480[a]	GeneAmp® Thin-Walled Reaction Tubes, 0.5-mL	N801-0537
	GeneAmp® Thin-Walled Reaction Tubes with Flat Cap	N801-0737
DNA Thermal Cycler (TC1)[a]	GeneAmp® Thin-Walled Reaction Tubes, 0.5-mL	N801-0537

a. The DNA Thermal Cycler (TC1) and the DNA Thermal Cycler 480 thermal cyclers require mineral oil that can be obtained from Applied Biosystems (P/N 0186-2302)

Materials for Purifying Extension Products

Method	Material	Supplier
Ethanol Precipitation	Ethanol (EtOH), non-denatured, 95%	MLS
	Aluminum foil tape, adhesive-backed	3M (Scotch Tape P/N 431 or 439)[a]

a. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

Safety

Documentation User Attention Words

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

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

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

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

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

Chemical Hazard Warning

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

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling

chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- 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.
- Handle chemical wastes in a fume hood.
- Minimize contact with and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing).
- 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.

Site Preparation and Safety Guide

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

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current material safety data sheet (MSDS) before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

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

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

<table><tr><th>If you have...</th><th>Then...</th></tr><tr><td>The MSDS document number or the Document on Demand index number</td><td>Enter one of these numbers in the appropriate field on this page.</td></tr><tr><td>The product part number</td><td rowspan="2">Select Click Here, then enter the part number or keyword(s) in the field on this page.</td></tr><tr><td>Keyword(s)</td></tr></table>
c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or e-mail.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Documents on Demand” under “Technical Support.”</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
</table>

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>By telephone from Canada</td><td>
<table>
<tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr>
<tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr>
<tr><td>French</td><td>Press 2, then 2, then 1</td></tr>
</table>
</td></tr>
<tr><td>By telephone from any other country</td><td>See the specific region under “To Contact Technical Support by Telephone or Fax” under “Technical Support.”</td></tr>
</table>

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

Preparing the Templates 2

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Control DNA Templates

Using Control DNA

Include a control DNA template as one of the templates in a set of sequencing reactions. The results from the control can help determine whether failed reactions are the result of poor template quality or sequencing reaction failure.

Control DNA Sequence

We recommend M13mp18 as a single-stranded control and pGEM®-3Zf(+) as a double-stranded control. All Applied Biosystems DNA sequencing kits provide pGEM® control DNA.

The partial sequence of pGEM-3Zf(+) from the –21 M13 forward primer and M13 reverse primer, followed by the ensuing 1000 bases is shown in Appendix A, “Control DNA Sequences.”

An Additional Control Sold Separately

The BigDye™ terminator v3.0 sequencing standard provides an additional control to help in troubleshooting electrophoresis runs. It contains lyophilized sequencing reactions that require only resuspension and denaturation before use.

There are two existing forms of the v3.0 sequencing standard, as shown in the table below. Please use the correct sequencing standard for your instrument. Refer to the product inserts for instructions on using each sequencing standard.

Instrument	Kit	Part Number
ABI PRISM® 3700 DNA Analyzer	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard	4390309
ABI PRISM® 3100 Genetic Analyzer	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard	4390303
ABI PRISM® 310 Genetic Analyzer		
ABI PRISM® 377 DNA Sequencers[a]		
ABI PRISM® 373 DNA Sequencers with the BigDye™ Filter Wheel[b]		

a. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

b. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Template Preparation Methods

Single- and Double-Stranded Templates

Refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for information on preparing single- and double-stranded templates.

BAC DNA Templates

With larger DNA targets such as bacterial artificial chromosomes (BACs), the quality of DNA template is important to the success of the sequencing reaction. Two methods have given good sequencing results:

- Alkaline lysis[1], with extra phenol extraction and isopropanol precipitation if very clean DNA is desired
- Cesium chloride (CsCl) banding

Commercial Kits

Commercial kits are also available for BAC DNA preparation:

- QIAGEN-tip 100 (QIAGEN: P/N 10043, 25 reactions; 10045, 100 reactions)
- QIAGEN-tip 500 (QIAGEN: P/N 10063, 25 reactions; 10065, 100 reactions)

1. Marra, M., Weinstock, L.A., and Mardis, E.R. 1996. End sequence determination from large insert cloning using energy transfer fluorescent primers. *Genomic Methods* 6: 1118–1122.

PCR Templates

Cycle sequencing provides the most reproducible results for sequencing PCR templates. Although PCR fragments can be difficult to denature with traditional sequencing methods, cycle sequencing provides several chances to denature and extend the template, which ensures adequate signal in the sequencing reaction.

Importance of Purifying Product

For optimum results, purify the PCR product before sequencing. In general, any method that removes dNTPs and primers should work. We recommend Centricon®-100 columns (P/N N930-2119). The protocol for using these columns is provided in “Purifying PCR Fragments” on page 2-5.

Refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for information on sequencing PCR templates.

Direct Link PCR Sequencing

PCR protocols that limit amounts of primers and dNTPs allow the product of the reaction to be used for sequencing with no purification. This is usually carried out by setting up the PCR amplification with 5–10 pmol of primers and 20–40 µM dNTPs, so that most of the primers and dNTPs are exhausted during amplification.

If the yield of the desired PCR product is high and the product is specific, *i.e.*, it produces a single band when analyzed by agarose gel electrophoresis, the sample can be diluted before sequencing and will give good results. The dilution ratio depends on the concentration of your PCR product and needs to be determined empirically (start with 1:2 and 1:10 dilutions with deionized water).

When you limit concentrations of primers and dNTPs and dilute the PCR products, the PCR parameters have to be robust. Direct Link PCR sequencing is most useful in applications where the same target is being amplified and sequenced repeatedly and PCR conditions have been optimized.

Purifying PCR Fragments

To purify PCR fragments by ultrafiltration:

Step	Action
1	Assemble the Centricon-100 column according to the manufacturer's recommendations.
2	Load 2 mL deionized water onto the column.
3	Add the entire sample to the column.
4	Spin the column at 3000 × *g* in a fixed-angle centrifuge for 10 minutes. **Note** The manufacturer recommends a maximum speed of 1000 × *g*, but 3000 × *g* has worked well in Applied Biosystems laboratories. If you are following the manufacturer's guidelines, increase the time to compensate.
5	Remove the waste receptacle and attach the collection vial.
6	Invert the column and spin it at 270 × *g* for 2 minutes to collect the sample. This should yield approximately 40–60 µL of sample.
7	Add deionized water to bring the purified PCR fragments to the original volume.

DNA Quantity

Quantitating DNA

For most purified PCR products, using 1 µL for each reaction (A, C, G, and T) will work. If possible, quantitate the amount of purified DNA by measuring the absorbance at 260 nm or by some other method. If you do not quantitate the amount of DNA beforehand, you run the risk of using too much DNA, which can affect the final sequencing result.

Template Quantity

The table below shows the amount of template to use in a cycle sequencing reaction.

Template	Quantity
PCR product:	
100–200 bp	2–5 ng
200–500 bp	5–10 ng
500–1000 bp	10–20 ng
1000–2000 bp	20–50 ng
>2000 bp	50–150 ng
Single-stranded	100–400 ng
Double-stranded	200–800 ng
Cosmid, BAC	0.5–1.0 µg

Note In general, higher DNA quantities give higher signal intensities.

The ranges given in the table above should work for all primers. You may be able to use even less DNA, especially when sequencing with the –21 M13 primer. The amount of PCR product to use in sequencing will also depend on the length and purity of the PCR product.

Performing Cycle Sequencing 3

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Introduction

Overview

The cycle sequencing protocols used for the ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS, are the same as those used for the ABI PRISM® BigDye™ Primer original Ready Reaction Cycle Sequencing Kit. They have been optimized for Applied Biosystems thermal cyclers.

Optimizing Cycle Parameters

The standard cycling conditions used in this manual have been optimized for the –21 M13 forward primer and work well for most primers. If you obtain low signal with the M13 reverse primer, you can improve your sequencing data by altering the cycling conditions as follows:

- Standard conditions use 55 °C as the annealing temperature in the three-step cycle. Lower the annealing temperature to 45–48 °C.
- The number of three-step versus two-step cycles can also affect signal strength. Increase the number of three-step cycles to 20 instead of the standard 15 (increasing the total number of cycles to 35).

Cycle Sequencing Single- and Double-Stranded DNA

Overview

This section describes how to prepare reactions and perform cycle sequencing on a variety of templates, including M13, plasmids, and PCR products.

Preparing the Reactions

Three Options

The flexibility of the BigDye™ primers v3.0 allows three options for cycle sequencing:

Reaction Type	Template	Cycle
1X	• PCR product • plasmid • M13	standard
0.5X	• PCR product • plasmid • M13	standard
High-sensitivity (2X)	• BACs • extra long PCR products • other large DNA	modified

Procedures

The type of tube required depends on the thermal cycler that you are using. Refer to “Materials for Cycle Sequencing” on page 1-10.

IMPORTANT Prepare separate tubes for each of the four reactions (A, C, G, and T).

1X Reactions

Step	Action				
1	Aliquot the following reagents into four PCR tubes:				
	Reagent	**A** (µL)	**C** (µL)	**G** (µL)	**T** (µL)
	Ready Reaction Premix	4	4	4	4
	DNA Template (see page 2-6 for quantity)	1	1	1	1
	Total Volume	5	5	5	5
2	**If using the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:**				
	a. Add 20 µL of light mineral oil. b. Spin to layer the oil over the aqueous reaction.				

