Advances in Fast PCR Contribute to a Fast Resequencing Workflow

Fast PCR and Fast BigDye® Cycle Sequencing using AmpliTaq Gold® Fast PCR Master Mix, UP and the Veriti™ Thermal Cycler

- Reduce time to results – complete your sequencing experiments in about 4 hours
- Use less DNA template – Fast procedures make efficient use of reagents and sample
- Maintain high data quality – improve time efficiencies without sacrificing sequencing read-length or accuracy

Introduction

Amplification of DNA samples by PCR is an important first step for many studies using resequencing workflows. Researchers have long faced challenges of maintaining high-quality sequence results, while simultaneously reducing analysis times and the consumption of reagents, plastics, and limited DNA samples.

Fast PCR is a new technology that dramatically decreases run times by using a combination of protocol changes, fast instruments, and fast reagents. These innovations enable a faster ramp rate and ensure thermal uniformity at top speeds, which decreases both PCR and cycle sequencing run times, and increases the number of runs that can be performed each day.

With Fast PCR technologies, resequencing workflows that once could take 11 hours or more to perform can now be completed in about 4 hours. This decrease in protocol time increases productivity, allowing you to focus your time on results interpretation and decision-making. Additionally, Fast procedures reduce the amount of sample and reagents needed to perform experiments, saving money and reducing sample consumption. Here, a protocol is presented for Fast PCR combined with fast cycle sequencing (see Fast Resequencing Workflow on page 2).

Fast PCR Products Specifically for Sequencing

Applied Biosystems has designed products for Fast PCR specifically optimized for superior performance in subsequent sequencing reactions. AmpliTaq Gold® Fast PCR Master Mix, UP is designed to produce amplicons ideally suited for the generation of high quality sequencing data. When compared to the

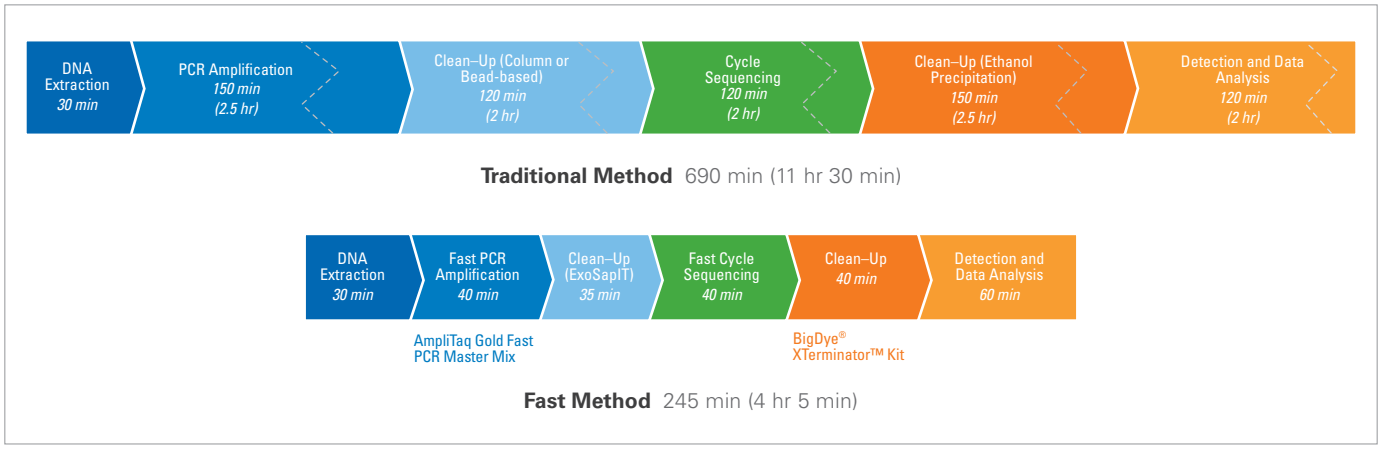


Figure 1. Estimated Time Requirements for Traditional vs. Fast Resequencing. Applied Biosystems products and protocols speed time to results by reducing the time required for each step in the process at each step. The Fast PCR and Fast Resequencing Workflow can be accomplished during a single workday, empowering you to do more in less time.

