User Bulletin

ABI PRISM® 310 Genetic Analyzer

April 16, 2001

SUBJECT: POP-4 Polymer Sequencing Protocols for the 310 Genetic Analyzer

Introduction

Overview

In an effort to further enhance the versatility and ease-of-use of the ABI PRISM® 310 Genetic Analyzer, Applied Biosystems is introducing two new sequencing protocols using the POP-4™ Performance Optimized Polymer (POP-4 polymer). The new protocols have been developed to provide accurate and well-resolved sequencing data in shorter run times than are presently attainable with POP-6™ Performance Optimized Polymer (POP-6 polymer) on the 310 Genetic Analyzer.

Additionally, the new protocols enable users to easily switch between fragment analysis and sequencing applications by eliminating the need to change capillary and polymer types.

In This User Bulletin

The following topics are discussed in this User Bulletin:

For More Information

If you would like more information, the following documents may be helpful:

Manual	Part Number
ABI PRISM 310 Genetic Analyzer User's Manual	903565
Automated DNA Sequencing Chemistry Guide **Note** This manual can be downloaded from our website: http://www.appliedbiosystems.com/ab/techsupp/310.html	4305080
ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit Protocol	4390037

Applied Biosystems

About POP-4 Polymer

Description

POP-4 polymer is a liquid polymer used to electrophorese DNA under denaturing conditions. It is similar to POP-6 polymer, however, POP-4 polymer contains a different polymer concentration. This different concentration results in a less viscous separation medium for POP-4 polymer than for POP-6 polymer.

Due to the different polymer formulations between POP-6 and POP-4 polymers, slight local mobility differences will be expected for the same data generated with both polymers. These mobility differences are most evident within the first 50 base pairs.

Advantages of POP-4 Polymer

The advantages of POP-4 polymer are:

- Low viscosity
- Faster run speeds
- High resolution
- Denaturing run conditions

Run Conditions and Specifications

The run conditions and performance specifications for sequencing with POP-4 polymer on the 310 Genetic Analyzer are listed below.

Module	Capillary	Run Time (Minutes)	Temp.	Resuspension Solution	Performance
P4StdSeq(1 mL) E	Lt = 47-cm, 50-µm i.d. Ld = 36-cm, 50-µm i.d.	52	50 °C	Hi-Di™ Formamide (P/N 4311320)	≥ 98.5% basecalling out to 525 nt
P4RapidSeq(1 mL) E		38			≥ 98.5% basecalling out to 425 nt

Sample Preparation

Overview

After performing cycle sequencing as described in the *ABI PRISM 310 Genetic Analyzer User's Manual*, unincorporated dye terminators must be completely removed before the samples can be analyzed by electrophoresis. In sequencing reactions, excess dye terminators may obscure data in the beginning of the sequence and can interfere with basecalling.

Purification Methods

To purify cycle sequencing extension products, you may use one of the following methods:

- Spin column
- Precipitation with ethanol (EtOH)
- Precipitation with ethanol (EtOH) and sodium acetate (NaOAc)
- Precipitation with isopropanol (anhydrous)

Spin Column Purification is Recommended

We recommend spin column purification for the POP-4 polymer sequencing protocols described in this User Bulletin. Spin column purification removes a greater proportion of unincorporated dye terminators. Precipitation methods are sometimes cheaper and faster, however, they remove less of the unincorporated dye terminators that can obscure data in the beginning of the sequence.

Note Procedures for spin column purification are given below. If you would like to use the alcohol precipitation procedures, please refer to the *ABI PRISM 310 Genetic Analyzer User's Manual*.

Spin Column Purification

Recommendations

We recommend 1-mL Centri-Sep™ spin columns.

If you are located...	Use the following part number...
in North America	Princeton Separations, P/N CS-900 for 32 columns
	Princeton Separations, P/N CS-901 for 100 columns
outside North America	Applied Biosystems, P/N 401763 for 32 columns
	Applied Biosystems, P/N 401762 for 100 columns

Tips for optimizing spin column purification:

- Use one column for each sample.
- Do not process more columns than you can handle conveniently at one time.
- Hydrate the spin columns for at least 2 hours.

  IMPORTANT It is essential that columns be hydrated for no less than 2 hours when purifying extension products created with ABI PRISM® BigDye™ terminator chemistry.

