

# BAC Fingerprinting on the Applied Biosystems 3730/3730x1 DNA Analyzer<sup>1</sup>

## Introduction

Many researchers today in genomics research have found large genomic fragment libraries, such as bacterial artificial chromosomes (BACs), to be versatile and practical tools in facilitating their research. When BAC libraries are arranged into maps that reflect the DNA sequence in a chromosome, they provide maximal information and utility. New BAC libraries require a rapid, efficient method for characterization and assembly of clones into contigs, which are then arrayed into physical maps of the chromosome. One widely used technique to generate these physical maps is to fingerprint fragments of BACs, generated from restriction enzymatic digests, with fluorescence-labeled dideoxynucleotides ([F]ddNTPs).

The ABI PRISM® SNaPshot® Multiplex Kit is an effective, easy, and cost-effective solution for BAC fingerprinting. The high throughput required by the BAC fingerprinting technique has led many researchers to inquire about the possibility of running the SNaPshot kit on a high-throughput instrument, such as the Applied Biosystems 3730/3730x1 DNA Analyzers, although

<sup>1</sup>Certain customers of Applied Biosystems have demonstrated that the Applied Biosystems 3730/3730x1 DNA Analyzer series instruments are capable of running the following applications. There has been no optimization or testing performed by Applied Biosystems to validate these applications and Applied Biosystems does not support such applications. However, we will be happy to refer you to such customers if you wish to get additional documents discussing such applications.

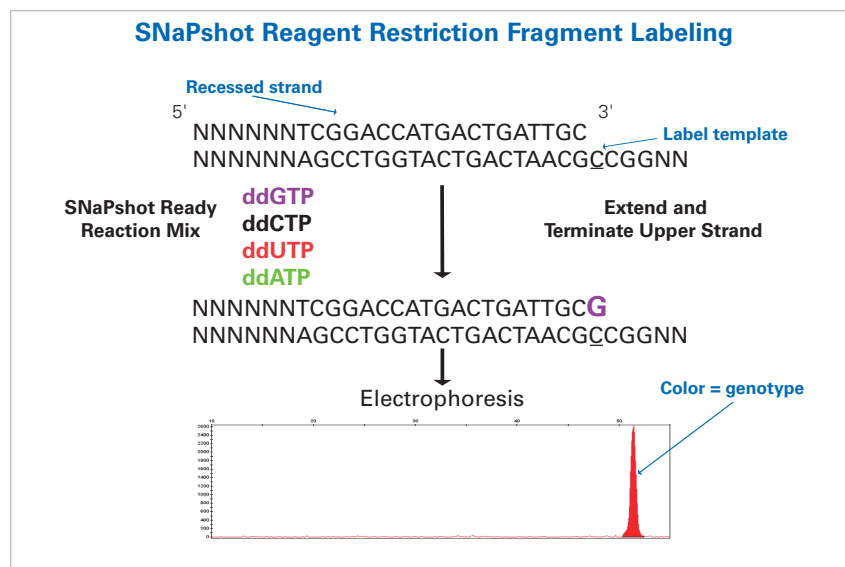


Figure 1. SNaPshot reagent labeling of restriction fragments.

it currently does not have supported run modules on these platforms.

In this Application Note, we will demonstrate how researchers can use the Any5Dye dye set feature in Data Collection Software v2.0 or higher to allow them to analyze [F]ddNTP fragments on their 3730/3730x1 instruments. The process of BAC fingerprinting presented in this Application Note was mainly developed in Dr. Jan Dvorak's laboratory at the University of California, Davis. More information about this technique can be obtained using the links provided on the next page.

## SNaPshot Kit Components for BAC Fingerprinting

The SNaPshot kit consists of the following three components:

### 1. The SNaPshot Multiplex Kit:

The kit offers a one-tube, single-base

extension/termination reagent for labeling. SNaPshot chemistry is based on the dideoxy single-base extension of an unlabeled oligonucleotide primer or primers. For BAC fingerprinting, the chemistry extends from the 3' recessed end of restriction fragments, with the 5' overhang serving as template (Figure 1). Four fluorescent dyes are used to distinguish each nucleotide.

### 2. GeneScan™-500 LIZ® Size Standard:

The labeled fragments are visualized on an Applied Biosystems DNA analyzer. A fifth dye, the GeneScan-500 LIZ Size Standard, is used in all samples as an internal size ladder for sizing precision. Fragments up to 500 base pairs (bp) can be sized within an hour, far less time than other methods require, making this process fast and economically feasible.