0.5X Reactions

Dilute 5X Sequencing Buffer (P/N 4305605, 600 reactions; 4305603, 5400 reactions) with four parts deionized water to 1X for use in this procedure.

Step	Action				
1	Dilute each Ready Reaction Premix (A, C, G, T) 1:1 with 1X Sequencing Buffer in a separate tube (*e.g.*, 2 µL of A Mix and 2 µL of 1X Sequencing Buffer).				
2	Aliquot the following reagents into four PCR tubes for each DNA template:				
	Reagent	**A** (µL)	**C** (µL)	**G** (µL)	**T** (µL)
	Diluted Ready Reaction Premix	4	4	4	4
	DNA Template (see page 2-6 for quantity)	1	1	1	1
	Total Volume	5	5	5	5
3	**If using the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:**				
	a. Add 20 µL of light mineral oil. b. Spin to layer the oil over the aqueous reaction.				

High-Sensitivity (2X) Reactions

Aliquot the following reagents into four PCR tubes:

Reagent	A (µL)	C (µL)	G (µL)	T (µL)
Ready Reaction Premix	8	8	8	8
DNA Template (see page 2-6 for quantity)	2	2	2	2
Total Volume	10	10	10	10

Note Use the GeneAmp® PCR System 9600 or 9700 for cycle sequencing high-sensitivity reactions. The use of other thermal cyclers is not supported.

If the majority of your applications require high sensitivity, please contact your local Field Applications Specialist or Technical Support for more options.

Cycle Sequencing on the System 9700, 9600, or 2400

Note The total time required for the cycling in this protocol is approximately 1 hour and 15 minutes.

To sequence single- and double-stranded DNA on the GeneAmp® PCR System 9700 (in 9600 emulation mode), 9600, or 2400:

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 5 µL.
2	Repeat the following for 15 cycles: • Rapid thermal ramp[a] to 96 °C • 96 °C for 10 sec. • Rapid thermal ramp to 55 °C • 55 °C for 5 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
3	Repeat the following for 15 cycles: • Rapid thermal ramp to 96 °C • 96 °C for 10 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
4	Rapid thermal ramp to 4 °C and hold until ready to precipitate.

a. Rapid thermal ramp is 1 °C/sec.

Cycle Sequencing on the TC1 or 480

Note The total time required for the cycling in this protocol is approximately 1 hour and 45 minutes.

To sequence single- and double-stranded on the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:

Step	Action
1	Place the tubes in a thermal cycler preheated to 95 °C.
2	Repeat the following for 15 cycles: • Rapid thermal ramp[a] to 95 °C • 95 °C for 30 sec. • Rapid thermal ramp to 55 °C • 55 °C for 30 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
3	Repeat the following for 15 cycles: • Rapid thermal ramp to 95 °C • 95 °C for 30 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
4	Rapid thermal ramp to 4 °C and hold until ready to precipitate.

a. Rapid thermal ramp is 1 °C/sec.

Cycle Sequencing BAC DNA

Preparing Sequencing Reactions

Follow the procedures given for preparing reactions in “Cycle Sequencing Single- and Double-Stranded DNA” (see “Preparing the Reactions” on page 3-3).

Performing Cycle Sequencing

Note The total time required for the cycling in this protocol is approximately 1 hour and 45 minutes.

To sequence BAC DNA on the GeneAmp PCR System 9700 (in 9600 emulation mode), 9600, or 2400:

Step	Action
1	Place the tubes in a thermal cycler, set the volume at 10 µL, and begin thermal cycling with the following parameters: • Rapid thermal ramp[a] to 95 °C • 95 °C for 5 min.
2	Repeat the following for 20 cycles: • Rapid thermal ramp to 95 °C • 95 °C for 30 sec. • Rapid thermal ramp to 50 °C • 50 °C for 15 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
3	Repeat the following for 15 cycles: • Rapid thermal ramp to 95 °C • 95 °C for 30 sec. • Rapid thermal ramp to 70 °C • 70 °C for 1 min.
4	Rapid thermal ramp to 4 °C and hold until ready to precipitate.

a. Rapid thermal ramp is 1 °C/sec.

Preparing the Samples for Loading 4

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Recommendations for Precipitation/Loading

For the 3700, 3100, 310, and 373 Instruments

In the ethanol precipitation method, reactions from four separate tubes are pooled together and precipitated using ethanol.

This method is recommended for the following instruments:

- ABI PRISM® 3700 DNA Analyzer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel installed.[1]

For the 3700, 3100, and 310 instruments, use this method to remove salts and other components of the reaction. For the 373 instruments, use this method to concentrate the samples.