Sequencing Parameter	Fast Method	Traditional Method
Auto Read Length	517	492
Continuous Read Length KB-QV30	513	506
KB-QV30 Count	510	503
Average Background (peak-under-peak) Detected	4.1%	4.6%

Table 1. Performance of Key Sequencing Parameters comparing Traditional vs. Fast Resequencing Workflows. Data shown represents the average of six amplicons (HM2-1, HM2-10, HM2-16, HM2-2, HM2-4, HM2-8) with an average length of 548 bp. The data demonstrates that the AmpliTaq Gold® Fast PCR Master Mix, UP outperforms the traditional master mix by yielding amplicons that produce lower background (peak-under-peak) percentages while maintaining longer read lengths and high KB-QV30, a measure of base-call quality.

standard AmpliTaq Gold® PCR Master Mix, the Fast PCR Master Mix produces similar and even higher quality templates for sequencing, yields longer continuous read lengths, lower background (percent of peak-under-peak products), and higher KB-QV30 basecall rates. Designed for use with your existing primers, AmpliTaq Gold Fast PCR Master Mix, UP replaces the current master mix in existing protocols for high-sensitivity and reproducible results – as few as 10 copies of a single gene can be detected in 10 ng of genomic DNA. When combined with the Veriti™ 96-Well Fast Thermal Cycler, and MicroAmp® Fast 96-Well Reaction Plates, the master mix allows for reduction in PCR reaction times to as little as 40 minutes for amplification of a 500 bp genomic DNA fragment.

Fast Sequencing Workflow in About 4 Hours

In addition to accelerating the PCR amplification step of the resequencing workflow, some of the same components designed for Fast PCR can also be used in rapid cycle sequencing procedures. Applied Biosystems has developed a modified Fast cycle sequencing protocol that utilizes the standard BigDye® Terminator Cycle Sequencing Kit v1.1 reagents along with the Veriti 96-Well Fast Thermal Cycler and MicroAmp Fast 96-Well Reaction Plates. This combination of reagents, consumables, and instrumentation produce equivalent, and often improved, sequencing results when combined with Fast PCR amplification (Table 2). In addition to the significant improvement in cycle sequencing time savings, sequencing reagents and template DNA quantity requirements are also reduced, relative to traditional cycle sequencing protocols, while maintaining high-quality sequencing reads. Now you can go from sample to sequencing results in about 4 hours. For a comparison of traditional and fast sequencing times, see Figure 1.

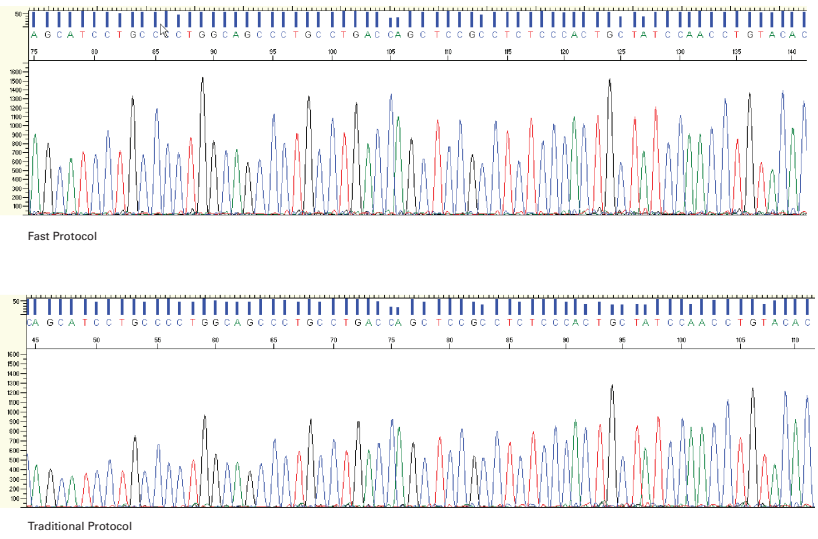


Figure 2. Electropherogram of Sequenced PCR Products Generated from the AmpliTaq Gold® Fast PCR Master Mix, UP and the AmpliTaq Gold® PCR Master Mix. A 600 base pair sample was cycle sequenced on the Veriti™ 96-Well Fast Thermal Cycler using BigDye® Terminator v1.1 Cycle Sequencing Kit and run on the Applied Biosystems 3130*x*/ DNA Analyzer demonstrating comparable results.