- Load the sample in the center of the column bed. Make sure that the sample does not touch the sides of the column and that the pipet tip does not touch the gel surface. If samples are not loaded properly, peaks from unincorporated dye terminators can result.
- Spin the column at 325–730 × *g* for best results. Use the following formula to calculate the best speed for your centrifuge:

  $g = 11.18 \times r \times (\text{rpm}/1000)^2$

  where:

  g = relative centrifugal force

  r = radius of the rotor in cm

  rpm = revolutions per minute

- Do not spin for more than 2 minutes.
- Perform the entire procedure without interruption to ensure optimal results. Do not allow the column to dry out.

Performing Spin Column Purification

To perform spin column purification:

Step	Action
1	Gently tap the column to cause the gel material to settle to the bottom of the column.
2	Remove the upper end cap and add 0.8 mL of deionized water.
3	Replace the upper end cap and vortex or invert the column a few times to mix the water and gel material.
4	Allow the gel to hydrate at room temperature for at least 2 hours. **Note** Hydrated columns can be stored for a few days at 2–6 °C. Longer storage in water is not recommended. Allow columns stored at 2–6 °C to warm to room temperature before use.
5	Remove any air bubbles by inverting or tapping the column and allowing the gel to settle.
6	Remove the upper end cap first, then remove the bottom cap.
7	Insert the column into the wash tube provided. Allow the column to drain completely by gravity. **Note** If flow does not begin immediately, apply gentle pressure to the column with a pipette bulb.
8	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes to remove the interstitial fluid.
9	Remove the column from the wash tube and insert it into a sample collection tube (*e.g.*, a 1.5-mL microcentrifuge tube).
10	Remove the extension reaction mixture from its tube and load it carefully onto the center of the gel material.
	If the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480 was used for thermal cycling: Remove the reactions from the tubes as described in the *ABI PRISM 310 Genetic Analyzer User's Manual*.
11	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes. **Note** If using a centrifuge with a fixed-angle rotor, place the column in the same orientation as it was in for the first spin. This is important because the surface of the gel will be at an angle in the column after the first spin.
12	Discard the column. The sample is in the sample collection tube.
13	Dry the sample in a vacuum centrifuge for 10–15 minutes, or until dry. Do not over-dry.

Electrophoresis on the 310 Genetic Analyzer

Requirements

To perform electrophoresis on the 310 Genetic Analyzer using POP-4 polymer, you will need the following:

- Filter Set E instrument (matrix) file
- POP-4 polymer Filter Set E sequencing run modules
- POP-4 polymer sequencing dye set/primer (mobility) files
- A basecaller compatible with POP-4 polymer

These components are described in greater detail below.

Note All of the components listed are supplied with v2.0 (P/N 4324228) and v2.1 (P/N 4324229) of the ABI PRISM® 310 Genetic Analyzer Data Collection software.

Instrument (Matrix) File

Data analysis requires a Filter Set E instrument (matrix) file made from the ABI PRISM® dRhodamine Matrix Standards (P/N 403046) or the ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421). See the *Automated DNA Sequencing Chemistry Guide* or the *ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit Protocol* for more information.

Run Modules

Sequencing	Polymer	Module	Capillary
Standard POP-4 polymer	POP-4 polymer, 1-mL syringe	P4StdSeq(1 mL) E	Lt = 47-cm, 50-µm i.d.
Rapid POP-4 polymer		P4RapidSeq(1 mL) E	Ld = 36-cm, 50-µm i.d.

Dye Set/Primer (Mobility) Files

Version	Chemistry	Polymer	Mobility File
Original and v2.0	ABI PRISM® BigDye™ terminator	POP-4 polymer	DT310POP4{BD}v1.mob
	ABI PRISM® dRhodamine terminator		DT310POP4{dRhod}v1.mob
	ABI PRISM® BigDye™ primer, forward		DP310POP4{BD-21M13}v2.mob
	ABI PRISM® BigDye™ primer, reverse		DP310POP4{BD-M13Rev}v1.mob
v3.0	ABI PRISM® BigDye™ terminator v3.0	POP-4 polymer	DT310POP4{BDv3}v1.mob
	ABI PRISM® BigDye™ primer v3.0, forward		DP310POP4{BDv3-21M13}v1.mob
	ABI PRISM® BigDye™ primer v3.0, reverse		DP310POP4{BDv3-M13Rev}v1.mob

Basecaller

A new basecaller has been created to accurately and reliably call bases generated using either POP-6 or POP-4 polymer data.