### 3. GeneMapper® Software v3.5:

Sizing information from GeneMapper Software v3.5 or higher is imported to downstream editing and contig assembly programs, such as FPC (FingerPrinted Contig) and GenoProfiler (see below), for further analysis.

#### BAC Fingerprinting Process

After the desired BACs are obtained, sample preparation begins with the following five steps (Figure 2):

1. Selective bacterial growth of single colonies
2. BAC purification
3. Restriction-endonuclease digestion
4. SNaPshot reagent labeling
5. Post-extension clean-up of the clones

Researchers have optimized these procedures and protocols in their laboratories. Protocols may differ as to the kind of restriction endonucleases (Table 1) and BAC DNA purification kits that are used, and whether enzymatic digestion and SNaPshot reagent labeling is performed in one tube or in separate reactions. We encourage researchers to optimize these procedures and protocols to suit their needs.

For more information about restriction endonuclease digestion and SNaPshot reagent labeling research and protocols, visit: <http://agronomy.ucdavis.edu/mcluo/fingerprinting.pdf>

#### Preparing the 3730/3730xl DNA Analyzer for Any5Dye runs

Before beginning the set-up procedures necessary for running SNaPshot kit samples on the 3730 or 3730xl DNA Analyzer, complete the following three steps:

1. Create a new run module with the indicated GeneMapper36\_POP7 settings (Figure 3).

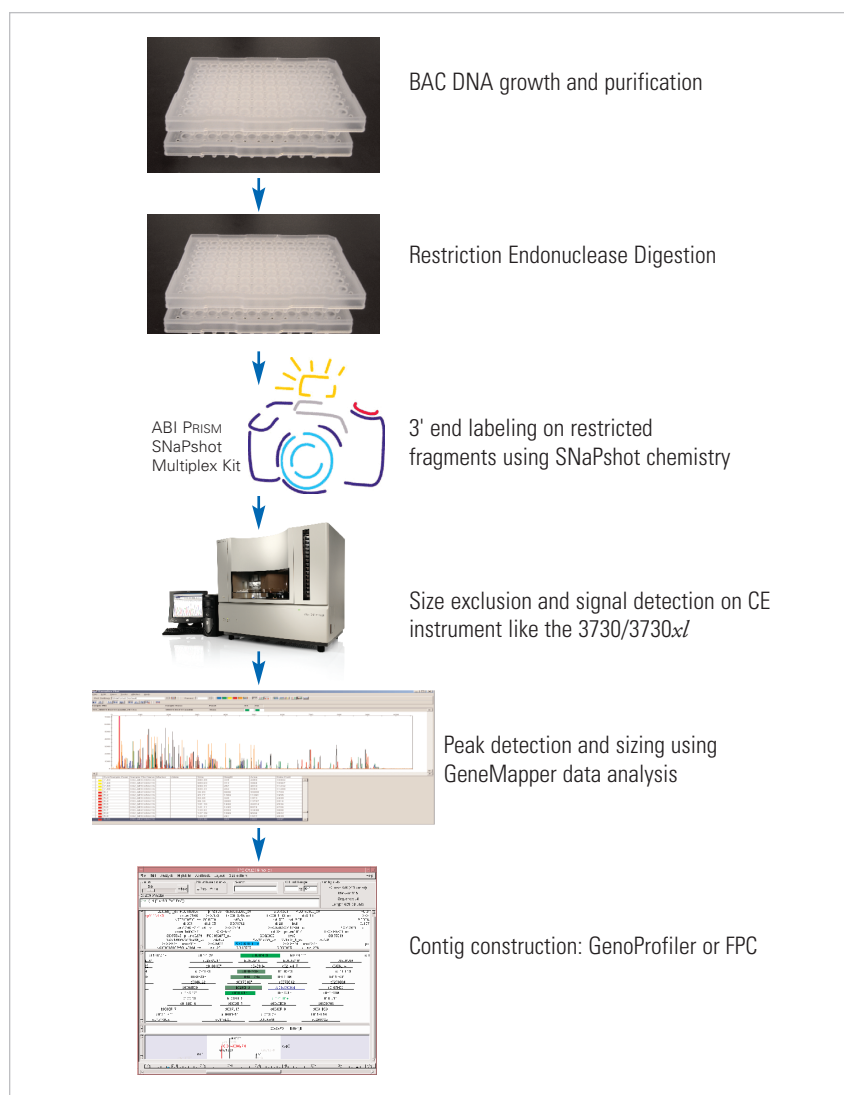


Figure 2. Flowchart of the BAC fingerprinting process.

