User Bulletin

Applied Biosystems 3730/3730*xl* DNA Analyzers and 3130/3130*xl* Genetic Analyzers

July 2005

SUBJECT: Using the SNaPshot® Multiplex System with the POP-7™ Polymer on Applied Biosystems 3730/3730*xl* DNA Analyzers and 3130/3130*xl* Genetic Analyzers

In This User Bulletin

This user bulletin includes the following topics:

Applied Biosystems

Overview

The SNaPshot Multiplex System with POP-7 Polymer Protocol

This user bulletin provides a detailed protocol for running the SNaPshot® Multiplex system with POP-7 polymer on the Applied Biosystems 3730/3730*xl* DNA Analyzers and the Applied Biosystems 3130/3130*xl* Genetic Analyzers.

Protocol Validation and Testing

Applied Biosystems has not performed optimization or testing to validate or support the use of the application described in this user bulletin on the 3730/3730*xl* DNA analyzers or the 3130/3130*xl* Genetic analyzers. However, several Applied Biosystems customers are currently running the SNaPshot Multiplex system and POP-7 Polymer as described in this protocol. If you want additional information about these applications, contact Applied Biosystems Technical Support. See "Appendix A" on page 14 for a table of run configurations using the SNaPshot Multiplex system on various Applied Biosystems instruments.

Customer Acknowledge-ment

We would like to acknowledge Dr. Sylvie Quiniou and Mary Duke at USDA-ARS, Mid-South Area Genomics Laboratory, Catfish Genetics Research Unit, Stoneville, MS (**http://www.ars.usda.gov**) for the protocols and data supplied for this User Bulletin as well as for the BAC Fingerprinting on 3730/3730*xl* Analyzers Application Note (PN 107AP04-01). Dr. Quiniou, a microbiologist at the Catfish Genetics Research Unit, which focuses on the genetic improvement of catfish in areas such as growth and disease resistance, has implemented the BAC fingerprinting application using the SNaPshot reagents-based chemistry system on the 3730 instrument as part of their research project. We would also like to acknowledge Mike Zianni and his staff at the Plant Microbe Genomics Facility, Ohio State University (**www.biosci.ohio-state.edu/~pmgf**), a core laboratory that provides DNA sequencing and genotyping services to the university and other research facilities in the state, for the protocols and data they supplied in this User Bulletin.

SNaPshot Multiplexing on the Applied Biosystems 3730/3730*xl* DNA Analyzers and 3130/3130*xl* Genetic Analyzers

The SNaPshot Multiplex Kit

The SNaPshot® Multiplex Kit, based on a single nucleotide primer extension (Figure 1), is part of a versatile system that can be used for a variety of applications:

- Low-to-medium throughput linkage and association studies
- Single locus fragment analysis
- BAC fingerprinting
- Single Nucleotide Polymorphism (SNP) genotyping
- Bacterial classification

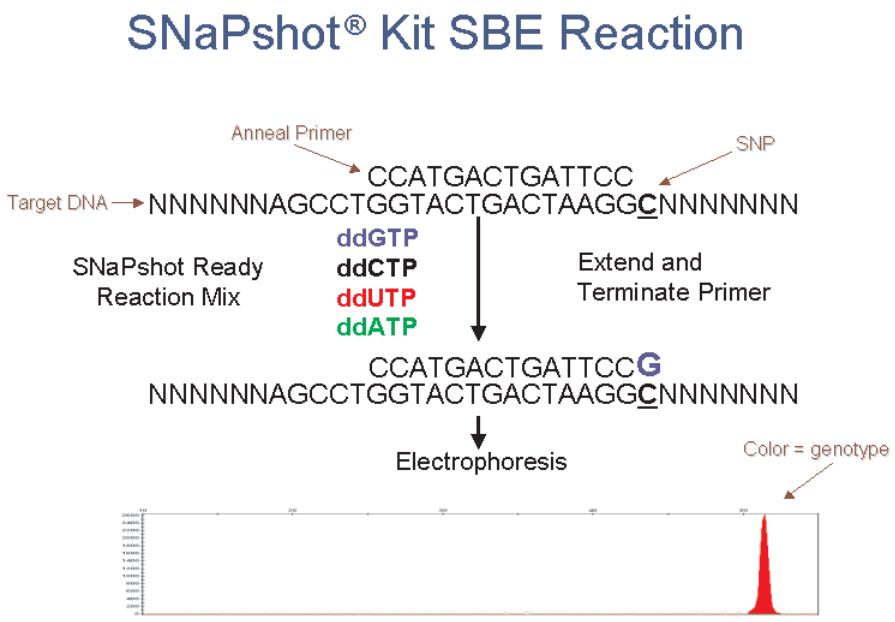

Figure 1 Single nucleotide primer extension

The SNaPshot reagent-based chemistry is based on the dideoxy single-base extension of an unlabeled oligonucleotide primer or primers. This method makes for easy conversion from manual PCR detection methods such as ethidium bromide gel-based chemistries.