See “Ethanol Precipitation for Microcentrifuge Tubes” on page 4-3 or “Ethanol Precipitation for 96-Well Reaction Plates” on page 4-5.

For the 377 Instrument

For the ABI PRISM® 377 DNA Sequencer (all models[2]), there are two options:

- Ethanol precipitation—samples are concentrated.

  See “Ethanol Precipitation for Microcentrifuge Tubes” on page 4-3 or “Ethanol Precipitation for 96-Well Reaction Plates” on page 4-5.

- Express Load—samples are loaded directly onto a gel without being precipitated

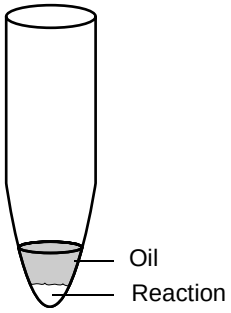
  See “Express Load Option for 36-Well Gels” on page 5-11.

IMPORTANT The Express Load option is only for the 377 instrument. It cannot be used for the 3700, 3100, 310, or 373 instruments.

1. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.
2. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

Ethanol Precipitation for Microcentrifuge Tubes

Procedure To precipitate in microcentrifuge tubes:

Step	Action
1	To a clean microcentrifuge tube, add: • 60 µL of non-denatured 95% ethanol OR • 120 µL of non-denatured 95% ethanol, if sequencing BAC DNA or other high-sensitivity reactions **Note** The use of sodium acetate is not necessary for precipitation. **⚠ WARNING CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Pipet the extension reactions from the bottom of each of the four tubes into the ethanol mixture. Mix thoroughly. **IMPORTANT To remove reactions run on the TC1 or DNA Thermal Cycler 480:** Place the pipette tip into the bottom of the reaction and carefully remove the reaction from the oil (see "Procedural Options" on page 4-4). Transfer as little oil as possible. 
3	Place the tube on wet ice or leave it at room temperature for 10–15 minutes to precipitate the extension products.
4	Spin the tube in a microcentrifuge for 10–20 minutes at maximum speed. Carefully aspirate or decant the supernatant and discard. At this point, a pellet may or may not be visible.

To precipitate in microcentrifuge tubes: *(continued)*

Step	Action
5	**Optional:** Rinse the pellet with 250 µL of 70% ethanol and spin for 5 minutes in a microcentrifuge. Again, carefully aspirate or decant the supernatant and discard. This may remove some of the salts from the pellet, but doing so is often not necessary. **Note** If you use sodium acetate, you must rinse the pellet. This will reduce the carryover of salt.
6	Dry the pellet in a vacuum centrifuge for 1–3 minutes, or until dry. Do not overdry.

Procedural Options

Removing the four reactions from under the oil can be difficult because of their small (5 µL) volumes. If this is a problem, try one of the following options:

- After cycling, add 5 µL of deionized water to each reaction for a total volume of 10 µL.

  If this is done, increase the amount of 95% ethanol used in step 1 of "Ethanol Precipitation for Microcentrifuge Tubes" on page 4-3 to 120 µL.

- Double the amount of Ready Reaction Premix and DNA template for each reaction (A, C, G, and T) to yield several loadings' worth (*i.e.*, a 2X reaction).

Ethanol Precipitation for 96-Well Reaction Plates

Standard Method

With the BigDye™ primers v3.0, the standard method is ethanol precipitation without the addition of salt. A final 70% ethanol wash is optional.

IMPORTANT Use non-denatured 95% ethanol rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

Precipitating in 96-Well Reaction Plates

To precipitate in 96-well reaction plates:

Step	Action
1	Remove the four 96-well reaction plates from the thermal cycler. Remove the caps from each tube.
2	Briefly centrifuge the plates to collect the samples at the bottoms of the tubes.
3	Using a multichannel pipet, pool the entire samples' volumes from each of the four plates (*i.e.,* containing the dideoxy A, C, G, and T reactions).
4	To the pooled reactions, add: • 60 µL of non-denatured 95% ethanol (EtOH) **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
5	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 431 or 439 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
6	Invert the plate a few times to mix.
7	Leave the plate on ice or at 4 °C for 15 minutes to precipitate the extension products.

To precipitate in 96-well reaction plates: *(continued)*

Step	Action
8	Place the plate in a table-top centrifuge with a plate adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: • 1400–2000 × *g*: 45 minutes • 2000–3000 × *g*: 30 minutes **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
9	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.
10	Place the inverted plate with the paper towel into the table-top centrifuge and spin at 50 × *g* for 1 minute.
11	Remove the plate and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Sample Electrophoresis 5

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Before You Begin

Important Reminders

- Be sure to use the correct dye set/primer (mobility) file for the BigDye™ primers v3.0. The dye base relationships are different than with the BigDye™ terminators v3.0 and dRhodamine terminators.
- For the ABI PRISM® 3700 DNA Analyzer, ABI PRISM® 3100 Genetic Analyzer, and ABI PRISM® 310 Genetic Analyzer, separate dye set/primer (mobility) files are used for the forward and reverse primers.
- We do not recommend using POP-5™ polymer on the 3700 instrument for BigDye primer v3.0 chemistry.
- For the ABI PRISM® 377 DNA Sequencers, a single dye set/primer (mobility) file is used for the forward and reverse primers.

