Applied Biosystems BigDye® XTerminator™ Purification Kit

Protocol

Applied Biosystems

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document.

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

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: LICENSE DISCLAIMER

This product is compatible with DNA sequencing or fragment analysis methods covered by patents owned or licensed by Applied Biosystems. No license under these patents to use these methods is conveyed expressly, by implication, or by estoppel. A license to use the DNA sequencing or fragment analysis methods for certain research and development activities is available from Applied Biosystems. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

TRADEMARKS:

Applera, Applied Biosystems, AB (Design), ABI PRISM, and BigDye are registered trademarks and Hi-Di, MicroAmp, POP-4, POP-6, POP-7, SAM, and XTerminator are trademarks of Applera Corporation or its subsidiaries in the US and/or certain other countries.

All other trademarks are the sole property of their respective owners.

Part Number 4374408 Rev. C
07/2007

Contents

Preface

This preface covers:

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.

Definitions

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

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 (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs," on page vi.)
- 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 in 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

The MSDSs for many chemicals supplied by Applied Biosystems are available to you free 24 hours a day. To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field of the MSDS Search page:
   a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.
3. To view, download, or print the document of interest:
   a. Right-click the document title.
   b. Select:
      - **Open** – To view the document
      - **Save Target As** – To download a PDF version of the document to a destination that you choose
      - **Print Target** – To print the document
4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
   a. Select **Fax** or **Email** below the document title.
   b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
   c. Enter the required information.
   d. Click **View/Deliver Selected Documents Now**.

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

Chemical Waste Hazards

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

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

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the 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

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.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**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

How to Obtain More Information

Related Documentation

The *BigDye® XTerminator™ Purification Kit Product Insert*, which is shipped with the kit, summarizes how to install BigDye® XTerminator™ Kit run modules and perform the purification protocol. Additional instrument-specific documents are listed in Table 3 on page 8.

Additional information is available at the BigDye XTerminator web page at:

www.appliedbiosystems.com/gameoverblobs

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" (x) below).

How to Obtain Support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- 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

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

BigDye® XTerminator™ Purification Kit Protocol

Overview

The BigDye® XTerminator™ Purification Kit is designed to sequester cycle-sequencing reaction components such as salt ions, unincorporated dye terminators, and dNTPs, to prevent their co-injection with dye-labeled extension products.

BigDye® XTerminator™ Solutions are added directly to finished sequencing reactions and vortexed. During vortexing, the BigDye XTerminator reagents capture and immobilize unwanted components. After vortexing, the reactions are briefly centrifuged to move the insoluble fraction of the reagent mixture and the captured reaction components to the bottom of the reaction well. The purified dye-labeled extension products remain in the supernatant and are injected directly from the supernatant into the DNA sequencer, where they are analyzed using specialized BigDye XTerminator run modules.

Supported Injection Configurations

Table 1 shows the instrument, plate, and method of sealing that allow for direct injection of samples purified with the BigDye XTerminator Purification Kit.

Table 1 Supported software, seals, and injection configurations for the BigDye® XTerminator™ Purification Kit

Plate Type and Reaction Volume/Well	Seals Supported for Direct Injection	Direct Injection Supported‡	Run Module
ABI PRISM® 3100/3100-Avant Genetic Analyzer with Data Collection Software v 2.0			
384-well, 5-µL	Septa	No	Standard
96-well, 10-µL	Septa	Yes	BDx
96-well, 20-µL	Septa	Yes	BDx

Table 1 Supported software, seals, and injection configurations for the BigDye® XTerminator™ Purification Kit *(continued)*

Plate Type and Reaction Volume/Well	Seals Supported for Direct Injection	Direct Injection Supported‡	Run Module
ABI PRISM® 3100/3100-Avant Genetic Analyzer with Data Collection Software v1.x			
384-well, 5-µL	Septa	No	Standard
96-well, 10-µL	Septa	No	Standard
96-well, 20-µL	Septa	No	Standard
Applied Biosystems 3130/3130*xl* Genetic Analyzer with Data Collection Software v3			
384-well, 5-µL	Septa	No	Standard
96-well, 10-µL	Septa	Yes	BDx
96-well, 20-µL	Septa	Yes	BDx
Applied Biosystems 3730/3730*xl* DNA Analyzer with Data Collection Software v2 or v3			
384-well, 5-µL	Heat seal	Yes	BDx
	Septa	No	Standard
96-well, 10-µL	Heat seal or septa	Yes	BDx
96-well, 20-µL	Heat seal or septa	Yes	BDx
ABI PRISM® 310 Genetic Analyzer			
384-well, 5-µL	Septa	No	Standard
96-well, 10-µL	Septa	No	Standard
96-well, 20-µL	Septa	No	Standard
Applied Biosystems 3700 DNA Analyzer			
384-well, 5-µL	Septa or heat seal	No	Standard
96-well, 10-µL	Septa or heat seal	No	Standard
96-well, 20-µL	Septa or heat seal	No	Standard

‡ For configurations where direct injection is not supported, transfer the supernatant to a new plate.

Note: MicroAmp™ Fast 96-Well Reaction Plates (PN 4346907) can be used with the BigDye XTerminator Purification Kit for sequencing reaction volumes 10 µL or less.

About the BigDye XTerminator Purification Kit

The BigDye XTerminator Purification Kit has two reagents:

- XTerminator Solution – Captures unincorporated dye terminators and free salts from the post cycle-sequencing reaction.
- SAM™ Solution – Improves BigDye XTerminator reagent performance and stabilizes the sample after purification.

Kit Size	Approximate Number of 20-µL Reactions	Volume of Each Kit Reagent (mL)		Part Number
		XTerminator™ Solution	SAM™ Solution	
2-mL	100	2	9	4376486
20-mL	1,000	20	90	4376487
50-mL	2,500	50	225	4376484
800-mL	40,000	800	3,600	4376485

Handling and Storage

If you plan to work with the BigDye XTerminator Purification Kit reagents for more than 30 minutes, keep them on ice with the caps closed between pipetting steps. Avoid freezing the reagents.

When stored at 4 °C, XTerminator Solution is stable through the expiration date on the package and bottle label.

When stored at room temperature or 4 °C, SAM Solution is stable through the date on the package and bottle label. Storage at room temperature may decrease the likelihood of precipitation.

About This Protocol

This protocol describes:

- Required and recommended materials for using the BigDye XTerminator Purification Kit.
- Instructions for preparing for and performing the purification protocol.
- Instructions for installing and updating run modules for the Data Collection software.

Sequencing Workflow

Figure 1 shows how using the BigDye XTerminator Purification Kit fits into a sequencing workflow.