Note For the protocols described in this User Bulletin, the Basecaller PPC file, in conjunction with the 310POP4.bcp file, is required for analyzing sequencing data generated with POP-4 polymer on the 310 Genetic Analyzer.

Resuspending and Loading the Samples

Recommendations

We recommend the following when resuspending samples for the POP-4 polymer sequencing protocols described in this User Bulletin:

- Due to its ease of use and increased signal strength, use deionized formamide for the resuspension solution.
- Use only the highest grade of deionized formamide, as this is essential for maintaining sequencing data integrity. Applied Biosystems provides a high-quality formamide (Hi-Di™ Formamide, P/N 4311320) for users of these POP-4 polymer sequencing protocols.
- Store deionized formamide at -20 °C in usable aliquots to prevent several freeze-thaw cycles. If the deionized formamide stored at -20 °C does not freeze, discard and use fresh deionized formamide for sample resuspension.
- Securely cover sample tubes with septa after resuspension with deionized formamide to limit the sample's exposure to air.

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

Resuspending and Loading Samples

To resuspend and load the samples:

Step	Action
1	Resuspend each sample pellet in 25 µL of deionized formamide (Hi-Di Formamide, P/N 4311320). **⚠ WARNING** **CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Securely seal each sample tube after resuspension with deionized formamide to limit the sample's exposure to air.
3	Vortex and spin the samples.
4	Heat the samples at 95 °C for 2 minutes, then chill on ice.
5	Vortex and spin the samples again.
6	Place on ice until ready to use.

To resuspend and load the samples: *(continued)*

Step	Action
7	Refer to the *ABI PRISM 310 Genetic Analyzer User's Manual* for guidelines on loading the samples.

Data Comparison: POP-6 and POP-4 Polymers

Sequencing Common Samples

For the sequencing protocols described in this User Bulletin, the same BigDye terminator v3.0 sequencing standards and plasmid samples were analyzed using both POP-6 and POP-4 polymers.

Note A new basecaller was used to analyze both sets of data. For more information about the new basecaller, see page 6.

Points to Note

- Read length is comparable across polymers using standard or rapid run modules.
- Due to the different polymer formulations between POP-6 and POP-4 polymers, slight local mobility differences will be expected for the same data generated with both polymers. These mobility differences are most evident within the first 50 base pairs.
- Data integrity is preserved (greater than 98.5% accuracy for the specified read length) for both polymers.

Examples

In Figures 1 and 2, lyophilized BigDye terminator v3.0 sequencing standards were resuspended in 25 µL of Hi-Di Formamide and analyzed with POP-4 polymer run modules on the 310 Genetic Analyzer.