Electrophoresis on the ABI PRISM 3700 DNA Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 3700 DNA Analyzer requires the following:

Run Modules

Configuration	Run Module
POP-6™ polymer, 50-cm	Seq1_1POP6DefaultModule

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-6 polymer	DP3700POP6{BDv3-21M13}v1.mob
	DP3700POP6{BDv3-21M13Rev}v1.mob

Note For the 3700 instrument, separate dye set/primer (mobility) files are used for the forward and reverse primers.

Standards

IMPORTANT Use Dye Set D.

Dye Set	Standards for Spectral Calibration
D	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309)

Note Refer to the product insert for instructions on using the standards for this instrument.

Performing Sample Electrophoresis

For information on how to perform sample electrophoresis on the 3700 instrument, refer to the following manuals:

- *ABI PRISM 3700 DNA Analyzer Sequencing Chemistry Guide* (P/N 4309125)
- *ABI PRISM 3700 DNA Analyzer User's Manual* (P/N 4306152)

Electrophoresis on the ABI PRISM 3100 Genetic Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 3100 Genetic Analyzer requires the following:

Run Modules

Configuration	Run Module
POP-6™ polymer, 36-cm	RapidSeq36_POP6DefaultModule
POP-6 polymer, 50-cm	StdSeq50_POP6DefaultModule

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-6 polymer	DP3100POP6{BDv3-21M13}v1.mob
	DP3100POP6{BDv3-21M13Rev}v1.mob

Note For the 3100 instrument, separate dye set/primer (mobility) files are used for the forward and reverse primers.

Standards

IMPORTANT Use Dye Set Z.

Dye Set	Standards for Spectral Calibration
Z	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303)

Note Refer to the product insert for instructions on using the standards for this instrument.

Performing Sample Electrophoresis

For information on how to perform sample electrophoresis on the 3100 instrument, refer to the following manuals:

- *ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide* (P/N 4315831)
- *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834)

Electrophoresis on the ABI PRISM 310 Genetic Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 310 Genetic Analyzer requires the following:

Filter Set E Run Modules

Configuration	Run Module
POP-4™ polymer, 1-mL syringe, 47-cm, 50-µm i.d. capillary, Ld = 36 cm	P4StdSeq (1 mL) E
POP-4 polymer, Rapid Sequencing, 1-mL syringe, 47-cm, 50-µm i.d. capillary, Ld = 36 cm	P4RapidSeq (1 mL) E
POP-6™ polymer, 1-mL syringe, 61-cm, 50-µm i.d. capillary	Seq POP6 (1 mL) E
POP-6 polymer, Rapid Sequencing, 1-mL syringe, 47-cm, 50-µm i.d. capillary	Seq POP6 Rapid (1 mL) E

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-4 polymer	DP310POP4{BDv3-21M13}v1.mob
	DP310POP4{BDv3-M13Rev}v1.mob
POP-6 polymer	DP310POP6{BDv3-21M13}v1.mob
	DP310POP6{BDv3-M13Rev}v1.mob

Note For the 310 instrument, separate dye set/primer (mobility) files are used for the forward and reverse primers.

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye primers v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note Refer to the product insert for instructions on using the standards for this instrument.

Setting the Data Analysis Range

The unincorporated dye primer peak at the beginning of the electropherogram (and the full-length PCR peak at the end of the electropherogram if sequencing PCR products) must be excluded from analysis for data to be normalized correctly.

Analysis of ABI PRISM 310 instrument data is especially sensitive to the location of the Peak 1 Location and Analysis Start Point. The software should pick these correctly in most cases. A miscalled Peak 1 Location and/or Analysis Start Point can result in spacing anomalies visible in the analyzed data.

Refer to page 6-15 of the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for more information on setting the data analysis range.

Resuspending the Samples for Sequencing with POP-6 Polymer

To resuspend the samples for sequencing with POP-6 polymer:

Step	Action
1	Resuspend each sample pellet in 25 µL of template suppression reagent (TSR, supplied with the polymer). **⚠ CAUTION CHEMICAL HAZARD**. **Template suppression reagent** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex and spin the samples.
3	Heat the samples at 95 °C for 2 minutes to denature, then chill on ice.
4	Vortex and spin the samples again. Place on ice until ready to use.
5	Refer to the *ABI PRISM 310 Genetic Analyzer User's Manual* (P/N 903565) for guidelines on loading the samples.