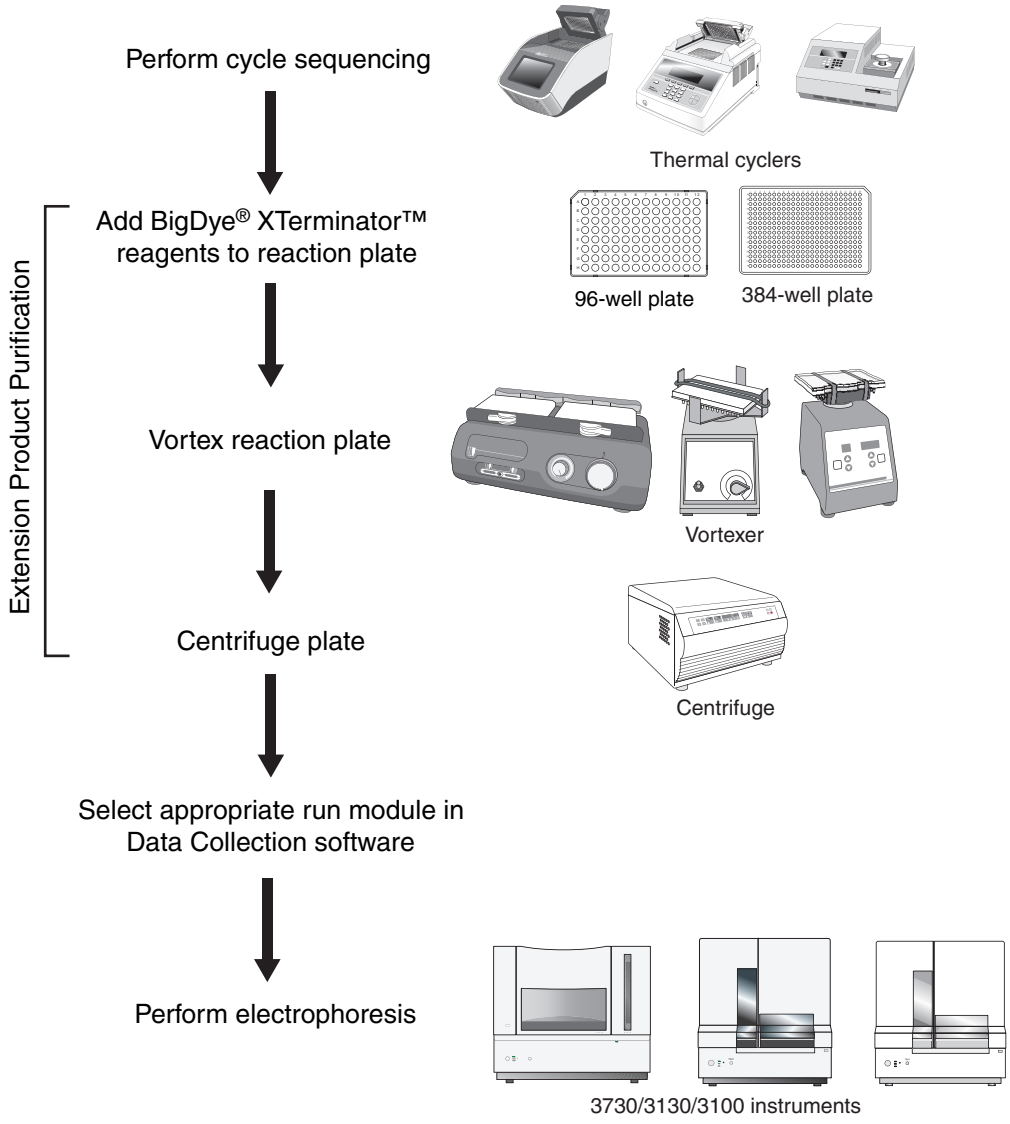


Figure 1 Sequencing workflow

For details about dye terminators, cycle sequencing, and electrophoresis, refer to the appropriate document or instrument user guide (see Table 3 on page 8).

Materials

Applied Biosystems Instruments

Table 2 Instruments and accessories from Applied Biosystems

Item	Source or Part Number
One of the following Applied Biosystems DNA or Genetic Analyzers and corresponding data collection software: • 3730*xl* DNA Analyzer with Data Collection Software v2.0 or 3.0 • 3730 DNA Analyzer with Data Collection Software v2.0 or 3.0 • 3130*xl* Genetic Analyzer with 3130 Data Collection Software v3.0 • 3130 Genetic Analyzer with 3130 Data Collection Software v3.0 • ABI PRISM® 3100 Genetic Analyzer with 3100 Data Collection Software v2.0 • ABI PRISM® 3100-*Avant* Genetic Analyzer with 3100-Avant Data Collection Software v2.0	Contact your Applied Biosystems sales representative.
Either of the following sequencing kits, as necessary: • BigDye® Terminator v3.1 Cycle Sequencing Kit • BigDye® Terminator v1.1 Cycle Sequencing Kit	Contact your Applied Biosystems sales representative.

Table 2 Instruments and accessories from Applied Biosystems

Item	Source or Part Number
MicroAmp™ *Fast* 96-Well Reaction Plates (0.1-mL) (10 plates).	4346907
MicroAmp™ *Fast* Optical 96-Well Reaction Plates with Barcode (0.1-mL). Available quantities are: • 20 • 200	 4346906 4366932
MicroAmp™ Optical 96-Well Reaction Plates. Available quantities are: • 20 • 500	 N8010560 4316813
MicroAmp™ Optical 96-Well Reaction Plates with Barcode. Available quantities are: • 20 • 500	 4306737 4326659
MicroAmp™ Optical 384-Well Reaction Plates with Barcode. Available quantities are: • 50 • 500 • 1000	 4309849 4326270 4343814
MicroAmp™ Fast Optical 48-Well Reaction Plates (20 plates)	4375816
Plate accessories, such as: • MicroAmp™ Clear Adhesive Films (100) • MicroAmp™ Adhesive Film Applicator • MicroAmp™ Multi Removal Tool • 96-Well Plate Septa (20) • MicroAmp™ Splash Free 96-Well Base • Heat Seal Film for Sequencing and Fragment Analysis Sample Plates	 4306311 4333183 4313950 4315933 4312063 4337570

Table 3 Applied Biosystems documents

Item	Part Number
One or more of the following, as applicable:	
• *Applied Biosystems 3730/3730xl DNA Analyzers Sequencing Chemistry Guide*	4331467
• *Applied Biosystems 3730/3730xl DNA Analyzer Getting Started Guide*	4359476
• *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide*	4352715
• *ABI PRISM® 3100/3100-Avant Genetic Analyzer User Guide*	4347102
BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol	4337035
BigDye® Terminator v1.1 Cycle Sequencing Kit Protocol	4337036

Vortexers and Accessories

The vortexing step of the BigDye XTerminator Purification Kit protocol is critical to achieving optimum performance. The use of one of the vortexers below is strongly recommended.

Select a vortexer and the indicated accessories based on the type of plates you plan to use and the number of plates you want to load at the same time:

- 96-well plates – Table 4
- 384-well plates – Table 5

The Digital Vortex-Genie 2, the IKA MS3 Digital Vortexer, the IKA Works 3 Vortexer, and the MixMate can vortex one plate at a time. The Taitec MicroMixer E-36 can vortex two plates at a time. The Union Scientific Vertical Shaker can accommodate up to six 96-well plates or eighteen 384-well plates at once.

If you select a vortexer that is not included in Table 4 or 5, best results are obtained from a vortexer with a minimum of 2000 rpm and a maximum orbital diameter of 4 mm.

Note: Additional supported vortexers are listed at the BigDye XTerminator web page at:

www.appliedbiosystems.com/gameoverblobs

Click the **Product Description** tab and look for the links in step 4 under the heading “Simple Purification Process”.

Table 4 Vortexers and adapters for 96-well plates

Vortexer and Source	Adapter or Accessories
Digital Vortex-Genie 2, Applied Biosystems, 120 V, 60 Hz (PN SI-A536) Digital Vortex-Genie 2, Applied Biosystems, 100 V, 50/60 Hz (PN SI-A586) Digital Vortex-Genie 2, Applied Biosystems, 230 V, 50 Hz, with: • No Plug (PN SI-A546) • European Plug (PN SI-A556) • British Plug (PN SI-A566) • Swiss Plug (PN SI-A576) All models above include shock-absorbing feet, Microplate Adapter for Applied Biosystems, and elastic bands. Scientific Industries, Inc. 70 Orville Drive Bohemia, NY 11716 Tel: (631) 567-4700 Fax: (631) 567-5896 info@scientificindustries.com www.scientificindustries.com	If you have another model of Digital Vortex-Genie 2, you need: • Shock-absorbing feet (PN 0K-0400-900) • Microplate adapter for Applied Biosystems (includes 2 elastic bands) (PN SI-0513) • Replacement elastic bands (2) for Microplate adapter for Applied Biosystems (PN 0K-0513-900) If you use the Recessed Platform (PN 504-0039-00): • Elastic bands (PN 568-0001-00)
IKA MS 3 Digital (PN 3319000) IKA® Works, Inc. 2635 North Chase Pkwy SE Wilmington, NC 28405-7419 This vortexer includes the MS 3.4 Microtiter Attachment (PN 3426400). Tel: (910) 452-7059 Fax: (910) 452-7693 usa@ika.net www.ika.net	From any office supply vendor: • Elastic bands, size 64 (90 mm × 6 mm) **Note:** For regions outside North America, an electrical plug adapter may be required.

Table 4 Vortexers and adapters for 96-well plates *(continued)*

Vortexer and Source	Adapter or Accessories
IKA Vortex 3 (PN 3340001) IKA® Works, Inc. 2635 North Chase Pkwy SE Wilmington, NC 28405-7419 Tel: (910) 452-7059 Fax: (910) 452-7693 usa@ika.net www.ika.net	From IKA Works: • VG 3.3 Universal attachment (Ident. No. 3342400) From any office supply vendor: • Elastic band, size 117B (3 mm × 178 mm)
Taitec MicroMixer E-36 (PN BNE36) Distributed in the US by: Bionexus Inc. 222 Madison Street, Suite 200 Oakland, CA 94607 Tel: (510)-625-8400 Fax: 510-625-8419 info@bionexus.net www.bionexus.net	From Applied Biosystems: • MicroAmp™ 96-Well Base (PN N8010531)
Union Scientific Vertical Shaker (PN 9816) Union Scientific Corp. 9633 Liberty Road Randallstown, MD 21133 Tel: (410) 496-0200 Fax: (410) 521-4590 CustomerService@UnionScientific.com www.unionscientific.com	From Applied Biosystems: • MicroAmp™ Splash Free 96-Well Base (PN 4312063)