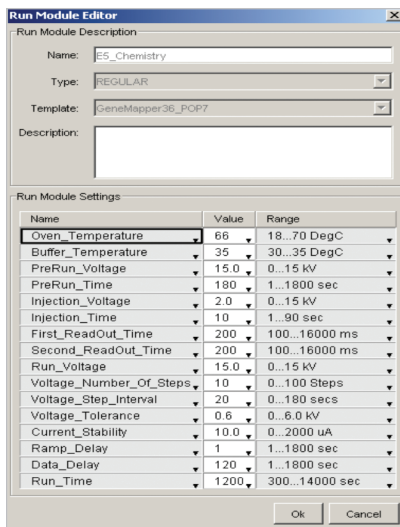
Restriction Endonuclease	Restriction Site	ddNTP	Fluorescent Dye Label	Restriction Fragment Color
EcoRI	G↓AATTC	A	dR6G	Green
BamHI	G↓GATCC	G	dR110	Blue
XbaI	T↓CTAGA	C	dTAMRA™	Yellow
XhoI	C↓TCGAG	T	dROX™	Red
HaeIII	GG↓CC	None		

Table 1. Example of possible six-base cutters for restriction endonucleases and dyes used in the SNaPshot Multiplex Kit.

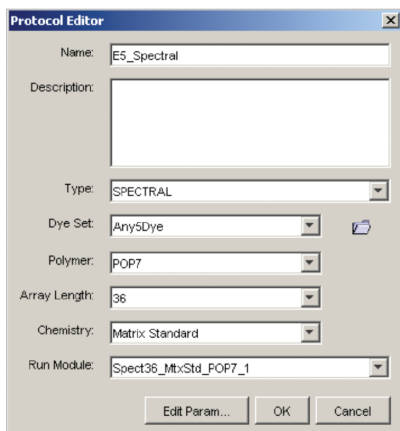
2. Create protocols for the spectral and regular run types with the Any5Dye dye set (Figure 4).

3. Install GeneMapper Software v3.5 or higher, if it is not already installed on your instrument computer. Your Service Engineer or Field Application Specialist can provide instructions, if needed.

**Note:** Additional information can be found in the 3730/3730xl DNA Analyzer User's Guide v2.0, which can be downloaded from the Applied Biosystems Web site at [www.appliedbiosystems.com](http://www.appliedbiosystems.com). To do so, enter the part number (4347118) in the Products window



**Figure 3.** Create a new Run Module, using the GeneMapper36\_POP7 setting as a template.



**Figure 4.** Create a new spectral protocol in Protocol Manager, using the Spect36\_MtxStd\_POP7\_1 setting.

of the Applied Biosystems home page. (You must be logged onto the myScience<sup>SM</sup> Research Environment to download or place an order.) GeneMapper Software v3.5 or higher is also available through either the Applied Biosystems Web site or your local sales representative.

### Creating a Spectral Protocol

1. From the left-hand navigation window of the 3730/3730x/ Analyzer Data Collection Software v2.0 or higher, select Protocol Manager.
2. From the Instrument Protocols area, select New. The Protocol Editor window should appear (Figure 4).

3. Choose a name for your spectral, i.e., E5\_Chemistry, in the Name field. (It is not necessary to fill in the Description field.) Then proceed as follows, making the appropriate selection from each field:

Field	Selection
Dye Set	Any5Dye
Polymer	POP7
Array Length	36
Chemistry	Matrix Standard
Run Module	Spect36_MtxStd_POP7_1

4. Select Edit Parameter at the bottom of the Protocol Editor window, and the Edit Spectral Params window will appear (Figure 5). 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 usual.

\*This parameter should be adjusted **only** if the spectral has failed and the condition number is not within the designated range. The capillary condition number is located in the Spectral Viewer.

- a. Choose the Any5Dye dye set and click on any well on the plate to display the condition number and quality score for a given capillary (Figure 6). The capillary condition number for each capillary may also be found in the Spectral Calibration Run Log [ E://Applied Biosystems/UDC/Data Collection/ Data/ga3730/ Instrument Number/ SpectralCalTmpFiles/ Any5Dye/SpectralCalResult-Run ].
- b. Determine the average condition number for the array by selecting several wells.
- c. Adjust the matrix condition number bounds to approximately  $\pm 1.5$  of the average condition number. A second spectral must then be run.

5. Prepare a spectral standard plate by following the protocol provided with the Matrix Standard Set DS-02 Kit (P/N 4322849), which was originally formulated for use on the ABI PRISM<sup>®</sup> 3700 DNA Analyzer. If the matrix-standard peaks are too strong (i.e., if pull-up or pull-down peaks are visible, (Figure 7), dilute the standards 2:1 with Hi-Di<sup>™</sup> Formamide and repeat the spectral run. When the spectral calibration has been completed (> 90% of passing caps), the instrument is ready for SNaPshot kit labeled samples.