Note Although not recommended on a routine basis, you can keep samples prepared in TSR frozen for several weeks before running on the ABI PRISM 310 Genetic Analyzer with no detectable loss in resolution or base calling.

Resuspending the Samples for Sequencing with POP-4 Polymer

Deionized formamide is the recommended resuspension solution for sequencing with POP-4 polymer on the 310 genetic analyzer.

IMPORTANT Use only the highest grade of deionized formamide, such as Applied Biosystems Hi-Di™ formamide (P/N 4311320). Deionized formamide should be stored at –20 °C in usable aliquots to prevent several freeze-thaw cycles. If the deionized formamide stored at –20 °C does not freeze, discard it and use fresh deionized formamide for sample resuspension.

To resuspend the samples for sequencing with POP-4 polymer:

Step	Action
1	Resuspend each sample pellet in 25 µL of deionized formamide. **IMPORTANT** Securely seal each sample tube after resuspension with deionized formamide to limit the sample's exposure to air. **⚠ WARNING CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex and spin the samples.
3	Heat the samples at 95 °C for 2 minutes to denature, then chill on ice.
4	Vortex and spin the samples again. Place on ice until ready to use.
5	Refer to the *ABI PRISM 310 Genetic Analyzer User's Manual* (P/N 903565) for guidelines on loading the samples.

Note Although extended sample storage at room temperature is not recommended on a routine basis, you can keep securely sealed samples prepared in deionized formamide at room temperature for up to 48 hours on the ABI PRISM 310 Genetic Analyzer autosampler with no detectable loss in resolution or base calling. Freezing of samples resuspended in deionized formamide is not advised.

Electrophoresis on the ABI PRISM 377 DNA Sequencers

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 377 DNA Sequencers (all models[1]) require the following:

Filter Set E Run Modules

Configuration[a]	Run Module
36-cm wtr, 1200 scans/hr, any comb	Seq Run 36E-1200
36-cm wtr, 2400 scans/hr, any comb	Seq Run 36E-2400
48-cm wtr, 1200 scans/hr, any comb	Seq Run 48E-1200

a. Any plate check and prerun module can be used on the ABI PRISM 377 DNA Sequencers.

Dye Set/Primer (Mobility) Files

Gel Formulation	Dye Set/Primer (Mobility) File
4.5% acrylamide (29:1) or 5% Long Ranger™ gel	DP377{BDv3-21M13}v1.mob
	DP377{BDv3-M13Rev}v1.mob

Note For the ABI PRISM 377 DNA Sequencers, a single dye set/primer (mobility) file is used for the forward and reverse primers.

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye primers v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note Refer to the product insert for instructions on using the standards for this instrument.

1. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

Using the Lane Guide Kit

If you are using the BigDye chemistries v3.0 on the 377 instrument in conjunction with the ABI PRISM® Lane Guide™ Lane Identification Kit, refer to that kit's protocol (P/N 4313804) for instructions on resuspending and loading samples.

Using Long-Read Gel and Buffer Formulations

For longer sequencing read lengths follow the gel and buffer formulations described in the user bulletin entitled *Achieving Longer High Accuracy Reads on the 377 Sequencer* (P/N 4315153).

Resuspending and Loading the Samples

Running Ethanol-Precipitated Samples

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Prepare a loading buffer by combining the following in a 5:1 ratio (5 parts deionized formamide to 1 part EDTA with blue dextran):
• Deionized formamide
• 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL)
⚠ WARNING CHEMICAL HAZARD. Formamide is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
⚠ CAUTION CHEMICAL HAZARD. EDTA may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</td></tr>
<tr><td>2</td><td>Resuspend each sample pellet in loading buffer as follows:
<table>
<tr><th>Template</th><th>Reaction</th><th>Volume (µL), 36-well</th><th>Volume (µL), 48- or 64-well</th></tr>
<tr><td rowspan="2">PCR product, plasmid, M13</td><td>1X</td><td>6</td><td>4</td></tr>
<tr><td>0.5X</td><td>2–4</td><td>1–2</td></tr>
<tr><td>BAC, large DNA</td><td>2X</td><td>2–4</td><td>1–2</td></tr>
</table></td></tr>
<tr><td>3</td><td>Vortex and spin the samples.</td></tr>
<tr><td>4</td><td>Heat the samples at 95 °C for 2 minutes to denature. Place on ice until ready to load.</td></tr>
<tr><td>5</td><td>Load each sample into a separate lane of the gel as follows:
<table>
<tr><th>Template</th><th>Reaction</th><th>Volume (µL), 36-well</th><th>Volume (µL), 48- or 64-well</th></tr>
<tr><td rowspan="2">PCR product, plasmid, M13</td><td>1X</td><td>0.75–2.0</td><td>0.5–1.5</td></tr>
<tr><td>0.5X</td><td>2–3</td><td>1–1.5</td></tr>
<tr><td>BAC, large DNA</td><td>2X</td><td>2–3</td><td>1–1.5</td></tr>
</table></td></tr>
</table>