The SNaPshot Multiplex System

The SNaPshot Multiplex system consists of the following:

- SNaPshot® Multiplex Kit including the master mix with fluorescently labeled ddNTPs and enzymes
- GeneScan™ 120 LIZ® Size Standard
- GeneMapper® Software v3.7 or higher
- Capillary electrophoresis instruments

See “Appendix B” on page 15 for a list of materials and part numbers.

Preparing the 3730/3730*xl* and 3130/3130*xl* Analyzers for Any5Dye and E5 Dye Set Runs

Before beginning the setup procedures necessary for running SNaPshot Kit samples on the 3730/3730*xl* or 3130/3130*xl* analyzers with POP-7 polymer, complete the following steps:

- Install GeneMapper Software v3.7 or higher. Your Applied Biosystems service engineer or field application specialist can provide instructions for software installation, if needed.
- Create protocols for the Spectral Calibration and Regular run types with the Any5Dye dye set for running on 3730/3730*xl* or with the E5 dye set for running on 3130/3130*xl*. (See “Creating the Spectral Calibration Protocol” on page 5.)
- Create a new Run Module with *GeneMapper36_POP7* settings for running on 3730/3730*xl* or with *FragmentAnalysis36_POP7* settings for running on 3130/3130*xl.* (See “Creating Run Modules” on page 8.)

  IMPORTANT! The GeneMapper Software must be installed on the instrument computer.

Additional information can be found in the *Applied Biosystems 3730/3730xl DNA Analyzers User Guide for use with Data Collection Software v2.0* (PN 4347118) and the *Applied Biosystems 3130/3130/xl Genetic Analyzers Getting Started Guide* (PN 4352715).

Both documents can be ordered from the Applied Biosystems Web site at **www.appliedbiosystems.com**.

Creating the Spectral Calibration Protocol

Create the spectral calibration protocol before you process the run. This protocol uses the Any5Dye dye set with the 3730/3730*xl* analyzer, which is available only with Data Collection Software v2.0 or higher, or the E5 dye set with the 3130/3130*xl* analyzer, which is available with Data Collection Software v3.0.

To create a spectral calibration protocol:

1. In the left-hand navigation window of the Data Collection software, select **Protocol Manager**.
2. In the Instrument Protocols area, select **New** to open the Protocol Editor window.

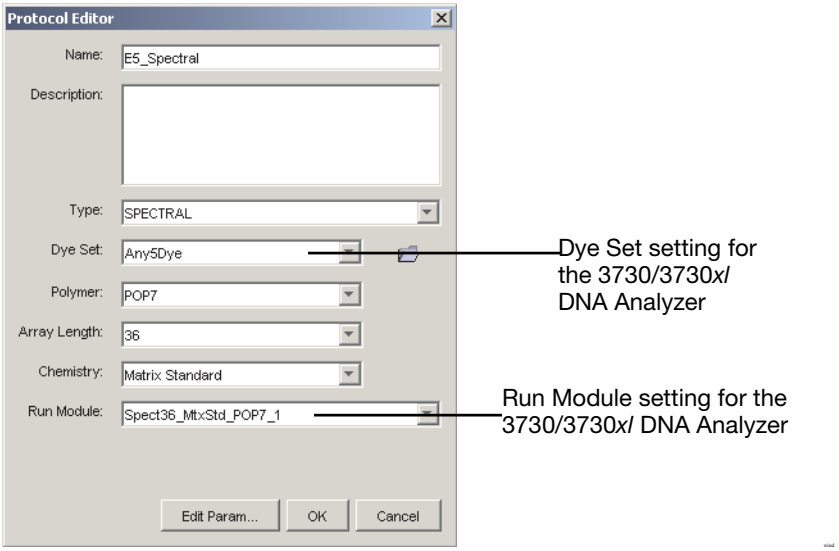

Figure 2 A spectral protocol in Protocol Manager, using the Any5Dye dye set and the *Spect36_MtxStd_POP7_1* run module on the 3730/3730*xl* DNA Analyzer