Fast and Traditional Methods Produce Equivalent Results

Using the Fast PCR and Fast Sequencing protocols and products as described, results similar to the 11-hour, traditional workflows are achieved in less than half the time. Figure 2 shows electropherograms of sequenced amplicons generated using the fast AmpliTaq Gold Fast PCR Master Mix, UP and protocol versus the traditional AmpliTaq Gold PCR Master Mix and protocol that demonstrate very similar results.

The Fast Resequencing Workflow

The following pages provide an outline of the Fast Resequencing Workflow. Specific protocol recommendations for the fast PCR amplification step and fast cycle sequencing step are provided in detail. Additionally, recommendations for DNA extraction, sample clean-up, capillary electrophoresis, detection, and data analysis are provided, yielding an easy-to-follow complete workflow for successful, fast resequencing. For a complete listing of support protocols for the Fast Resequencing Workflow, please visit www.appliedbiosystems.com/fastsolutions.

1. DNA Extraction.

The Fast Resequencing Workflow begins with DNA extraction. The extraction method can vary and is usually dependent upon the type and total number of samples to be processed. The **MELT™ Total Nucleic Acid Isolation System** uses magnetic bead technology for the isolation of DNA and RNA from many different sample types. This technology efficiently removes PCR inhibitors, is easily scaled for large reactions, and is suitable for automation.

Also available from Applied Biosystems is the **RecoverAll™ Total Nucleic Acid Isolation Kit**. Designed to extract total nucleic acid from formaldehyde or formaldehyde-fixed, paraffin-embedded (FFPE) tissues, up to four 20 µm sections, or up to 35 mg of unsectioned core samples, can be processed per reaction.

2. Primer Design, Fast PCR Amplification & Clean-Up.

After DNA extraction, the region of interest is amplified by PCR. The **VariantSEQr® Resequencing System** is a collection of PCR primers for identifying sequence variations in hundreds of human genes quickly and easily. It includes pre-designed PCR primer sets and protocols. VariantSEQr primer pairs have been designed for more than 420,000 amplicons, representing nearly 16,000 human genes. **AmpliTaq Gold® Fast PCR Master Mix, UP** quickly produces amplicons ideal for subsequent cycle sequencing reactions. The AmpliTaq Gold Fast PCR Master Mix, UP is designed for fast cycling conditions when used with **MicroAmp® Fast 96-well Reaction Plates**, and the **Veriti™ 96-Well Fast Thermal Cycler**. After PCR, ExoSAP-IT® (USR Corporation, P/N 78200) is recommended for sample purification prior to cycle sequencing.

3. Fast Cycle Sequencing.

For fast sequencing reactions, the **BigDye® Terminator v1.1 Cycle Sequencing Kit** is combined with the **MicroAmp Fast 96-Well Reaction Plates** and the **Veriti 96-Well Fast Thermal Cycler**. The **BigDye Terminator v1.1 Cycle Sequencing Kit** is designed for specialty applications that require optimal basecalling adjacent to primers and for sequencing short PCR product templates with rapid electrophoresis run modules. Specific protocol modifications for fast cycle sequencing reactions are found on p. 6 of this Application Note.

4. Clean-Up.

Fluorescently labeled dideoxy terminators, dNTPs, and salts should be removed prior to capillary electrophoresis (CE) analysis of the cycle sequencing products. Bisulfite sequencing reactions can be purified using Centri-Sep™ columns (96-well plate) or ethanol precipitation. Both of these purification methods, which require careful attention to detail, result in some loss of sample during processing. The **BigDye® XTerminator™ Purification Kit** is a simple alternative that reduces time, sample loss and efficiently removes unincorporated BigDye terminators.

5. Detection.

Detection by capillary electrophoresis is performed after cycle sequencing reactions and sample clean-up. The Applied Biosystems 3130 or 3130*x*/ Genetic Analyzers were used to generate the sequencing data provided in this Application Note. The **3130 Genetic Analyzer** is a 4-capillary electrophoresis instrument for low- to medium-throughput laboratories. This high performance system has sophisticated automation capabilities that save time, reduce costs and increase productivity. The **3130*xl* Genetic Analyzer** is a 16-capillary electrophoresis instrument for medium-throughput laboratories. The Rapid Sequencing (BDx_RapidSeq) run module and the POP-6™ Polymer should also be used when performing the Fast Resequencing Workflow.

6. Data Analysis.

Either the **Sequence Scanner v1.0**, the **Sequencing Analysis Software v5.3.1** with **KB™ Basecaller v1.4**, or **Variant Reporter™ Software** are appropriate for data analysis.