Table 5 Vortexers and adapters for 384-well plates

Vortexer and Source	Adapter or Accessories
Digital Vortex-Genie 2 See Table 4 on page 9 for recommended models and source.	If you have another model of Digital Vortex-Genie 2, you need: • Shock absorbing feet (PN 0K-0400-900) • Microplate adapter for Applied Biosystems (includes 2 elastic bands) (PN SI-0513) • Replacement elastic bands (2) for Microplate adapter for Applied Biosystems (PN 0K-0513-900) If you use the Recessed Platform (PN 504-0039-00): • Elastic bands (PN 568-0001-00)
IKA MS 3 Digital (PN 3319000) This vortexer includes the MS 3.4 Microtiter Attachment (PN 3426400). See Table 4 on page 9 for source.	From any office supply vendor: • Elastic bands, size 64 (90 mm × 6 mm) **Note:** For regions outside North America, an electrical plug adapter may be required.
Union Scientific Vertical Shaker (PN 9816) See Table 4 on page 9 for source.	None
Taitec MicroMixer E-36 (PN BNE36) See Table 4 on page 9 for source.	None

Table 5 Vortexers and adapters for 384-well plates *(continued)*

Vortexer and Source	Adapter or Accessories
MixMate, 120 V, 50/60 Hz (PN 022674200) MixMate, 240 V, 50/60 Hz (PN 022674226) Eppendorf USA One Cantiague Road P.O. Box 1019 Westbury, NY 11590-0207 Tel: (800)-645-3050 info@eppendorf.com www.eppendorf.com	None

Other Materials and Accessories

Table 6 Other materials and accessories

Item	Source
Swinging-bucket centrifuge	Major laboratory supplier
Troughs (if using multichannel pipettors)	Major laboratory supplier
ALPS 300™ Automated Laboratory Plate Sealer (optional) **Note:** You can also use Applied Biosystems MicroAmp™ Clear Adhesive Films to seal your plates.	ABgene Inc., USA 565 Blossom Road Rochester, NY 14610 Tel: (585) 654-4800 Fax: (585) 654-4810 infoUSA@abgene.com abgene.com

Table 6 Other materials and accessories *(continued)*

Item	Source
Wide-orifice (>1.0 mm) pipette tips such as:	
• Rainin Wide-Orifice LTS 250-µL Tip 960/10 (PN RT L250W)	Rainin Instrument, LLC 7500 Edgewater Drive P.O. Box 2160 Oakland, CA 94621-0060 Tel: (510) 564-1600 Fax: (510) 564-1617 Rainin Instrument, LLC Rainin Road, Box 4026 Woburn, MA 01888-4026 Tel: (781) 935-3050 Fax: (510) 564-1617 pipets@rainin.com www.rainin.com
• Axygen wide-bore tips (PN T-205-WB-C-R)	Axygen Scientific Inc. 33210 Central Ave. Union City, CA 94587 Tel: (510) 494-8900 Fax: (510) 494-0700 info@axygen.com www.axygen.com

Before You Begin Purification

The BigDye® XTerminator™ Purification Kit consists of two reagents: XTerminator Solution and SAM™ Solution. The insoluble phase of the reagent mixture separates if left undisturbed during sample preparation. It is important to deliver a homogeneous mixture of the reagents to each well so that correct proportions of the reagents are present.

The pipetting method (sequential or premix) affects the steps required to keep the two phases of the BigDye XTerminator reagents adequately mixed. Protocols for sequential, premix, and robotic pipetting follow.

Software

IMPORTANT! If you are doing direct injection, verify that the appropriate software run module for your system is installed and updated (see Appendix A, "Run Modules," on page 37). You can download the run modules from **www.appliedbiosystems.com**.

The BigDye XTerminator run modules adjust the injection height to removed only the supernatant containing the purified dye labeled extension products to inject in the sequencer. In addition, the injection times and voltages are optimized for the higher signal usually seen with samples purified with the BigDye XTerminator Purification Kit.

Equipment and Materials

- Obtain the required materials (see Table 2 on page 6)
- Select a vortexer (see Tables 4 and 5, starting on page 9).
  - If you use the Digital Vortex-Genie 2, read "Mixing with the Scientific Industries Digital Vortex-Genie 2" on page 47.
  - If you use the Union Scientific Vertical Shaker, read "Mixing with the Union Scientific Vertical Shaker" on page 50.
  - If you use the IKA MS3 Digital, read "Mixing with the IKA MS 3 Digital Vortexer" on page 52.
  - If you use the Taitec MicroMixer, read "Mixing with the Taitec MicroMixer" on page 55.
- Select an instrument and software (see Table 2 on page 6)
- Identify the procedure to follow. If you use:

– Sequential pipetting: Follow the procedure in "Performing Purification – Sequential Pipetting," below.
– Premix pipetting: Follow the procedure in "Performing Purification – Premix Pipetting" on page 20.
– Robotic pipetting: Follow the procedure in Appendix E, "Performing Purification – Robotic Pipetting."

Performing Purification – Sequential Pipetting

Guidelines for Sequential Pipetting

These guidelines apply to single- and multi-dispense pipettes.

- Use wide-bore pipette tips (tips with an orifice >1.0 mm) whenever pipetting the XTerminator Solution.
- Use conventional pipette tips for pipetting the SAM Solution.
- Agitate the XTerminator Solution for at least 10 seconds using a standard laboratory vortexer at maximum speed before pipetting.

  IMPORTANT! XTerminator Solution allowed to stand for more than 2 minutes must be reagitated.

- When pipetting the XTerminator Solution, place the tip of the pipettor below the surface of the liquid.

  Note: Applied Biosystems recommends that you aspirate no more than the volume of XTerminator Solution that you can pipette in approximately 2 minutes.

- Dispense at the fastest speed setting and touch off during dispensing.

Performing Purification

This procedure gives instructions for purification using sequential addition of the BigDye XTerminator Purification Kit reagents. The SAM Solution is added first, followed by the XTerminator Solution.

WARNING **CHEMICAL HAZARD. SAM Solution** is a flammable liquid and vapor. It may be harmful if absorbed through the skin, inhaled or swallowed. Exposure may cause eye, skin, and respiratory tract irritation, liver damage, and central nervous system depression. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. After cycle sequencing is complete, centrifuge the reaction plate for 1 minute.

2. To each well of the reaction plate, add the volume of SAM Solution specified below using a conventional pipette tip.

   Make sure there are no particulates in the SAM Solution before pipetting. If particulates are present, heat the SAM Solution to 37 °C and mix to resuspend. Cool to room temperature before using.

Plate Type and Reaction Volume/Well	Volume of SAM™ Solution/Well (µL)
384-well, 5-µL	22.5
96-well, 10-µL	45
96-well, 20-µL	90

   IMPORTANT! For 384-well reactions with reaction volume less than 5 µL, add water to bring the volume to 5 µL before adding SAM Solution. For 96-well reactions with reaction volume less than 10 µL, add water to bring the volume to 10 µL before adding SAM Solution.

3. Add the XTerminator Solution:

   a. Vortex the XTerminator Solution bulk container at maximum speed for at least 10 seconds, until it is homogeneous.

   b. Use a wide-bore pipette tip to aspirate the XTerminator Solution.

      IMPORTANT! Avoid pipetting from the top of the liquid.

   c. Into each well, add the volume of XTerminator Solution specified below:

Plate Type and Reaction Volume/Well	Volume of XTerminator™ Solution/Well (µL)
384-well, 5-µL	5
96-well, 10-µL	10
96-well, 20-µL	20

   d. Discard the pipette tip.

4. Seal the plate using:
   - A heat seal at 160 °C for 1.5 seconds (see Table 6 on page 12 and the note below).

     or
   - MicroAmp™ Clear Adhesive Films (follow the procedure in Appendix C).

   Verify that each well is sealed.

   IMPORTANT! If you use a 3730/3730*xl* instrument and plan to use direct injection without a septa mat, only Applied Biosystems Heat Seal Film for Sequencing and Fragment Analysis Sample Plates (PN 4337570) is supported.

5. Place the plate in a recommended vortexer (see Tables 4 and 5, starting on page 9). Use the appropriate adapter, if recommended.

6. Vortex the reaction plate for 30 minutes, using the following conditions:

Vortexer	Plate Type	Speed
Digital Vortex-Genie 2	96-well	1800 rpm
	384-well	2000 rpm
Eppendorf MixMate	384-well	2600 rpm
IKA MS3 Digital	Either	2000 rpm‡
IKA Vortex 3	Either	Setting 5§
Taitec MicroMixer E-36	Either	Maximum
Union Scientific Vertical Shaker#	Either	Setting 100

‡ Set the vortexer to Mode B. See "Mixing with the IKA MS 3 Digital Vortexer" on page 52 for instructions.

§ Use the maximum setting that does not cause the vortexer to "walk" across the bench.

Add any additional plates to meet mass requirements. (See "Mixing with the Union Scientific Vertical Shaker" on page 50 for information.)

It is recommended to pause vortexing after 1 minute and examine the wells to verify that the contents are well mixed.

7. In a swinging-bucket centrifuge, spin the plate at 1000 × *g* for 2 minutes.

   At this point you can analyze the samples or store the sealed plates for later analysis (see "Storage of Purified Samples" on page 27).