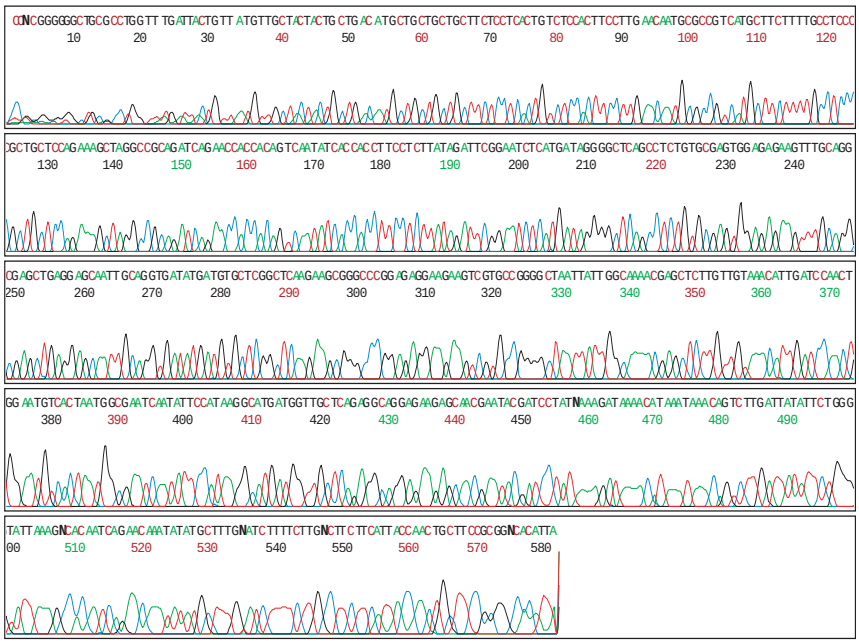

Figure 1 Standard POP-4 polymer run module

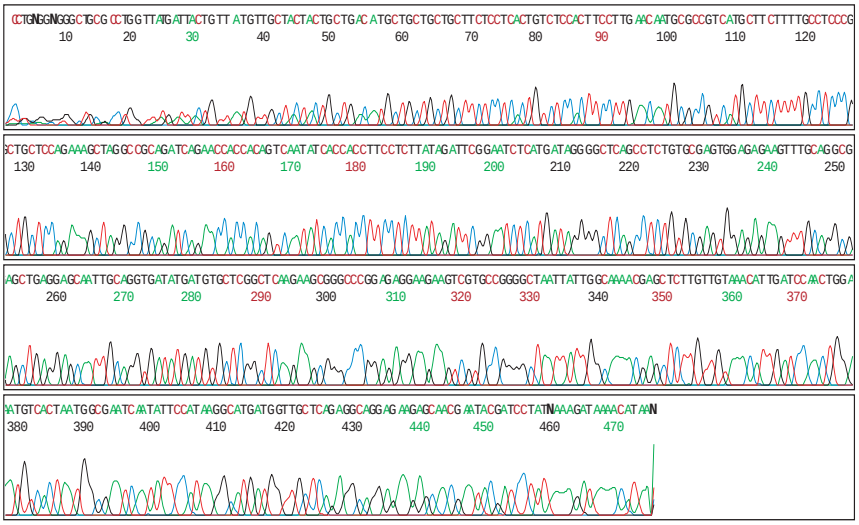

Figure 2 Rapid POP-4 polymer run module

In Figures 3 through 6, 200 ng of pGEM®-3Zf(+) plasmid DNA with 3.2 pmoles of -21M13 forward primer was sequenced using the BigDye terminator v3.0 chemistry. Excess terminators were removed using Centri-Sep columns. Following lyophilization, the samples were resuspended in 25 µL of Hi-Di Formamide for POP-4 polymer or Template Suppression Reagent (TSR) for POP-6 polymer. The samples were then analyzed with both POP-6 and POP-4 polymer run modules on the 310 Genetic Analyzer.