BigDye® XTerminator™ Kit Cleans-Up Better than Centri-Sep™ Columns

Centri-Sep columns have long been used to remove dye terminators from cycle sequencing reactions. Now, the BigDye® XTerminator™ Purification Kit is available. This kit provides a fast, simple purification method for DNA sequencing reactions that removes unincorporated BigDye® terminators. Clean-up is complete in less than 40 minutes and requires less than 10 minutes of labor. In the figure below, the two clean-up methods are compared. Two samples were prepared and sequenced according to the Fast Resequencing Workflow. After cycle sequencing, the reactions were purified using either the BigDye XTerminator Purification Kit or Centri-Sep columns. The electropherogram generated from the sample cleaned-up using BigDye XTerminator Kit (top) shows that more usable data is generated, compared to the electropherogram from the Centri-Sep column purified sample (bottom). As you can see, the sequence from the BigDye XTerminator sample can be read starting at base number 9, compared to base number 43 for the Centri-Sep sample.

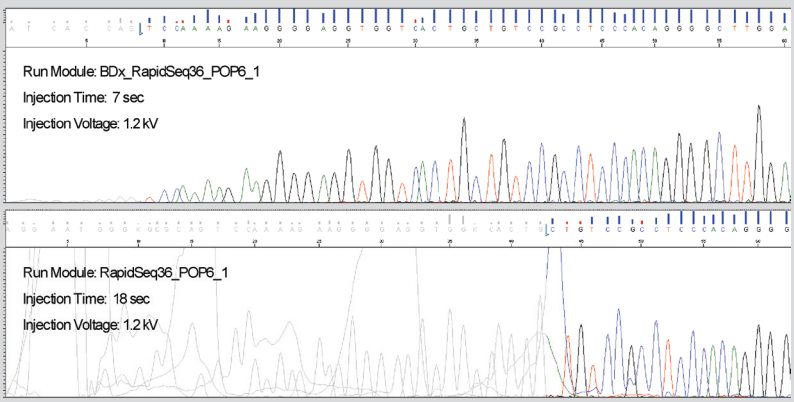
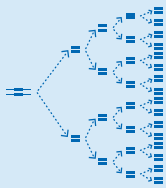


Figure 4. Comparison of Sequencing Data using the BigDye® XTerminator™ Kit and Centri-Sep™ for Cycle Sequencing Reaction Clean-up. Two 600 bp samples were sequenced using the fast cycle sequencing protocol. Reactions were cleaned-up after sequencing using either the BigDye Xterminator Kit (top) or Centri-Sep columns (bottom). KB basecaller was used to analyze the data and SeqScanner Software was used to view the data.

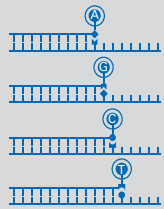
Fast Resequencing Workflow

1. DNA Extraction

2. Primer Design, Fast Amplification & Clean-Up



3. Fast Cycle Sequencing

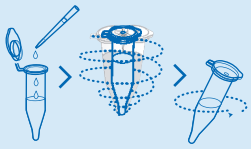
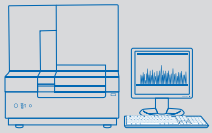
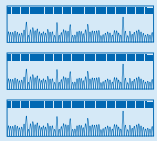


Description		
DNA is isolated from various sample types including blood, cultured cells, and fresh, frozen or FFPE (formalin-fixed-paraffin embedded) tissue	• Primers are designed to amplify target regions by PCR. • Fast PCR is performed • Reaction is cleaned-up to remove unincorporated primers, dNTPs & salts	• Produces sequencing products for subsequent detection and analysis on Applied Biosystems Genetic Analyzers • Utilizes optimized fast protocols, MicroAmp Fast 96-Well Reaction Plates, and the Veriti Thermal Cycler
Technical Considerations		

• Minimum input amounts vary with sample type and method used. Minimums generally are:
  • 100 cells
  • 1 mg tissue
  • ≤80 µm FFPE tissue
	• Fast cycle times are utilized
	• VariantSEQr Resequencing System can be used to select primers for regions of interest: more than 420,000 primer pairs representing approximately 16,000 human genes.
	• Produces PCR template ideal for sequencing
		• BigDye Terminator v1.1 Kit is optimal for basecalling adjacent to the primer and for short amplicon sequencing with rapid run modules *(Note: The BigDye Terminator v1.1 kit was used for the experiments illustrated in this Application Note)*
		• BigDye Terminator v3.1 kit is suggested for a wide range of applications, such as de novo sequencing and resequencing on PCR products, plasmids, and BACs.
		• Applied Biosystems kits include buffers optimized for use with BigDye® Terminator Kits