8.	Place the reaction plate in the instrument. **IMPORTANT!** Do not heat-denature or use Hi-Di™ Formamide with samples containing BigDye XTerminator reagents. • **For 384-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant*, 3130/3130*xl* or 310 Genetic Analyzer instrument** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. • **For 96-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant* or 3130/3130*xl* instrument, with Data Collection software v2.0 or later**– Remove the adhesive film or heat seal, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant* instrument with Data Collection software v1.x or 310 Genetic Analyzer** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument.
9.	In the Data Collection Software Plate Editor, select the appropriate run module: • **For most plate and instrument combinations** – Select the BigDye XTerminator run module for your instrument and plate type (see Appendix A, "Run Modules"). • **If you transferred the supernatant to a clean plate after vortexing** – Select a standard run module.
10.	Run the plate.

Performing Purification – Premix Pipetting

If you use premix pipetting:

- Prepare a mixture of the two BigDye® XTerminator™ reagents (referred to here as "premix").
- Perform the purification, according to the procedure on page 23.

  For purification using robotic pipetting, see Appendix E, "Performing Purification – Robotic Pipetting."

Guidelines for Preparing the Premix

These guidelines apply to single- and multi-dispense pipettes.

- Use wide-bore pipette tips (tips with an orifice >1.0 mm) for pipetting the XTerminator Solution.
- Use conventional pipette tips for pipetting the SAM Solution.
- Agitate the XTerminator Solution for at least 10 seconds using a standard laboratory vortexer at maximum speed before pipetting.

  IMPORTANT! XTerminator Solution that is allowed to stand for more than 2 minutes must be revortexed.

Preparing the Premix

WARNING **CHEMICAL HAZARD. SAM Solution** is a flammable liquid and vapor. It may be harmful if absorbed through the skin, inhaled or swallowed. Exposure may cause eye, skin, and respiratory tract irritation, liver damage, and central nervous system depression. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Based on your plate and reaction size, calculate the volumes of XTerminator Solution and SAM Solution needed.

Plate Type and Reaction Volume/Well	Volume/Well (µL)	
	XTerminator™ Solution	SAM™ Solution
384-well, 5 µL	5	22.5
96-well, 10 µL	10	45
96-well, 20 µL	20	90

Multiply the number of reactions to be performed by the volume of XTerminator Solution and SAM Solution needed per reaction, adding 10 to 20% extra volume for reagent trough dead volume. The ratio of SAM Solution to XTerminator Solution should be 4.5 to 1 (v/v).

For example, for one 96-well, 10-µL reaction plate with 10% extra volume:

- **XTerminator Solution:**
  10 µL/reaction × 96 reactions × 1.10 = 1056 µL
- **SAM Solution**:
  45 µL/reaction × 96 reactions × 1.10 = 4752 µL

2.	Combine the SAM Solution and the XTerminator Solution: a. Vortex the XTerminator Solution bulk container at maximum speed for at least 10 seconds, until it is homogeneous. b. Using a wide-bore pipette tip or a graduated cylinder, transfer the appropriate volume of XTerminator Solution to a clean container. **IMPORTANT!** Insert the pipette tip well below the surface of the liquid before aspirating. c. Using a conventional pipette tip or graduated cylinder, add the appropriate volume of SAM Solution to the container with the XTerminator Solution. Make sure there are no particulates in the SAM Solution before pipetting. If particulates are present, heat the SAM Solution to 37 °C and mix to redissolve. Cool to room temperature before using. d. Mix the reagents until homogenous.
3.	Depending on your schedule: • Continue to "Performing Purification" on page 23. *or* • Store the premix in a clean, capped container at 4 °C for up to 5 days.

Guidelines for Pipetting the Premix

- Use conventional pipette tips when pipetting the premix.
- When pipetting from a bottle, keep the premix agitated using a rocking motion.

  IMPORTANT! Using a stir bar on a stir plate does not keep the premix properly suspended.

- When pipetting from a trough, keep the premix agitated by:
  - Rocking the trough back and forth lengthwise to create a wave motion.

    or
  - Placing the pipette tips 1 to 2 mm above the trough bottom and moving them gently from side-to-side.
- Agitate the XTerminator premix before each aspiration.

Performing Purification

This procedure provides instructions for purification by single-step addition of the premix and automated pipettors.

1.	Be sure the premix is well mixed, then transfer it to the trough or reservoir.
2.	After cycle sequencing is complete, centrifuge the reaction plate for 1 minute.

3. Into each well of the reaction plate, use a conventional pipette tip to add the volume of premix specified below:

Plate Type and Reaction Volume/Well	Volume of Premix/Well (µL)
384-well, 5-µL	27.5
96-well, 10-µL	55
96-well, 20-µL	110

Add more premix to the trough or reservoir as necessary.

IMPORTANT! For 384-well reactions with reaction volume less than 5 µL, add water to bring the volume to 5 µL before adding SAM Solution. For 96-well reactions with reaction volume less than 10 µL, add water to bring the volume to 10 µL before adding SAM Solution.

IMPORTANT! Dispense the premix within 1 minute of aspiration to avoid separation of the reagents in the pipette tip.

4. Seal the plate using:
   - A heat seal at 160 °C for 1.5 seconds (see Table 6 on page 12 and the note below).

     or
   - MicroAmp™ Clear Adhesive Films (follow the procedure in Appendix C).

   Verify that each well is sealed.

   IMPORTANT! If you use a 3730/3730xl instrument and plan to use direct injection without a septa mat, only Applied Biosystems Heat Seal Film for Sequencing and Fragment Analysis Sample Plates (PN 4337570) is supported.

5. Place the plate in a recommended vortexer (see Tables 4 and 5, starting on page 8). Use the appropriate adapter, if recommended.

6. Vortex the reaction plate for 30 minutes using the following conditions:

Vortexer	Plate Type	Speed
Digital Vortex-Genie 2	96-well	1800 rpm
	384-well	2000 rpm
Eppendorf MixMate	384-well	2600 rpm
IKA MS3 Digital	Either	2000 rpm‡
IKA Vortex 3	Either	Setting 5§
Taitec MicroMixer E-36	Either	Maximum
Union Scientific Vertical Shaker#	Either	Setting 100

‡ Set the vortexer to Mode B. See "Mixing with the IKA MS 3 Digital Vortexer" on page 52 for instructions.

§ Use the maximum setting that does not cause the vortexer to "walk" across the bench.

Add any additional plates to meet mass requirements. (See "Mixing with the Union Scientific Vertical Shaker" on page 50 for information.)

It is recommended to pause vortexing after 1 minute and examine the wells to verify that the contents are well mixed.

7. In a swinging-bucket centrifuge, spin the plate at 1000 × *g* for 2 minutes.

   At this point you can analyze the samples or store the sealed plates for later analysis (see "Storage of Purified Samples" on page 27).

8.	Place the reaction plate in the instrument. **IMPORTANT!** Do not heat-denature or use Hi-Di™ Formamide with samples containing BigDye XTerminator reagents. • **For 384-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-Avant, 3130/3130*xl* or 310 Genetic Analyzer instrument** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. • **For 96-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-Avant or 3130/3130*xl* instrument, with Data Collection software v2.0 or later**– Remove the adhesive film or heat seal, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-Avant instrument with Data Collection software v1.x or 310 Genetic Analyzer** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument.
9.	In the Data Collection Software Plate Editor, select the appropriate run module: • **For most plate and instrument combinations** – Select the BigDye XTerminator run module for your instrument and plate type (see Appendix A, "Run Modules"). • **If you transferred the supernatant to a clean plate after vortexing** – Select a standard run module.
10.	Run the plate.

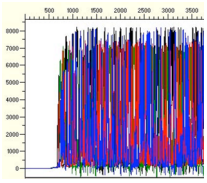
Storage of Purified Samples

Samples purified with the BigDye® XTerminator™ Purification Kit can be stored under the following conditions:

- **Room temperature storage –** Sample plates sealed with heat seal film, adhesive film, or septa for up to 48 hours at room temperature (20 to 25 °C).
- **Refrigerated storage** – Sample plates sealed with heat seal film or adhesive film for up to 10 days at 4 °C.
- **Frozen storage** – Sample plates sealed with heat seal film or adhesive film for up to 10 days at –20 °C.