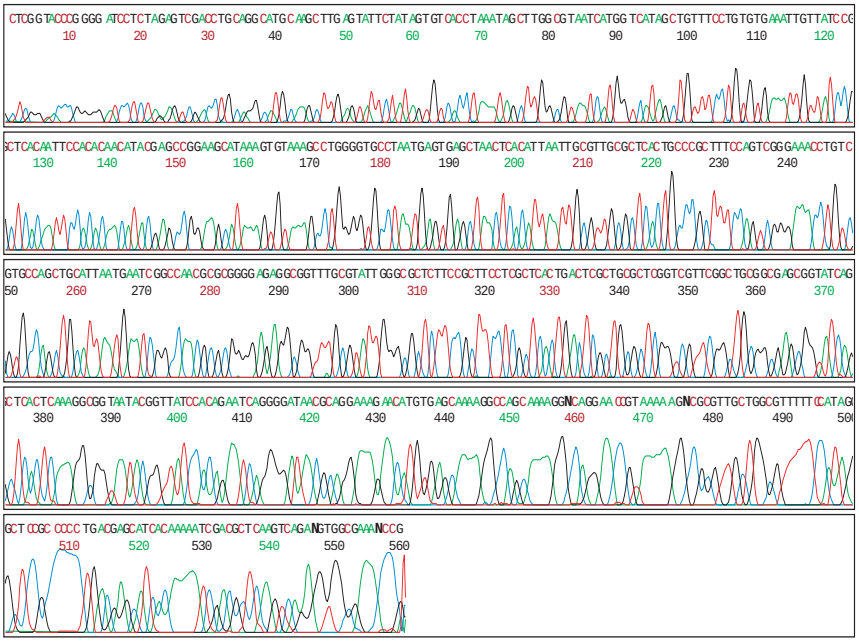

Figure 3 Standard POP-4 polymer run module

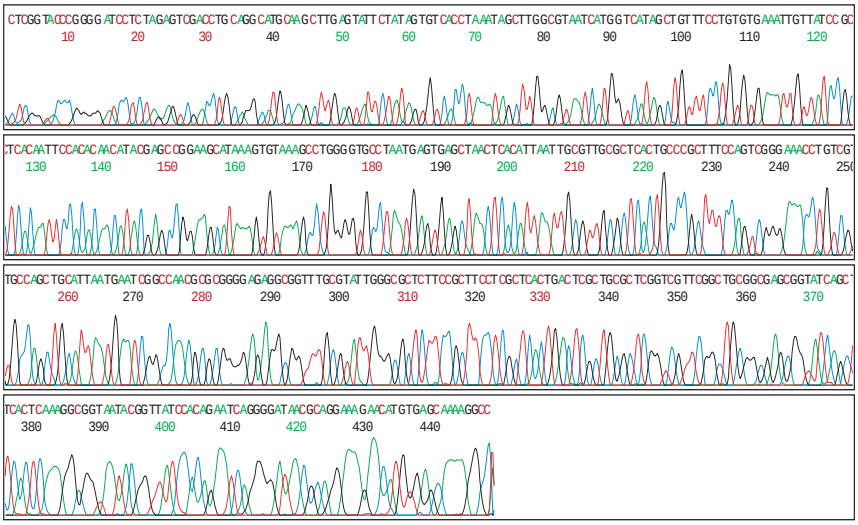

Figure 4 Rapid POP-4 polymer run module

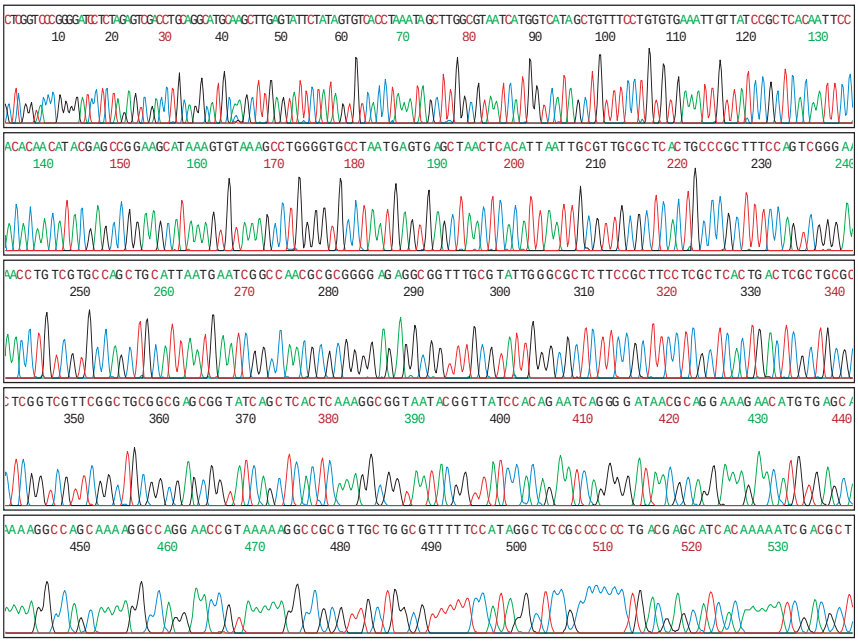

Figure 5 Standard POP-6 polymer run module

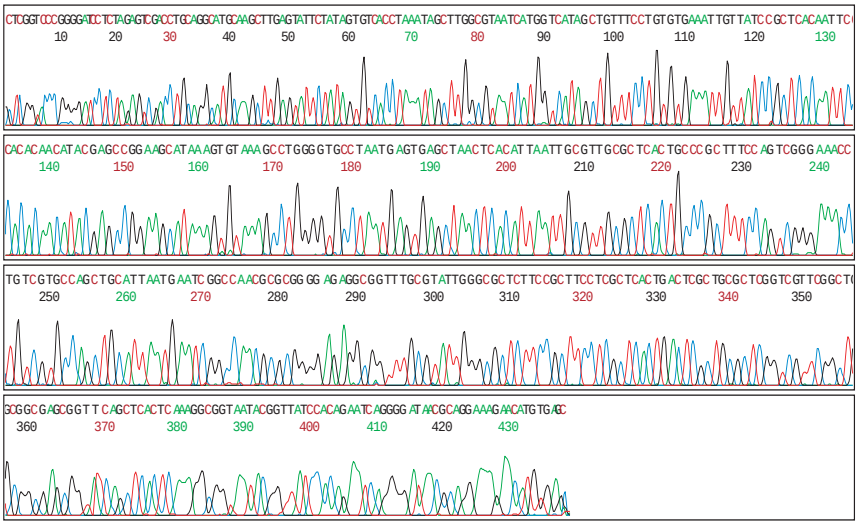

Figure 6 Rapid POP-6 polymer run module

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