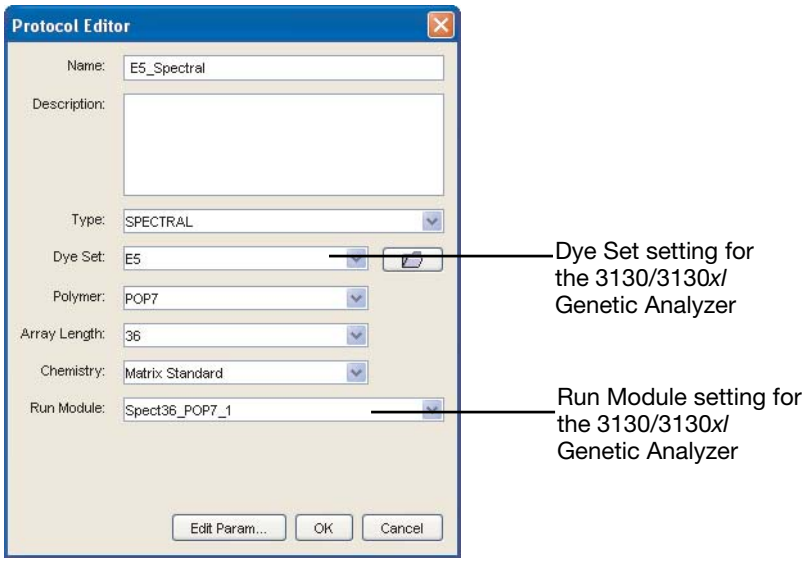

Figure 3 A spectral protocol in Protocol Manager, using the E5 dye set and the Spect36_POP7_1 run module on the 3130/3130xl Genetic Analyzer

3. Enter a name for your spectral calibration in the Name field (*E5_Spectral* in the example shown in Figure 3 on page 5). Use Table 1 below to make the appropriate selection for your instrument from each field listed.

   Note: Entering information in the Description field is optional

Table 1 Protocol Editor selections for the 3730/3730*xl* and the 3130/3130*xl* analyzers

Field	3730/3730xl Setting	3130/3130xl Setting
Type	Spectral	Spectral
Dye Set	Any5Dye	E5
Polymer	POP7	POP7
Array Length	36	36
Chemistry	Matrix Standard	Matrix Standard
Run Module	Spect36_MtxStd_POP7_1	Spect36_POP7_1

4. Select **Edit Parameter** at the bottom of the Protocol Editor window to open the Edit Spectral Params window.

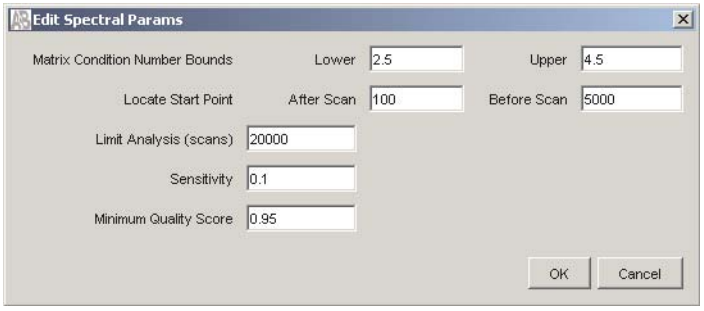

Figure 4 The Edit Spectral Params window

   – For the 3730/3730*xl* DNA analyzers, the Matrix Condition Number Bound can be set to 2.5 (minimum) and 4.5 (maximum). The Minimum Quality Score should not be lower than 0.9. A minimum quality score of 0.95 is common.
   – For the 3130/3130*xl* Genetic analyzers, use the default condition numbers.

5. Click **OK** to save any changes to the parameters and click **OK** again to save the newly created Spectral Protocol.

6. Prepare a spectral calibration matrix plate by following the protocol provided with the Matrix Standard Set DS-02 Kit (PN 4323014 for the 3130 series systems and PN 4322849 for the 3730 series systems). PN 4322849 is formulated for use with the ABI PRISM® 3700 DNA Analyzer, but it can also be used on the 3730/3730*xl* instrument.

7. Follow instructions in the instrument user manual to perform a spectral calibration run. When the spectral calibration run is complete and passes (>90% of passing caps), the instrument is ready to run SNaPshot samples. (See "Creating Regular Run Protocols" on page 9.)

   If the spectral calibration fails, check the Spectral Calibration Run Log for the error source. Any errors with spectral calibration in each capillary are also detailed in the log file. The file is located at the following address:

   E://AppliedBiosystems/UDC/DataCollection/Data/ga3x30/Instrument Number/SpectralCalTmpFiles/Any5Dye/SpectralCalResult-Run

Correcting Spectral Calibration Failures

If the spectral calibration failure is due to matrix standard peaks being too strong (i.e., if pull-up or pull-down peaks are visible (Figure 5), dilute the standards 2:1 with Hi-Di™ Formamide and perform spectral calibration again.