Express Load Option for 36-Well Gels

Step	Action
1	Combine the four reactions (A, C, G, T) with 5 µL of 5 mM EDTA (25 µL total volume). **⚠ CAUTION CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex briefly, then spin in a microcentrifuge.
3	Prepare a loading buffer by combining the following in a 5:1 ratio (5 parts deionized formamide to 1 part EDTA with blue dextran): • Deionized formamide • 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL) **⚠ WARNING CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Combine 4 µL of each 25-µL reaction/EDTA mixture with 4 µL of loading buffer. Store the remaining reaction/EDTA mixture at –15 to –25 °C. This mixture can be concentrated by ethanol precipitation if the Express Load procedure does not yield enough signal.
5	Heat the samples at 98 °C for 5 minutes with the lids open to denature and concentrate. Place on ice until ready to load.
6	Load 2.5 µL of each sample into a separate lane of the gel.

Electrophoresis on the 373 Instrument with BigDye Filter Wheel

Requirements

General guidelines are provided below for running the ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel[2] installed. For more detailed instructions, please refer the user's manual for your 373 instrument or to the user bulletin entitled *Using the ABI PRISM 373 BigDye Filter Wheel* (P/N 4304367).

Gel

For 48-cm well-to-read (wtr), we recommended 5% Long Ranger™ gel.

New Dye Set/Primer (Mobility) Files

Gel Formulation	Dye Set/Primer (Mobility) File
5% Long Ranger gel, 48-cm wtr[a]	DP373{BDv3-21M13}v1.mob
	DP373{BDv3-M13Rev}v1.mob

a. If you are running other wtr lengths, these are being tested. Please call Technical Support.

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye primers v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
A (For use with the BigDye Filter Wheel)	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note For instructions on using the matrix standards (P/N 4390421) for the 373 instruments, contact Technical Support.

2. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Resuspending and Loading the Samples

To resuspend and load the samples:

Step	Action				
1	Prepare a loading buffer by combining the following in a 5:1 ratio (5 parts deionized formamide to 1 part EDTA with blue dextran): • Deionized formamide • 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL) **⚠ WARNING** **CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION** **CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.				
2	Resuspend each sample pellet in loading buffer as follows:				
		Volume (µL)			
	Template	**18 or 24 well**	**32 or 36 well**	**48-well**	**64-well**
	PCR product, plasmid, M13	3–4	3–4	3–4	2–4
	BAC, large DNA	3	3	2	2
3	Vortex and spin the samples.				
4	Heat the samples at 95 °C for 2 minutes to denature. Place on ice until ready to load.				
5	Load each sample into a separate lane of the gel as follows:				
		Volume (µL)			
	Template	**18 or 24 well**	**32 or 36 well**	**48-well**	**64-well**
	PCR product, plasmid, M13	1–3	1–3	1–2	1–2
	BAC, large DNA	3	3	2	2

Control DNA Sequences

A

Appendix Summary

In This Appendix The following topics are covered in this appendix:

–21 M13 Forward Primer

Sequence The pGEM®-3Zf(+) sequence below is the sequence of the –21 M13 forward primer, followed by the ensuing 1000 bases.

TGTAAAACGACGGCCAGT (–21 M13 primer)

GAATTGTAAT	ACGACTCACT	ATAGGGCGAA	TTCGAGCTCG	40
GTACCCGGGG	ATCCTCTAGA	GTCGACCTGC	AGGCATGCAA	80
GCTTGAGTAT	TCTATAGTGT	CACCTAAATA	GCTTGGCGTA	120
ATCATGGTCA	TAGCTGTTTC	CTGTGTGAAA	TTGTTATCCG	160
CTCACAATTC	CACACAACAT	ACGAGCCGGA	AGCATAAAGT	200
GTAAAGCCTG	GGGTGCCTAA	TGAGTGAGCT	AACTCACATT	240
AATTGCGTTG	CGCTCACTGC	CCGCTTTCCA	GTCGGGAAAC	280
CTGTCGTGCC	AGCTGCATTA	ATGAATCGGC	CAACGCGCGG	320
GGAGAGGCGG	TTTGCGTATT	GGGCGCTCTT	CCGCTTCCTC	360
GCTCACTGAC	TCGCTGCGCT	CGGTCGTTCG	GCTGCGGCGA	400
GCGGTATCAG	CTCACTCAAA	GGCGGTAATA	CGGTTATCCA	440
CAGAATCAGG	GGATAACGCA	GGAAAGAACA	TGTGAGCAAA	480
AGGCCAGCAA	AAGGCCAGGA	ACCGTAAAAA	GGCCGCGTTG	520
CTGGCGTTTT	TCCATAGGCT	CCGCCCCCCT	GACGAGCATC	560
ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	GAAACCCGAC	600
AGGACTATAA	AGATACCAGG	CGTTTCCCCC	TGGAAGCTCC	640
CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	CTTACCGGAT	680
ACCTGTCCGC	CTTTCTCCCT	TCGGGAAGCG	TGGCGCTTTC	720
TCATAGCTCA	CGCTGTAGGT	ATCTCAGTTC	GGTGTAGGTC	760
GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	CCCCCCGTTC	800
AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	ATCGTCTTGA	840
GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA	880
GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	TATGTAGGCG	920
GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	ACTACGGCTA	960
CACTAGAAGG	ACAGTATTTG	GTATCTGCGC	TCTGCTGAAG	1000