Applied Biosystems Solutions		
• MELT™ Total Nucleic Acid Isolation System • RecoverAll™ Total Nucleic Acid Isolation Kit	• VariantSEQr® Resequencing System • Veriti™ 96-Well Fast Thermal Cycler* • AmpliTaq Gold® Fast PCR Master Mix, UP • MicroAmp® Fast 96-Well Reaction Plates • Clear Adhesive Film	• Veriti™ 96-Well Fast Thermal Cycler • BigDye® Cycle Sequencing Kit, v1.1 • MicroAmp Fast 96-Well Reaction Plates • Clear Adhesive Film
Benefits		
• Choice of DNA purification kits are available • All produce high quality DNA ready for sequencing, even from blood or FFPE tissue	• Saves 3 hours, 15 minutes over traditional methods • VariantSEQr system simplifies primer design • AmpliTaq Gold Fast PCR Master Mix, UP provides delivers high-yield amplicons ideal for sequencing • Veriti 96-well Fast Thermal Cycler combined with Fast reagents and plastics dramatically shortens cycling times	• Saves 1 hour, 20 minutes over traditional methods • Small 10 µL reaction volumes provide reagent savings

*The Veriti 96-Well Fast Thermal Cycler (0.1 mL) will produce the fastest PCR results, however you may also run use the standard Veriti 96-Well Thermal Cycler (0.2 mL) and receive rapid results similar to what is shown in this protocol.

4. Sample Clean-Up	5. Detection	6. Data Analysis
		
Description		
Remove unincorporated terminator dyes and other bi-products of cycle sequencing	After cycle sequencing, detection and primary data analysis are performed using a capillary electrophoresis system, such as the Applied Biosystems 3130 or 3130*xl* Genetic Analyzer	Software determines each base of the DNA sequence and compares it to a reference sequence for identification of mutations or variants
Technical Considerations		
• Greatly reduce dye blobs while minimizing sample loss • Minimizes handling	• Polymer designed for use with short amplicons and quick time to results • Run module optimized for BigDye XTerminator reactions and designed for quick results • For laboratories with higher sample throughput requirements, the 3730 (48-capillary) or the 3730*xl* (96-capillary) Genetic Analyzer can be considered. (Note: In this Application Note, all protocol recommendations and data are for the 3130 or 3130*xl* systems.)	Research goals of the user define the appropriate software for the experiment • For basecalling and sequence trimming use Sequence Analysis Software v5.3.1 • To view data and generate reports, use Sequence Scanner v1.0 • For mutation detection or comparison to a reference, use Variant Reporter Software v1.0
Applied Biosystems Solutions		
• BigDye® XTerminator™ Purification Kit	• 3130 or 3130*xl* Genetic Analyzers • POP-6™ Polymer • Rapid Sequencing (BDx_RapidSeq) Run Module	• Variant Reporter™ software (Use to compare samples with or without a reference sequence) or SeqScape® software (Use to compare samples with a reference sequence) • Sequencing Analysis Software with KB Basecaller
Benefits		
• Saves 1 hour, 10 minutes over traditional methods • BigDye® XTerminator™ Kit scavenges all unincorporated dye terminators and stabilizes samples before analysis (see sidebar)	• Saves 1 hour over traditional methods • Minimal setup, simple operation • Continuous, unattended 24-hour operation • Long term reliability with extremely low maintenance requirements	• Fast analysis for large projects • Basecalls, applies mobility files, and assigns QVs • Improved mixed base calling • Accurate data analysis • Configurable variant reports

The Fast Resequencing Protocol

Materials for Fast PCR Amplification

- AmpliTaq Gold® Fast PCR Master Mix, UP
- Veriti™ 96-Well Fast Thermal Cycler (0.1 mL)
- MicroAmp® Fast 96-Well Reaction Plates
- MicroAmp® Clear Adhesive Film