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

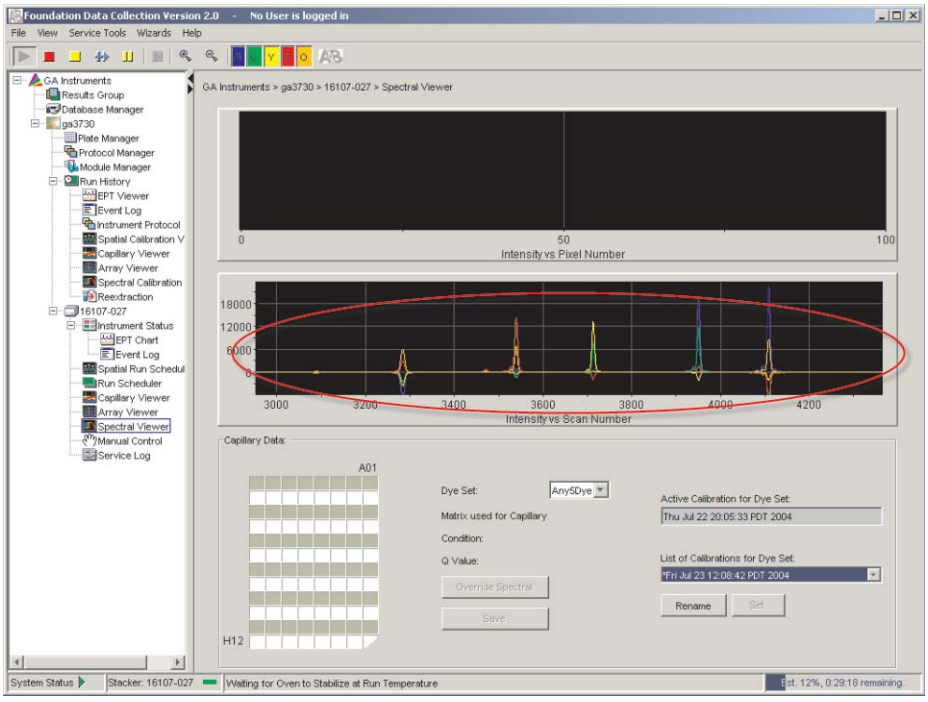

Figure 5 Failed spectral calibration showing pull-up and pull-down peaks

If the spectral calibration failure is due to the condition number not being within the designated range, adjust the Matrix Condition Number Bound‡ by following the steps below.

Note: The capillary condition number for each capillary is found in the Spectral Calibration Run Log.

1. Determine the average condition number for the array by selecting several wells.
2. Adjust the matrix condition number bounds to approximately ±1.5 of the average condition number.
3. Run spectral calibration immediately after you correct the reason for failure. Make new matrix standards or, if the elapsed time between the two spectral runs is less than one hour, use the same matrix standards.

Creating Run Modules

To create a Run Module:

1. Launch the Data Collection Software v3.0 for the 3130 Series Systems or v2.0 or higher for the 3730 Series Systems.
2. Select **Module Manager** and then **New** to open the Run Module Editor window.

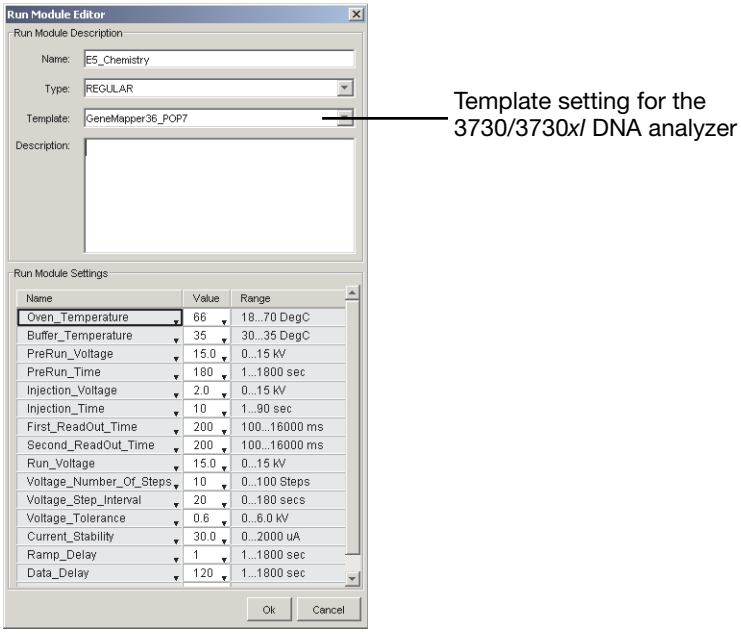

Figure 6 The Run Module Editor window using the *GeneMapper36_POP7* template on the 3730/3730*xl* DNA analyzer

‡ This parameter is adjusted only if the spectral calibration failed and the condition number is not within the designated range. The capillary condition number is located in the Spectral Viewer.