Troubleshooting

Observation	Possible Cause	Recommended Action
Dye artifacts (blobs) at beginning of electropherogram	Incomplete mixing during purification due to: • Non-recommended vortexer used	Use recommended mixing equipment.
	• Vortexer speed too low • Mixing time insufficient • Mixing not uniform across plate	• Follow mixing guidelines in Appendix D. • Stop vortexer after 1 minute and examine wells to verify that mixing is complete.
	Insufficient SAM Solution	Use 4.5 parts SAM™ Solution for every 1 part XTerminator™ Solution.
	SAM Solution cloudy	See “SAM Solution forms precipitates” on page 34.
	Leaking plate seal	• If using clear adhesive film, follow the sealing protocol in Appendix C. • If using a heat seal, increase the time and temperature for the heat-sealing step.
	Insufficient DNA template in reaction	Increase DNA template concentration (see Appendix B).
	Insufficient BigDye® XTerminator™ reagent due to: • Incorrect volume added	Use a volume of XTerminator Solution equal to the volume of the reaction to be purified.
	• BigDye XTerminator reagent not properly suspended during dispensing • Pipette tip with incorrect bore size used	Follow guidelines for mixing and dispensing XTerminator Solution on page 15.
	• XTerminator Solution pipetted from the very top of the container	When pipetting XTerminator Solution, be sure the pipette tip is below the surface of the liquid.

Observation	Possible Cause	Recommended Action
Offscale signal 	Excessive DNA injected due to: • Extension product concentration too high	• Reinject samples as soon as possible. Second injections from samples with offscale data frequently give acceptable onscale data. • Decrease DNA template concentration (see Appendix B).
	• Injection time too long • Injection voltage too high	Decrease injection time and/or voltage.
Weak signal	Insufficient DNA injected due to: • Extension product concentration too low	Increase DNA template concentration (see Appendix B).
	• Failed sequencing reaction	Repeat sequencing reaction.
	• Injection time too short • Injection voltage too low	Increase injection time and/or voltage.
	Incorrect injection height due to: • Non-BigDye XTerminator Purification Kit run module used	Use the correct BigDye XTerminator Purification Kit run module (see Appendix A).
	• Excessive XTerminator Solution causing incorrect bed height	Use correct amount of XTerminator Solution.
	• BigDye XTerminator Purification Kit run module offset not updated after autosampler recalibration	Run the Update BDx Utility to update run module offset values (see Appendix A).
	• Non-supported plate	Use supported plates.

Observation	Possible Cause	Recommended Action
Weak signal	Incomplete desalting due to: • Insufficient XTerminator Solution used	Use a volume of XTerminator Solution equal to the volume of the reaction to be purified.
	• Incomplete mixing	• Follow guidelines for mixing and dispensing XTerminator Solution on page 15. • Follow mixing guidelines in Appendix D. • When pipetting XTerminator Solution, be sure the pipette tip is below the surface of the liquid.
	Insufficient volume in well due to: • Insufficient SAM Solution used during purification	Use 4.5 parts SAM Solution for every 1 part XTerminator Solution.
	• Pipetting with wide-bore pipette tips created by manually cutting conventional pipette tips	Purchase and use wide-bore (orifice >1.0 mm) pipette tips.
	• Leakage of seal during purification	• If using clear adhesive film, follow the sealing protocol in Appendix C. • If using a heat seal, increase the time and temperature for the heat-sealing step.
	Capillary electrophoresis system issues, such as: • Old array • Expired polymer • Old buffer • Wrong mobility file	Troubleshoot capillary electrophoresis system.
	XTerminator Solution overheated	Do not expose BigDye XTerminator reagents to temperatures above 25 °C.
	Hi-Di™ Formamide used or sample heat denatured	Follow the protocol and do not use Hi-Di™ Formamide or heat denature the sample.

Observation	Possible Cause	Recommended Action
Delayed analysis start point (Slow migrating samples/late starts)	Unlabeled DNA template injected into capillary, interfering with proper separation of extension products due to: • Too much template in sequencing reaction • High-molecular-weight template used in sequencing reaction	• Reinject samples as soon as possible. Second injections from samples with offscale data frequently give acceptable onscale data. • Decrease template concentration in DNA sequencing reaction (see Appendix B).
	• Injection time too long • Injection voltage too high	Decrease injection time and/or voltage.
Poor resolution/short read lengths	Offscale signal	See “Offscale signal” on page 29.
	Degradation of purified extension products due to: • Samples stored for >48 hours at room temperature • Samples stored for >10 days in refrigerator or freezer	Prepare fresh sample and analyze within 48 hours or store appropriately.
	• Stored samples improperly sealed	Seal samples with adhesive or heat seal film for storage.
	• Insufficient SAM Solution used during purification	Use 4.5 parts SAM Solution for every 1 part XTerminator Solution.
	• Old or improperly sealed septa used	Use fresh septa mat when running plates on DNA sequencer.
	Insufficient signal strength	See “Weak signal” on page 29.
	Capillary electrophoresis system issues, such as: • Old array • Expired polymer • Old buffer • Wrong mobility file	Troubleshoot capillary electrophoresis system.
	Injection of high-molecular-weight template DNA	See “Delayed analysis start point (Slow migrating samples/late starts)”, above.

Observation	Possible Cause	Recommended Action
Loss of small extension products	Over-exposure of purified extension products to BigDye XTerminator reagent due to: • Mixing during purification in great excess of recommended time	Follow the protocol.
	• Sample stored for >48 hours at room temperature • Sample stored for >10 days in refrigerator or freezer.	Prepare fresh sample and analyze within 48 hours, or store appropriately.
	Degradation of BigDye XTerminator reagents due to: • Exposure of reagents to high temperature (>30 °C), even briefly, before or during purification • Exposure of reagents to room temperature for more than 24 hours	Repurify samples and ensure that samples remain at room temperature during processing.
	• Reagent shelf-life exceeded	Discard and use fresh BigDye XTerminator reagents.
	Protocol not optimized for small molecule recovery.	• Use POP-6™ polymer for capillary electrophoresis. • Use the BigDye® Terminator v.1.1 Cycle Sequencing Kit. • Load reaction plates as soon as possible after centrifuging.

Observation	Possible Cause	Recommended Action
No signal	Incorrect injection height due to: • Non-BigDye XTerminator Purification Kit run module used	Use the correct BigDye XTerminator Purification Kit run module (see Appendix A).
	• Excessive XTerminator Solution, causing incorrect bed height	Use correct amount of XTerminator Solution.
	• BigDye XTerminator Purification Kit run module offset not updated after autosampler recalibration	Run the Update BDx Utility to update run module offset values (see Appendix A).
	• Nonsupported plate	Use supported plates.
	Insufficient volume in well due to: • Insufficient SAM Solution used during purification	Use 4.5 parts SAM Solution for every 1 part XTerminator Solution.
	• Leakage of seal during purification	• If using clear adhesive film, follow the sealing protocol in Appendix C. • If using a heat seal, increase the time and temperature for the heat-sealing step.
	Degraded BigDye XTerminator reagent due to extreme exposure to heat	See “Loss of small extension products” on page 32.
	Extremely delayed analysis start point; slow migrating samples	See “Delayed analysis start point (Slow migrating samples/late starts)” on page 31.
	Failed sequencing reaction	Repeat the sequencing reaction.
	Capillary electrophoresis system issues, such as: • Old array • Expired polymer • Old buffer • Wrong mobility file	Troubleshoot the capillary electrophoresis system.
	Hi-Di™ Formamide used, or sample heat denatured	Follow the protocol and do not use Hi-Di™ Formamide or heat denature the sample.

Observation	Possible Cause	Recommended Action
SAM™ Solution cloudy	SAM Solution forms precipitates	• Gently warm SAM Solution to 25 to 37 °C and mix to redissolve. Allow to cool to room temperature before using. Warm SAM Solution produces poor results. **Note:** If SAM Solution is mixed vigorously, allow foam to settle before using. • Store SAM Solution at room temperature.
Leaking wells	Plate poorly sealed during purification.	• If using clear adhesive film, follow the sealing protocol in Appendix C. • If using a heat seal, increase the time and temperature for the heat-sealing step.
Nonuniform purification results across plate	Nonuniform mixing across plate due to: • Non-recommended vortexer used	Use recommended mixing equipment.
	• Vortexer speed too low	• Follow mixing guidelines in Appendix D. • Stop vortexer after 1 minute and examine wells to verify that mixing is complete.
	Nonuniform dispensing of BigDye XTerminator reagent across plate due to: • XTerminator Solution not completely suspended during dispensing • Pipette tip with incorrect bore size used • Premix not properly suspended during dispensing	Follow guidelines for mixing and dispensing XTerminator Solution (see page 15) or premix (see pages 20 and 22).
XTerminator Solution and/or SAM™ Solution frozen	Reagents stored improperly.	Gently thaw reagents in refrigerator. Allow reagents to thaw completely before mixing.

Observation	Possible Cause	Recommended Action
XTerminator Solution too thick to pipette	Improper pipetting technique due to: • XTerminator Solution not completely suspended during dispensing • Pipette tip with incorrect bore size used	Follow guidelines for mixing and dispensing XTerminator Solution on page 15.
	Evaporation	• Discard and use fresh XTerminator Solution. • Seal the XTerminator Solution storage bottle properly. • Implement evaporation control for extended dispensing.