Materials for Fast Cycle Sequencing

- MicroAmp® Fast 96-Well Reaction Plates
- MicroAmp® Clear Adhesive Film
- BigDye® Terminator™ v1.1 Cycle Sequencing Kit
- 5X Sequencing Buffer
- VariantSEQr® Resequencing System (PCR primers with M13 tails)
- M13 forward and reverse primers (prepare to 3.2 µmol concentration)
- BigDye XTerminator™ Purification Kit (includes SAM™ solution)
- Plate vortex

For a complete listing of support protocols for the Fast Resequencing Workflow, please visit www.appliedbiosystems.com/fastsolutions

Protocol for Fast PCR Amplification

Prepare and run the reaction as described in AmpliTaq Gold® Fast PCR Master Mix, UP Quick Reference Card. The Veriti Thermal Cycler cycling conditions are as follows.

Activation of Enzyme	PCR			PCR Final (Step)	
Hold	Cycle (35 Cycles)			Hold	Hold
	Denature	Annealing	Extension		
95˚C	96˚C	Primer TM*	68˚C	72˚C	4˚C
10 Min	3 sec	3 sec	See Table Below	10 sec	∞

* User Primer TM calculator found on an Applied Biosystems Thermal Cycler, or go to http://www.appliedbiosystems.com/support/techtools/calc/

Recommended Extension Times:

Length (Kb)	Extension Time
0.5	5 sec
1.0	15 sec
1.5	30 sec

Protocol for Fast Cycle Sequencing

Fast Cycle Sequencing is performed with modifications to the BigDye Terminator v1.1 Kit Protocol as follows:

Prepare the reaction mixtures for a 1/2 dilution BigDye® Terminator v1.1 Kit reaction:

1. For each reaction, add the following reagents to a separate well in a MicroAmp™ Fast 96-Well reaction plate:

Reagents	Amount
BigDye® Terminator v1.1	2 µL
Forward or Reverse primers	1 µL
DI water	4 µL
5X Sequencing Buffer	1 µL
DNA	2µL (2 – 10 ng of total DNA)
Total	10 µL

Note: *All reagents, except the sample DNA, can be made into a Master Mix; then 8 µL of Master Mix solution is added to each well.*

2. Vortex and spin briefly.

3. Set the Veriti Thermal Cycler parameters as follows:

Denaturation	Cycle Sequencing			Hold
Hold	Cycle (25 Cycles)			Hold
	Denature	Annealing	Extension	
96˚C	96˚C	50˚C	60˚C	4˚C
1 Min	10 sec	3 sec	75 sec	∞

* Use rapid thermal ramp (1˚C/sec) for each new temperature.

4. Place the reaction plate into the thermal cycler, cover with the compression pad, close the cover, select the program you just created along with the correct reaction volume; run.

5. When the run is finished, remove the reaction plate and centrifuge the contents briefly.

Note: *For more information, please see the BigDye® Terminator v1.1 Cycle Sequencing Kit Protocol. For a complete listing of support protocols for the Fast Resequencing Workflow, please visit www.appliedbiosystems.com/fastsolutions*

Purify Sequencing Products

1. After cycle sequencing, centrifuge the reaction plate briefly, then pipette the correct volume of BigDye XTerminator reagents into each plate well:

Reagents	Amount
SAM™ Solution	45 µL
BigDye® XTerminator™ Solution	10 µL

Note: *Vortex the XTerminator Solution container thoroughly before using. Use a wide-bore pipette tip when pipetting the solution into the wells.*

Note: *For large scale preparations, SAM and BigDye XTerminator solutions can be premixed and stored for up to 24 hours at 4 °C.*

2. *See options for this step below.*

   2a. Seal the reaction plate by covering the wells with a Clear Adhesive Film. Apply firm pressure by dragging an applicator (P/N 4333183) across the film: side-to-side and top to bottom. To ensure a very firm seal, place the plate in a thermal cycler, close the lid and program the thermal cycler to hold at 4 °C for 3-5 minutes. The heat and pressure from the thermal cycler lid completes the sealing process.

   Note: Place the compression pad on top of the reaction plate before closing the lid of the thermal cycler.