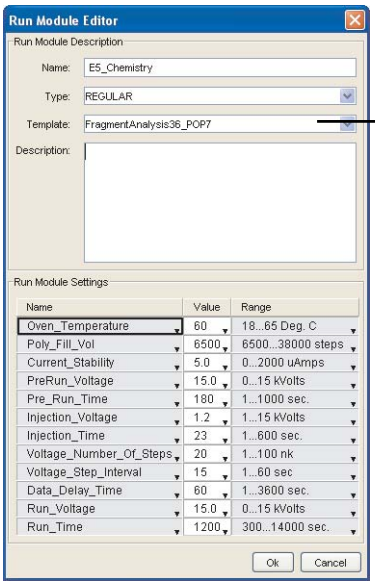

Figure 7 The Run Module Editor window using the *FragmentAnalysis36_POP7* template on the 3130/3130*xl* Genetic analyzer

3. Fill in the appropriate fields in the Run Module setting fields, as follows:

Field	3730/3730*xl* Instrument Setting	3130/3130*xl* Instrument Setting
Name	E5 Chemistry (for example)	E5 Chemistry (for example)
Type	Regular	Regular
Template	GeneMapper36_POP7	FragmentAnalysis36_POP7

Note: For SNaPshot reagent-based chemistry, if the desired fragments appear before the default time of 1,200 seconds, the Run_Time may be shortened. The Plant Microbe Genomics Facility at Ohio State University shortened the Run_Time to 600 seconds on a 36-cm capillary array.

Creating Regular Run Protocols

To create Regular Run Protocols, follow theses steps:

1. Open the Data Collection software and select **Protocol Manager**.
2. Click the **New** button in the Instrument Protocols area. The Protocol Editor window opens (see Figure 8 and Figure 9 on page 10).

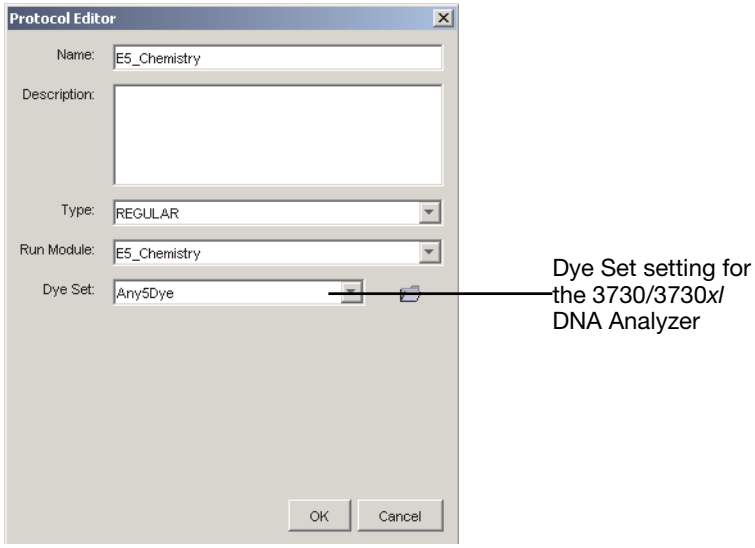

Figure 8 The Protocol Editor window in the Data Collection software on the 3730/3730*xl* DNA analyzer