Appendix A: Run Modules

Overview

This appendix provides:

- A list of BigDye® XTerminator™ Purification Kit run modules
- Instructions for installing, updating, and uninstalling BigDye XTerminator Purification Kit run modules

The BigDye XTerminator run modules adjust the injection height to removed only the supernatant containing the purified dye labeled extension products to inject in the sequencer.

When analyzing purified samples, select the run module appropriate to your system components from Table 1.

Note: If you transfer the supernatant to a clean plate after centrifuging, refer to the instrument User Guide for the appropriate run module.

Table 1 BigDye® XTerminator™ Purification Kit run modules

Instrument	Array length (cm)	Polymer	KB QV20 LOR	Appropriate Software Run Module
3730/3730*xl* DNA Analyzer	50	POP-7™	900	BDx_XLRSeq50_POP7
			850	BDx_LongSeq50_POP7
			700	BDx_FastSeq50_POP7
	36	POP-7™	700	BDx_StdSeq36_POP7
			550	BDx_RapidSeq36_POP7

Table 1 BigDye® XTerminator™ Purification Kit run modules

Instrument	Array length (cm)	Polymer	KB QV20 LOR	Appropriate Software Run Module
ABI PRISM® 3100/3100-*Avant*, 3130/3130*xl* Genetic Analyzer	80	POP-7™	950	BDx_LongSeq80_POP7
		POP-4™	700	BDx_LongSeq80_POP4
	50	POP-7™	850	BDx_StdSeq50_POP7
		POP-6™	600	BDx_StdSeq50_POP6
		POP-4™	600	BDx_StdSeq50_POP4
		POP-7™	700	BDx_FastSeq50_POP7
	36	POP-7™	600	BDx_RapidSeq36_POP7
		POP-6™	500	BDx_RapidSeq36_POP6
		POP-7™	500	BDx_UltraSeq36_POP7
		POP-4™	400	BDx_UltraSeq36_POP4

Installing Run Modules

Applied Biosystems provides BigDye XTerminator Purification Kit run modules for the 3100/3100-*Avant,* 3130/3130*xl,* and 3730/3730*xl* Data Collection Software. Use these run modules whenever you run a plate containing BigDye XTerminator reagents. The modules adjust the sample injection height to prevent injection failures.

IMPORTANT! If you pipette the purified supernatant from a reaction plate to a new reaction plate, use a standard run module instead of a BigDye XTerminator run module to inject from the new plate.

1. Download the Support Files Installer:
   a. Go to **www.appliedbiosystems.com**
   b. Click **Support**, then **Software Downloads**.
   c. In the list, select **BigDye® XTerminator™ Purification Kit**.
   d. Click the PC link next to the BigDye XTerminator Support Files Installer to download the installer file.
2. Power on the instrument (solid green light state) and the data collection computer.

3. Quit the data collection software if it is running.
4. Double-click **BDx_Installer.exe**, then follow the prompts in the Support Files Installer wizard.

Updating Run Modules

You need to update the run modules with new calibration values whenever the sequencer autosampler is recalibrated. You run the Update BDx Utility to update the BDx run modules.

1. Quit the Data Collection software, but do not shut down the data collection computer.
2. Power on the instrument (solid green light state).
3. Select **Start ▸ Programs ▸ Applied Biosystems ▸ BDx Utility ▸ Update BDx Utility**.

Uninstalling Run Modules

Note: Uninstalling the BDx run modules also removes any instrument protocols and plate records that use a BDx run module. Run folders containing ab1 files that have been extracted to the hard drive are not removed.

1. Power on the instrument (solid green light state) and the data collection computer.
2. Quit the data collection software if it is running.
3. Select **Start ▸ Programs ▸ Applied Biosystems ▸ BDx Utility ▸ Uninstall BDx**.
4. Follow the prompts in the Uninstaller wizard.

Appendix B: DNA Quantity Guidelines

DNA sequencing reactions purified with the BigDye® XTerminator™ Purification Kit result in high signal strength when analyzed on a DNA sequencer. When you prepare sequencing samples for purification with the BigDye XTerminator reagents, you may need to decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on scale during analysis.

When you plan to purify samples with BigDye XTerminator kit, use the following table as a guide to the amount of template DNA for the initial cycle sequencing.

- Start at the lower end of the range. If low signal strength is observed, increase the quantity.
- If offscale signal is observed, decrease the template concentration.

IMPORTANT! If you decrease the template concentration, also decrease the amount of any template controls proportionately. For example, if you run a pGEM control, you should dilute it 1:2 or 1:4 and add only 1 to 2 µL.

Table 1 DNA template quantity guidelines

Template Type	DNA Quantity/Reaction (ng)
PCR products	
100 to 200 bp	0.5 to 3
200 to 500 bp	1 to 10
500 to 1000 bp	2 to 20
1000 to 2000 bp	5 to 40
>2000 bp	10 to 50
Other types of template	
Single-stranded DNA	10 to 50

Table 1 DNA template quantity guidelines *(continued)*

Template Type	DNA Quantity/Reaction (ng)
Double-stranded DNA	50 to 300
Cosmid or BAC DNA	200 to 1,000
Bacterial genomic DNA	1,000 to 3,000

Appendix C: Plate Sealing Procedure

IMPORTANT! Always handle the MicroAmp™ Clear Adhesive Film by the end-tabs.

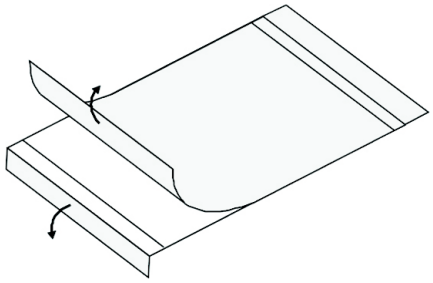
To use the Clear Adhesive Film for the BigDye® XTerminator™ purification protocol:

1. Place the loaded reaction plate in the appropriate Splash Free Support Base and wipe away any drops of XTerminator™ Solution or SAM™ Solution that may have spilled on the sealing surface of the plate.

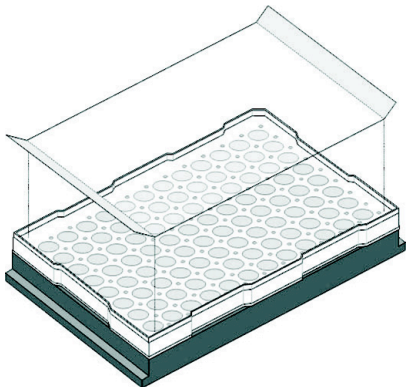
   Note: Any liquid on the surface of the plate before the Clear Adhesive Film is applied causes inadequate sealing between the film and plate and may result in leakage.

2. Remove a single Clear Adhesive Film from the bag. Notice the two ends of the film are without adhesive.

3. Peel back the white protective backing from the center sealing surface in one swift movement, taking care not to touch the center sealing surface with your fingers.



4. With the Clear Adhesive Film positioned over the reaction plate, gently lower the film onto the plate. Handle only the non-adhesive ends of the film.

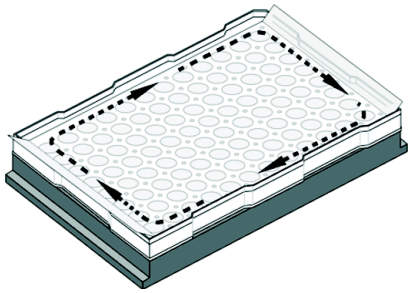


5. While applying firm pressure, drag the applicator slowly across the Clear Adhesive Film, side to side and top to bottom, to ensure good contact between the film and the plate over the entire surface of the reaction plate.

   Note: Applicators are available separately (PN 4333183).

6. Repeat step 5. With firm pressure, drag the applicator at least 5 times side to side and 5 times top to bottom, with firm pressure, over the Clear Adhesive Film for a leak-free seal.

7. Using firm pressure, run the edge of the applicator along all four sides of the outside border of the Clear Adhesive Film following the lines in the figure below.



8. Using a paper tissue and firm pressure, run a finger across each column and row of the plate to ensure each well is tightly sealed.

   Optionally, place the plate in a thermal cycler, close the lid and program the thermal cycler to hold 20 °C for 5 minutes.

   Ensure the sample block temperature is less than 25 °C.

   The heat and pressure from the thermal cycler lid completes the sealing process.

   Continue with the purification protocol.

Appendix D: Mixing Guidelines

Mixing with the Scientific Industries Digital Vortex-Genie 2

- If needed, replace the stock rubber feet with the shock-absorbing feet (see Table 4 on page 9). To remove the stock rubber feet, turn the vortexer upside down and remove the feet using a 1/4-in. hex driver. Use the 1/4-in. hex driver to install the shock-absorbing feet.
- If needed, attach the Microplate Adapter for Applied Biosystems (PN SI-0513) to the vortexer. When installing the adapter, push from the center to avoid bending the adapter.
- Secure the plate to the adapter using the elastic bands as shown in Figure 1 on page 48.
- Be sure that the plate is well secured to the platform before starting the vortexer.
- Take care not to disrupt the plate sealing film when attaching the plate to the platform. If the seal is disturbed, remove the seal and replace with a fresh seal.
- If you use the Digital Vortex-Genie 2 with the recessed platform adapter (PN SI-0513), the position of the elastic band varies, depending on the type of plate you use:
  - For 96-well plates, see Figure 2 on page 48.
  - For 384-well plates, see Figure 3 on page 49.