OR

   2b. Terminate the reaction. After cycle sequencing, centrifuge the reaction plate briefly, and then transfer reactions from the MicroAmp Fast 96-Well Reaction Plate to a standard MicroAmp 96-Well Reaction Plate using a multi-channel pipettor. Pipette the correct volume of BigDye XTerminator reagents into each plate well (see above table) and seal the reaction plate by covering the wells with MicroAmp Clear Adhesive Film and applying firm pressure by dragging an applicator across the film, side to side, and top to bottom to ensure good contact over the entire surface of the reaction plate.
   Note: *The process described in this step is recommended if fast adapter plates are not being used.*

3. Vortex the reaction plate for 30 minutes.

4. Centrifuge the reaction plate for 2 minutes at 1000 x g.
   Note: For more information, please reference the Applied Biosystems BigDye® XTerminator™ Purification Kit Protocol. (For a complete listing of support protocols for the Fast Resequencing workflow, please visit www.appliedbiosystems.com/fastsolutions).

Running on the Genetic Analyzer

1. Set up the 3130 or 3130*xl* instrument with a 36 cm array and POP-6™ polymer using Data Collection v3.0 Install Array Wizard (Chapter 1 of the Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide).

2. Run a Spatial and BigDye® Terminator v 1.1 (E) Spectral Calibration Standard (Chapter 2 and 3 of the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide).

3. Create a new Run Module using the BDx_RapidSeq36_POP6 Run Module as a template.

Edit the following parameters:	
Injection Time	3 – 7 sec*
Injection Voltage	1.2 kV
*Depends on the amount of DNA used	

4. Set up the Results Group, Instrument Protocol, Analysis Protocol and Plate Record, according to Chapter 4 of the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide.

   Note: Make sure that you choose the modified BDx_RapidSeq36_POP6 Run Module and Dye Set E when you create your Instrument Protocol.

5. Seal the reaction plate using a 96-well plate septa, and assemble the plate assembly, according to Chapter 3 (pp. 33-34) of the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide.

   Note: Use the 96-well Fast reaction plate assembly for 3130/3130xl Analyzer for option 2a. For option 2b, use the standard plate assembly

6. Place the reaction plate in the Applied Biosystems Genetic Analyzer, link the plate record to the plate on the instrument and start the run, according to Chapter 7 of the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide.

   Note: For more information, please reference the Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide (P/N 4352715 Rev C). (For a complete listing of support protocols for the Fast Resequencing workflow, please visit www.appliedbiosystems.com/fastsolutions).

ORDERING INFORMATION

Description	Quantity	Part Number
INSTRUMENTS		
Veriti™ 96-Well Fast Thermal Cycler	1 instrument	4375305
3130 Genetic Analyzer	1 instrument	3130-01
3130*x*/ Genetic Analyzer	1 instrument	3130XL
REAGENTS, KITS AND ASSAYS		
MELT™ Total Nucleic Acid Isolation Kit	100 rxns	AM1983
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	40 purifications	AM1975
VariantSEQr® Resequencing System, www.appliedbiosystems.com/variantseqr for ordering information		
AmpliTaq Gold® Fast PCR Master Mix, UP	25 rxns	4390937
AmpliTaq Gold® Fast PCR Master Mix, UP	250 rxns	4390939
AmpliTaq Gold® Fast PCR Master Mix, UP	2,500 rxns	4390941
BigDye® Terminator v1.1 Cycle Sequencing Kit	1,000 rxns	4337451
BigDye® Terminator v3.1 Cycle Sequencing Kit (Please see www.appliedbiosystems.com for additional BigDye® Terminator kit sizes)	1,000 rxns	4337456
5X Sequencing Buffer	11 mL	4305603
BigDye® XTerminator™ Purification Kit	2 mL	4376486
POP-6™ Polymer	7,000 µL	4316357
CONSUMABLES		
MicroAmp® Fast 96- Well Reaction Plate, 0.1 mL	10 plates	4346907
MicroAmp® Clear Adhesive Film	100 films	4306311
MicroAmp® Adhesive Film Applicator	5 applicators	4333183
MicroAmp™ Optical Film Compression Pad	5 pads	4312639
SOFTWARE		
Variant Reporter™ Software v1.0, 30-day demo	1 CD	4385270
Variant Reporter™ Software v1.0, Initial License	1 software license	4385261
Sequencing Analysis Software v5.3.1 Initial License	1 software license	4360967
Sequencing Analysis Software v 5.3.1 Upgrade from v5.1.x and older	1 CD	4310991
SeqScape® Software v2.6 upgrade from v2.1.x and older	1 CD	4332045
SeqScape® Software v2.6, Initial License	1 license	4327091
Sequence Scanner Software, www.appliedbiosystems.com for free software download		

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

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