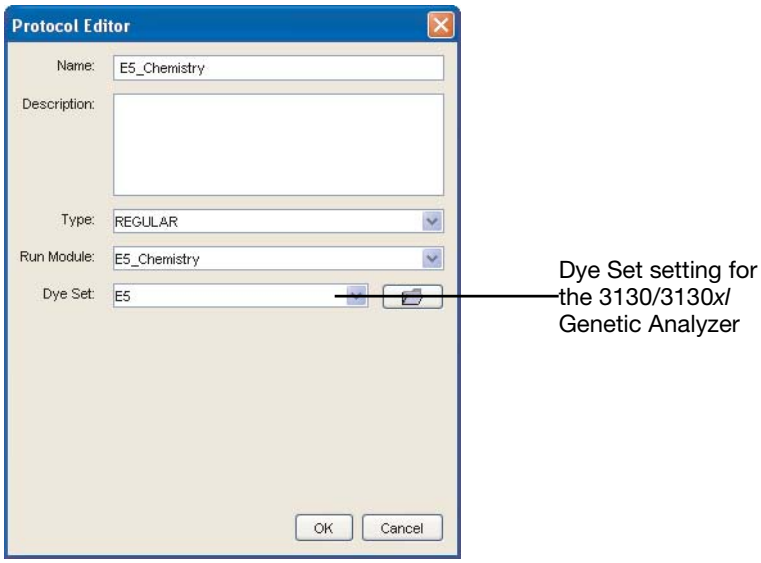

Figure 9 The Protocol Editor window in the Data Collection software on the 3130/3130*xl* Genetic analyzer

3. Enter the name of your new instrument protocol (i.e., E5_Chemistry) in the Name field. Entering information in the Description field is optional.
4. Select **Regular** in the Type field.
5. In the Run Module field, select the Run Module created above (i.e. E5_Chemistry) from the drop-down menu.
6. In the Dye Set field, select **Any5Dye** for running on the 3730/3730*xl* analyzer or **E5** for running on the 3130/3130*xl*. Then click **OK**.

7. Set up a sample sheet. Refer to instructions in the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide (PN 4352715) or the Applied Biosystems 3730/3730*xl* DNA Analyzer Getting Started Guide (PN 4359476).
8. Run the sample plates and analyze the results with GeneMapper Software v3.7 or higher for simultaneous sizing and genotyping (see Figure 10).

Figure 10 shows a multiplexed SNaPshot Kit reaction using POP-7 polymer and a 36-cm capillary array on the Applied Biosystems 3730 DNA analyzer. The data was analyzed by GeneMapper Software v3.7. The color bars represent bins used for allele calling. The GS120LIZ size standard was used for sizing.The analyzed data can then be exported in a tab-delimited Microsoft Excel® Software spreadsheet.

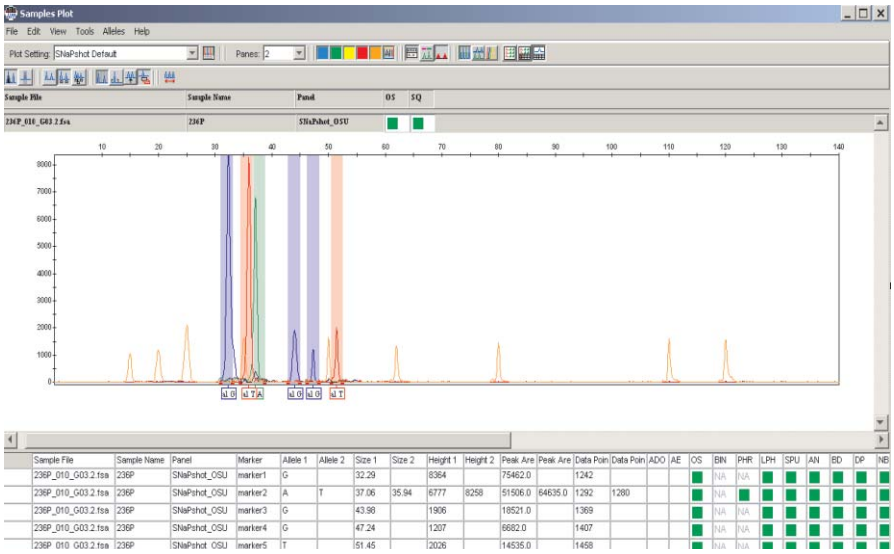

Figure 10 Multiplexed SNaPshot Kit reaction using POP-7 polymer and a 36-cm array on the 3730 DNA analyzer

Rapid Assessment of SNaPshot Primers for Multiplexing with the SNaPshot Primer Focus Kit

During the course of SNP validation, users often encounter two major obstacles:

- Determining which combination of SNP loci to multiplex
- Establishing tools to enable automatic data analysis

Using the SNaPshot Primer Focus kit enables rapid assessment of potential oligonucleotides used in the single-base extention step designed for a project. The chemistry uses single-base extension of a primer in the presence of fluorescently-labeled dideoxynucleotides, but without the presence of DNA template. You can preview all potential full length products (the four possible alleles are represented by four colors: A=green, C=black, G=blue, T=red), and you can calculate the mobility rate for each allele using this kit.