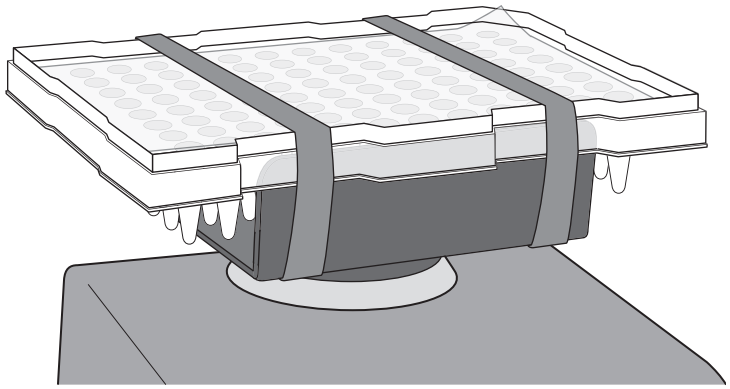


Figure 1 Elastic band positions for either type of plate with the Microplate Adapter for Applied Biosystems

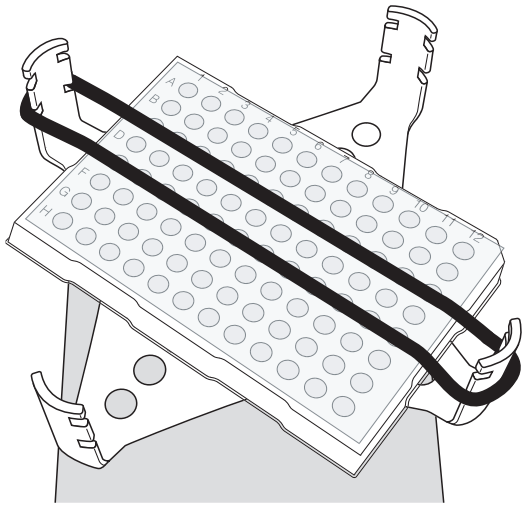


Figure 2 Elastic band position for 96-well plates with the recessed platform adapter

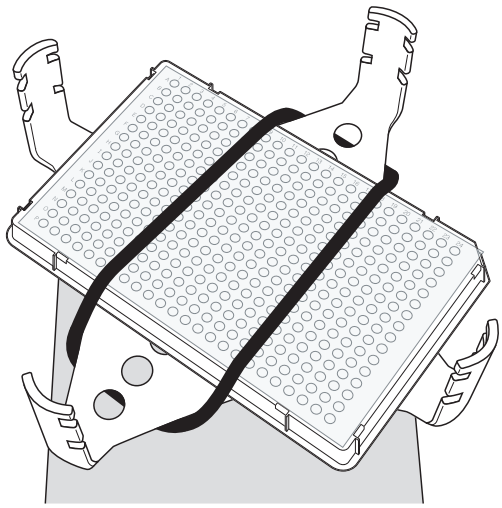


Figure 3 Elastic band position for 384-well plates with the recessed platform adapter

Mixing with the Union Scientific Vertical Shaker

Note: The Union Scientific vertical shaker can be noisy when it shakes. If the shaker is placed on the floor, it operates more quietly.

Loading Additional Plates

The Union Scientific vertical shaker is designed to operate optimally with a total sample weight (plates, samples, and bases) between 450 and 2500 g.

If the mass of your filled plates is less than 450 grams, add additional plates to the vertical shaker until the total mass is greater than 450 grams.

Use Table 1 to calculate the mass of your filled plates and any additional masses needed for the vertical shaker. See the example calculation after Table 1.

Table 1 Masses of reaction plates and reagents

Item		Mass (g)
MicroAmp™ Optical 384-Well Reaction Plates‡		
Empty		18
Reagent mass per well		0.033
Reagent mass per plate		12
Total mass of filled plate, including reagents		30
MicroAmp™ Optical 96-Well Reaction Plates		
Empty		22
MicroAmp™ Splash Free 96-Well Base		68
10-µL reactions	Reagent mass/well	0.065
	Reagent mass/plate	6
	Total mass of filled plate	28
20-µL reactions	Reagent mass/well	0.130
	Reagent mass/plate	12
	Total mass of filled plate	34

‡ 384-well plates are used without bases. 96-well plates require bases.

Example Calculation

Suppose you have three 96-well plates with 10-µL reactions.

The total mass of the plates, the reagents, and the splash-free bases is 288 grams [(3 × 68) + (3 × 28) = 288 grams].

162 more grams are required to make a total mass of 450 grams. (450 – 288 = 162 grams).

Any combination of plates and bases that total to at least 162 grams is sufficient, such as:

- Three empty bases [(3 × 68) = 204 grams]

  or

- Two plates, each with a base [(2 × 22) + (2 × 68) = 180 grams]

Mixing with the IKA MS 3 Digital Vortexer

IMPORTANT! The vortexer must be set to Mode B before use. See "Set the Vortexer Mode" on page 54.

- In regions outside North America, an electrical plug adapter may be required.
- The IKA Microtiter Attachment (PN MS 3.4) is supplied with the IKA MS 3 vortexer and is required for 96- or 384-well plates (see Figure 4).

  Follow the instructions in the MS 3 Users Manual to install the microtiter adapter.
- Follow the loading instructions appropriate for your plate:
  - For 96-well plates, secure the front and rear of the 96-well plate with elastic bands, as shown in Figure 5.
  - For 384-well plates, flex the plate slightly lengthwise and slip the front and rear of the plate under the tabs (see Figure 6).

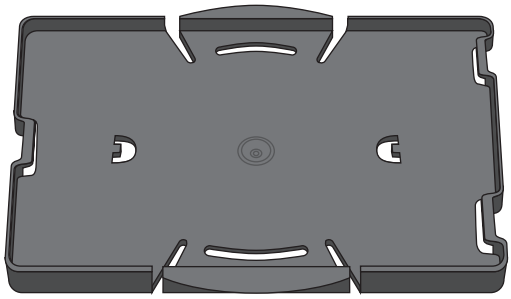


Figure 4 IKA MS3 Microtiter Attachment

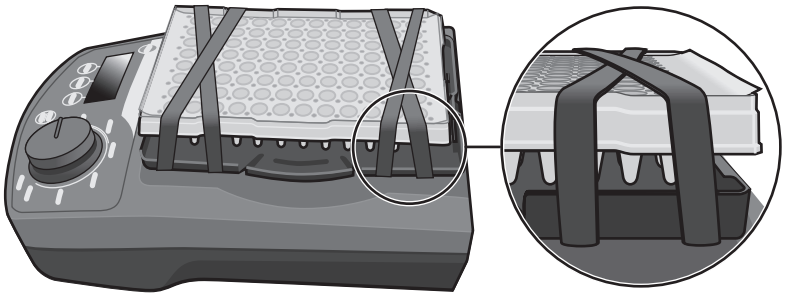


Figure 5 IKA MS 3 with a 96-well plate, showing placement of the elastic bands

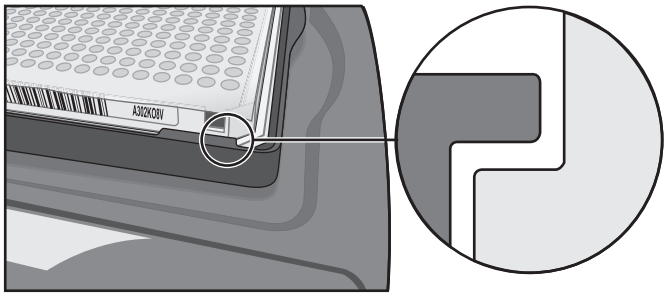


Figure 6 Close-up of the IKA MS3 with a 384-well plate, showing a tab holding the plate

Set the Vortexer Mode

The MS 3 has two speed ranges: Mode A (low-speed) and Mode B (high-speed). For the BigDye® XTerminator™ Purification Kit, you need to set the vortexer to Mode B.

Each time the vortexer powers on, it is in Mode A. To set the mode to Mode B each time you use the vortexer, hold down the **Start/Stop** button, then press the **Power** button (see Figure 7).

This changes the vortexer to Mode B, with a top speed of 3000 rpm.