M13 Reverse Primer

Sequence The pGEM®-3Zf(+) sequence below is the sequence of the M13 reverse primer, followed by the ensuing 1000 bases.

CAGGAAACAGCTATGACC (M13 reverse primer)

ATGATTACGC	CAAGCTATTT	AGGTGACACT	ATAGAATACT	40
CAAGCTTGCA	TGCCTGCAGG	TCGACTCTAG	AGGATCCCCG	80
GGTACCGAGC	TCGAATTCGC	CCTATAGTGA	GTCGTATTAC	120
AATTCACTGG	CCGTCGTTTT	ACAACGTCGT	GACTGGGAAA	160
ACCCTGGCGT	TACCCAACTT	AATCGCCTTG	CAGCACATCC	200
CCCTTTCGCC	AGCTGGCGTA	ATAGCGAAGA	GGCCCGCACC	240
GATCGCCCTT	CCCAACAGTT	GCGCAGCCTG	AATGGCGAAT	280
GGACGCGCCC	TGTAGCGGCG	CATTAAGCGC	GGCGGGTGTG	320
GTGGTTACGC	GCAGCGTGAC	CGCTACACTT	GCCAGCGCCC	360
TAGCGCCCGC	TCCTTTCGCT	TTCTTCCCTT	CCTTTCTCGC	400
CACGTTCGCC	GGCTTTCCCC	GTCAAGCTCT	AAATCGGGGG	440
CTCCCTTTAG	GGTTCCGATT	TAGTGCTTTA	CGGCACCTCG	480
ACCCCAAAAA	ACTTGATTAG	GGTGATGGTT	CACGTAGTGG	520
GCCATCGCCC	TGATAGACGG	TTTTTCGCCC	TTTGACGTTG	560
GAGTCCACGT	TCTTTAATAG	TGGACTCTTG	TTCCAAACTG	600
GAACAACACT	CAACCCTATC	TCGGTCTATT	CTTTTGATTT	640
ATAAGGGATT	TTGCCGATTT	CGGCCTATTG	GTTAAAAAAT	680
GAGCTGATTT	AACAAAAATT	TAACGCGAAT	TTTAACAAAA	720
TATTAACGCT	TACAATTTCC	TGATGCGGTA	TTTTCTCCTT	760
ACGCATCTGT	GCGGTATTTC	ACACCGCATA	TGGTGCACTC	800
TCAGTACAAT	CTGCTCTGAT	GCCGCATAGT	TAAGCCAGCC	840
CCGACACCCG	CCAACACCCG	CTGACGCGCC	CTGACGGGCT	880
TGTCTGCTCC	CGGCATCCGC	TTACAGACAA	GCTGTGACCG	920
TCTCCGGGAG	CTGCATGTGT	CAGAGGTTTT	CACCGTCATC	960
ACCGAAACGC	GCGAGACGAA	AGGGCCTCGT	GATACGCCTA	1000

Technical Support B

Technical Support

Contacting Technical Support

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Product	Hours
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Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6100**	**81 3 5566 6501**
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403044	Ready Reaction	100
403045	Ready Reaction	1000
4303143	Ready Reaction	5000
403041	Protocol	–

The ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

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4390161	-21 M13	100
4390163	M13 reverse	100
4390157	-21 M13	5000
4390159	M13 reverse	5000

The ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

P/N	Kit	Reactions
4390236	Ready Reaction	24
4390242	Ready Reaction	100
4390244	Ready Reaction	1000
4390246	Ready Reaction	5000
4390253	Ready Reaction	25000

The ABI PRISM® dGTP BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

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4313677	Lane Guide	1000
4313679	Lane Guide	5000
4313804	Protocol	–

ABI PRISM Matrix Standards

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4390309	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard	3700
4390303	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard	3100/377/ 373/310
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