Once the data is generated, the optimal combination of SNP markers are identified for multiplexing. In Figure 11 and Figure 12 on page 12, Focus kit data from a 3130*xl* Genetic Analyzer demonstrates the mobility of a 32-mer oligonucleotide by itself and in combination with a 36-mer oligonucleotide. The data shows that sufficient separation is obtained from the 4-nucleotide length difference between the two primers.

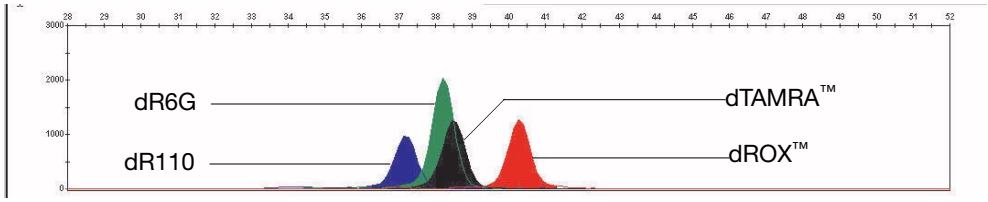

Figure 11 Primer Focus® Kit samples run with POP-7 polymer on a 3130*xl* Genetic Analyzer with a 36-cm capillary array. Mobility of a 32-mer oligonucleotide primer focus product is shown for each of the 4 dyes: dR110 (blue), dR6G (green), dTAMRA™ (yellow) and dROX™ (red).

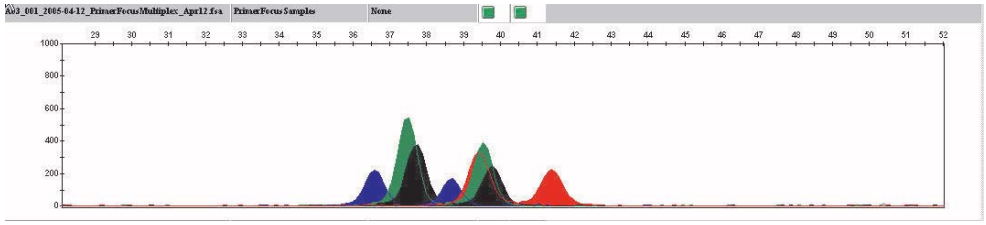

Figure 12 Multiplexed Primer Focus Kit samples run with POP-7 polymer on a 3130*xl* Genetic Analyzer with a 36-cm capillary array. Mobility of two primer focus oligonucleotide products, the 32-mer shown in Figure 11 and a 36-mer when mixed in equal amounts and run in the same capillary.

Data Analysis with GeneMapper Software v3.7 or higher

The workflow for analysis of data in GeneMapper software involves the following:

- Addition of appropriate samples to a new project
- Setting up analysis parameters
- Running the analysis
- Reviewing the results of the analyzed project

The Autoanalysis Option in the Data Collection software run on the 3130/3130*xl* Genetic Analyzers and the 3730/3730*xl* DNA Analyzers automates all steps from addition of samples to running the analysis; the user only reviews the analyzed results. GeneMapper software contains a SNaPshot Kit specific analysis method designed to recognize and analyze SNaPshot Kit data. Once the mobility of the extended products has been determined, bins representing the actual allele size range and dye color for a given SNP can be assigned. The software uses bins to make the actual allele call (see Figure 10 on page 11). Once these bins have been set up, the software automatically assigns genotypes to samples at the end of the analysis. These bins are saved and reapplied automatically to all subsequent projects that use the same set of SNPs enabling a streamlined workflow from sample loading, data collection, data analysis and genotype results generation. Please refer to the *GeneMapper® Software v3.7 User Guide* (PN 4359413) for more detailed information on data analysis.

Sizing Differences

In fragment analysis, a fragment is assigned a size based on its relative mobility to size standards as it migrates through the polymer. Any change in run conditions, such as capillary array length or polymer type, affects fragment mobility. Sizing differences resulting from mobility changes between various types of polymer are more apparent for sequences < 50 base pairs (bp). Due to the nature of the polymer, smaller fragments (< 50 bp) run on POP-7™ polymer on the 3130/*xl* Genetic Analyzers and on the 3730/*xl* DNA Analyzers may have slightly different mobilities.

To avoid sizing inconsistency with smaller fragments, Applied Biosystems recommends that genotyping projects be started and completed on the same instrument, using consistent run conditions. Avoid inconsistencies by creating single-base extention primers no shorter than 25 nucleotides (nts). In addition, the spacing should be at least 6-8 nts for primers that are less than 30 nts and 4-6 nts for primers that are greater than 30 nts. Alternatively, genotypes can be standardized to one platform by running representative alleles across all platforms and creating instrument-specific bin sets with standardized bin names in GeneMapper Software v3.7 or higher.