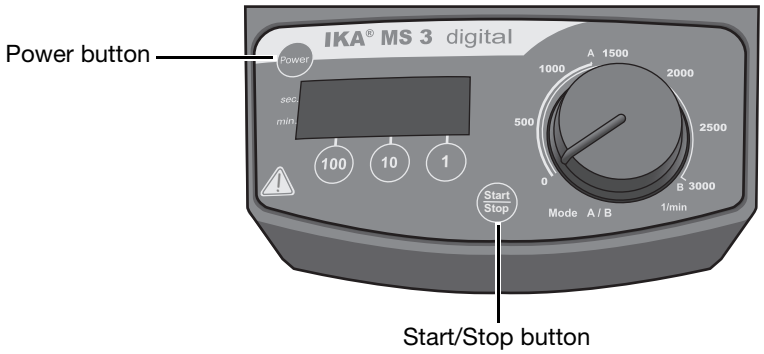


Figure 7 IKA MS 3 Digital front panel

Mixing with the Taitec MicroMixer

1. Load the plates
   - For 96-well plates, put the plate into a MicroAmp™ 96-Well Base (PN N8010531), then use the white levers to clamp the plate onto the MicroMixer.
   - For 384-well plates, use the white levers to clamp the plate directly onto the MicroMixer.
2. Set the Range switch to **High Speed**.
3. Turn the Speed dial all the way to the right, for maximum speed.

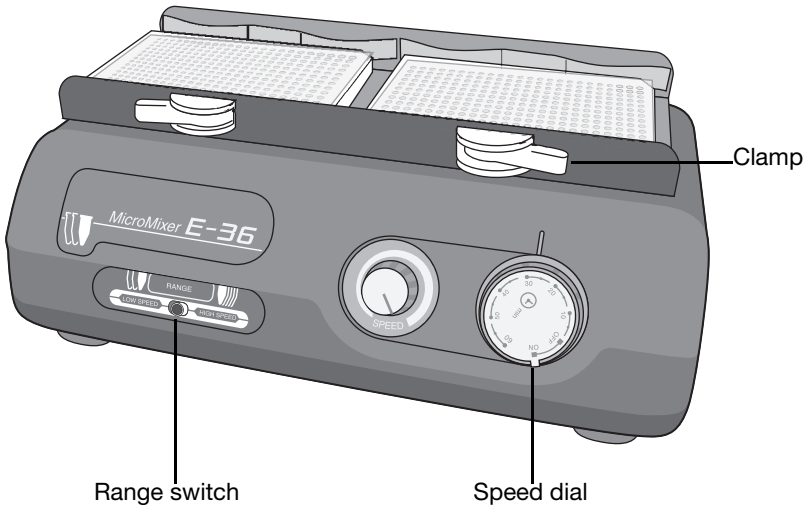


Figure 8 Taitec MicroMixer control panel

Appendix E: Performing Purification – Robotic Pipetting

Overview

This appendix provides materials and instructions for purification with the BigDye® XTerminator™ Purification Kit using robotic pipettors.

If you use robotic pipetting:

1. Prepare a mixture of the two BigDye® XTerminator™ reagents (referred to here as "premix").
2. Perform the purification, according to the procedure below.

Materials

Table 1 Robotic pipetting systems and accessories

Item	Source
Biomek FX/NX robotic pipetting system, with either: • 96-mandrel, 200-µL head • 384-mandrel, 30-µL head	Beckman Coulter 4300 N. Harbor Boulevard P.O. Box 3100 Fullerton, CA 92834-3100 Tel: (800) 742-2345 Fax: (800) 643-4366 www.beckmancoulter.com
Bubble Paddle Stirrer for Biomek FX/NX (PN VP OK21Kit)	V&P Scientific, Inc. 9823 Pacific Heights Boulevard Suite T San Diego, CA 92121 Tel: (858) 455-0643 Fax: (858) 455-0703 sales@vp-scientific.com www.vp-scientific.com

Table 1 Robotic pipetting systems and accessories *(continued)*

Item	Source
Pipette tips From Beckman Coulter: • For the 96-mandrel head, 96-well tips (PN 717252) • For the 384-µL mandrel head: – 384-well 30-µL tips (PN 719223) – 384-well 50-µL tips (PN A22288) From Axygen: • For the 96-mandrel head, 96-well tips (PN FX-250-L-R-S) • For the 384-mandrel head: – 384-well 30-µL tips (PN FX-384-L-R-S) – 384-well 50-µL tips (PN FX-384-XL-L-R-S)	Beckman Coulter (see above) Axygen Scientific Inc. 33210 Central Ave. Union City, CA 94587 Tel: (510) 494-8900 Fax: (510) 494-0700 info@axygen.com www.axygen.com

Performing Purification

1. Prepare the appropriate amount of premix, as described in "Preparing the Premix" on page 21.

2. Program the robotic pipettor with the mode, speed, and air gap:

   a. Set the mode:

Pipetting Head Channels	Wells/Plate	Mode
96	96	Single aspiration/ single dispense
96	384	Single aspiration/ multi-dispense
384	384	Single aspiration/ single dispense

   b. Set the aspiration speed to **30 µL/sec** and the dispense speed to **90 µL/sec**.

   c. Set the pipettor to preaspirate at least 5 µL of air (for blowout after dispensing).

3.	Continue programming the robotic pipettor: a. Set the position of the tips for aspiration, based on the stirring mode your pipettor supports: – If stirring can be stopped during aspiration, position the tips at least 2 mm above the trough bottom. – If stirring cannot be stopped during aspiration, position the tips at least 10 mm above the stirring mechanism. **Note:** Damage to the pipetting head can occur if the tips contact the stirring mechanism. Verify that sufficient clearance is maintained between the pipetting head and the stirring mechanism. b. Set the blowout volume. **Note:** If you use a 96-well pipetting head to dispense into 384-well plates, you can use multi-dispensing to dispense into all quadrants from a single aspiration, followed by a blowout step after the last dispense. c. Set the dispense height no more than 2 mm lower than the top of the reaction plate well and enable tip touch.
4.	Mix and then transfer the premix to the reservoir.
5.	Run the method to aspirate and dispense the premix. Add premix to the reservoir as needed.
6.	Seal the plate using: • A heat seal at 160 °C for 1.5 seconds (see Table 6 on page 12 and the note below). *or* • MicroAmp™ Clear Adhesive Films (follow the procedure in Appendix C). Verify that each well is sealed. **IMPORTANT!** If you use a 3730/3730*xl* instrument and plan to use direct injection without a septa mat, only Applied Biosystems Heat Seal Film for Sequencing and Fragment Analysis Sample Plates (PN 4337570) is supported.

7. Place the plate in a recommended vortexer (see Tables 4 and 5, starting on page 9). Use the appropriate adapter, if recommended.

8. Vortex the reaction plate for 30 minutes, using the following conditions:

Vortexer	Plate Type	Speed
Digital Vortex-Genie 2	96-well	1800 rpm
	384-well	2000 rpm
Eppendorf MixMate	384-well	2600 rpm
IKA MS3 Digital	Either	2000 rpm‡
IKA Vortex 3	Either	Setting 5§
Taitec MicroMixer E-36	Either	Maximum
Union Scientific Vertical Shaker#	Either	Setting 100

‡ Set the vortexer to Mode B. See "Mixing with the IKA MS 3 Digital Vortexer" on page 52 for instructions.

§ Use the maximum setting that does not cause the vortexer to "walk" across the bench.

Add any additional plates to meet mass requirements. (See "Mixing with the Union Scientific Vertical Shaker" on page 50 for information.)

Note: It is recommended to pause vortexing after 1 minute and examine the wells to verify that the contents are well mixed.

9. In a swinging-bucket centrifuge, spin the plate at 1000 × *g* for 2 minutes.

   At this point you can analyze the samples or store the sealed plates for later analysis (see "Storage of Purified Samples" on page 27).

10.	Place the reaction plate in the instrument. **IMPORTANT!** Do not heat-denature or use Hi-Di™ Formamide with samples containing BigDye XTerminator reagents. • **For 384-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant*, 3130/3130*xl* or 310 Genetic Analyzer instrument** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument. • **For 96-well plates and a:** – **3730/3730*xl* instrument** – If the plate is heat sealed, place it directly in the instrument. Otherwise, remove the adhesive film, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant* or 3130/3130*xl* instrument, with Data Collection software v2.0 or later**– Remove the adhesive film or heat seal, cover the plate with a septa mat, then place it in the instrument. – **3100/3100-*Avant* instrument with Data Collection software v1.x or 310 Genetic Analyzer** – Remove the adhesive film or heat seal, transfer 10 µL of the supernatant to a clean plate, cover the plate with a septa mat, then place it in the instrument.
11.	In the Data Collection Software Plate Editor, select the appropriate run module: • **For most plate and instrument combinations** – Select the BigDye XTerminator run module for your instrument and plate type (see Appendix A, “Run Modules”). • **If you transferred the supernatant to a clean plate after vortexing** – Select a standard run module.
12.	Run the plate.

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera businesses.

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: +1 650.638.5884

07/2007

Applied Biosystems

Part Number 4374408 Rev. C