Conclusion

In addition to multiplexing, the SNaPshot Multiplex Kit can also be used for the rapidly expanding field of fragment analysis and SNP genotyping. Researchers in these areas increasingly look for ways to improve throughput and cost-effectiveness without sacrificing data quality. One solution is to leverage the flexibility of the Applied Biosystems 3730/3730*xl* DNA analyzers and the 3130/3130*xl* Genetic analyzers to accommodate genotyping applications designed for the SNaPshot Multiplex Kit.

For more information, please contact your Sales Representative, Field Application Specialist, or Technical Support.

Appendix A

Throughput Results Using the SNaPshot Multiplex System on the Applied Biosystems 3730/3730*xl* DNA Analyzers, 3130/3130*xl* Genetic Analyzers, and 3100 Analyzers

Instrument (array & polymer)	Number of Capillaries	Run Time (min)	Number of Runs/24 hr	Number of Genotypes/Run	Number of Genotypes/Day
3730 (36-cm, POP-7)	48	33	43	10	20,640
3730*xl* (36-cm POP-7)	96	33	43	10	41,280
3130 (36-cm, POP-7)	4	35	41	10	1,640
3130 (22-cm, POP-4™)	4	20	72	10	2,880
3100-Avant (36-cm, POP-4)	4	24	60	10	2,400
3100-Avant (22-cm POP-4)	4	20	72	10	2,880
3130*xl* (36cm, POP-7)	16	35	41	10	6,560
3130*xl* (22-cm POP-4)	16	20	72	10	11,520
3100 (36-cm array POP-4)	16	24	60	10	9,600
3100 (22-cm array POP-4)	16	20	72	10	11,520

Appendix B

Ordering Information for Materials

Description	Comments	PN
ABI PRISM® SNaPshot® Multiplex Kit	5,000 rxns	4323163
ABI PRISM® SNaPshot® Multiplex Kit with protocol	1,000 rxns	4323154
ABI PRISM® SNaPshot® Multiplex Kit with protocol	100 rxns	4323151
SNaPshot® Primer Focus® Kit	100 rxns	4329538
3730/3730*xl* DNA Analyzer User's Guide v2.0 or higher		4347118
Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide		4352715
GeneMapper® Software v3.7	Initial License	4363138
GeneMapper® Software v3.7	Additional License	4363137
GeneScan™ 120 LIZ® Size Standard		4324287
48 capillary × 36 cm Capillary Array (3730)		4331247
96 capillary × 36 cm Capillary Array (3730*xl*)		4331244
36cm Applied Biosystems 3130 and 3100-Avant Capillary Array		4333464
36cm Applied Biosystems 3130*xl* and 3100 Capillary Array		4315931
Hi-Di™ Formamide		4311320
Matrix Standard Set DS-02 (dR110, dR6G, dTAMRA™, dROX™, LIZ® Dyes)	3700 DNA Analyzer	4322849
Matrix Standard Set DS-02 (dR110, dR6G, dTAMRA™, dROX™, LIZ® Dyes)	3130/3130*xl* Genetic Analyzers	4323014
POP-7™ Polymer for Applied Biosystems 3130 and 3130*xl* Genetic Analyzers	7 mL	4352759
POP-7™ Polymer for 3730/3730*xl* DNA Analyzers, Box of 15, 25 mL Bottles	15 bottles/box 420 mL	4335612

Appendix C

SNaPshot Reference Documents

Title	Part Number
ABI PRISM® SNaPshot™ ddNTP Primer Extension Kit Protocol	4312665
ABI PRISM® SNaPshot™ Multiplex Kit Protocol	4323357
ABI PRISM® SNaPshot™ Multiplex Kit Quick Reference Card	4323975
BAC Fingerprinting on the Applied Biosystems 3730/3730*xl* DNA Analyzer SNaPshot® Multiplex System Application Note	107AP04-01
GeneMapper® Software Version 4.0 SNaPshot Kit Analysis Getting Started Guide	4363078
GeneScan Analysis Software: Genetic Analysis: Product Bulletin	107PB05-01
Using ABI PRISM® Genotyper® Software Version 3.7 with the SNaPshot™ Multiplex Kits ABI PRISM® Genotyper® Software User Bulletin	4326549

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SNaPshot Kits -

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3730/3730xl and 3130/3130xl Analyzers -

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