### Creating a Run Module

1. Launch Data Collection Software v2.0.
2. Select Module Manager and then New. A Run Module Editor window will appear (Figure 3).
3. Fill in the appropriate fields in the Run Module Description area, as follows:

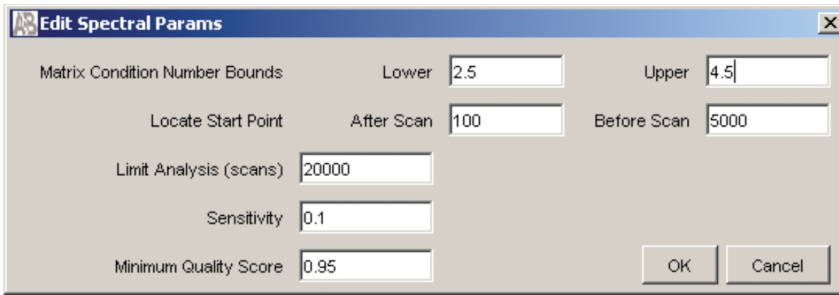
Field	Selection
Name	E5 Chemistry (for example)
Type	Regular
Template	GeneMapper36_POP7

### Creating a Regular Protocol

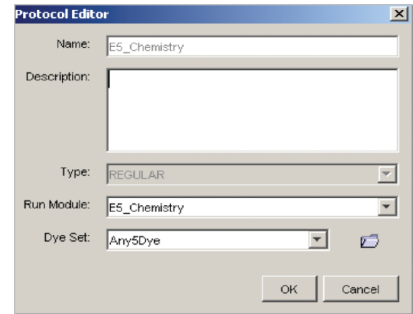
1. Select Protocol Manager in Data Collection Software v2.0 or higher.
2. Click the New button in the Instrument Protocols area, and the Protocol Editor window will appear (Figure 8).
3. Enter the name of your new instrument protocol (i.e., E5\_Chemistry), in the Name field. It is not necessary to fill in the Description field.
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 and click Ok.

### Begin Sample Runs on the 3730/3730x/ DNA Analyzer

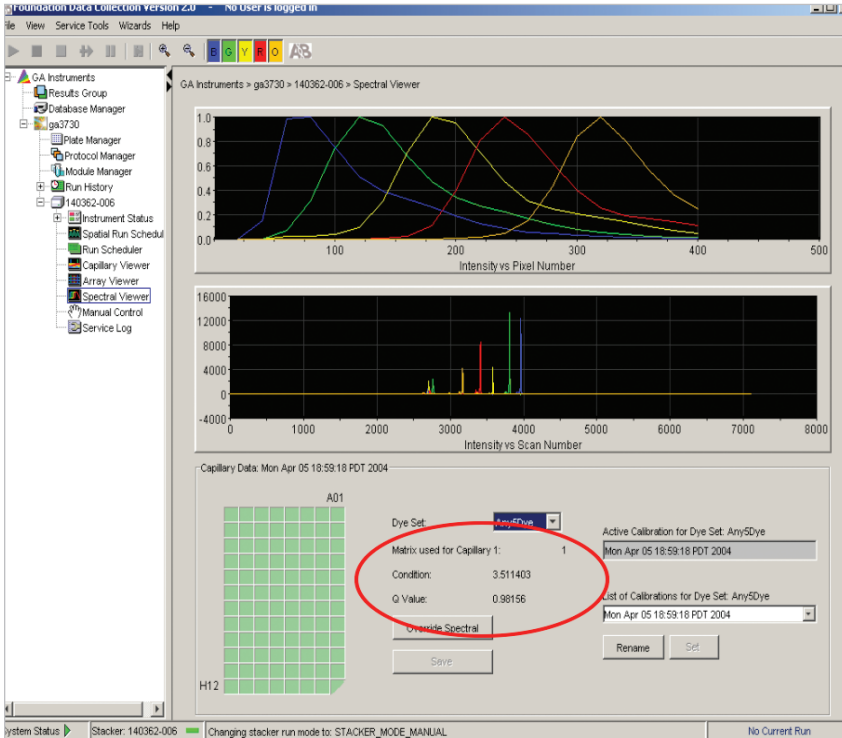
Run the sample plates using the newly created run modules and analyze the results with GeneMapper Software v3.5 or higher for simultaneous sizing and genotyping (Figures 9 and 10). The analyzed data can then be exported in a tab-delimited Excel<sup>®</sup> spreadsheet for further analysis or storage in a database.



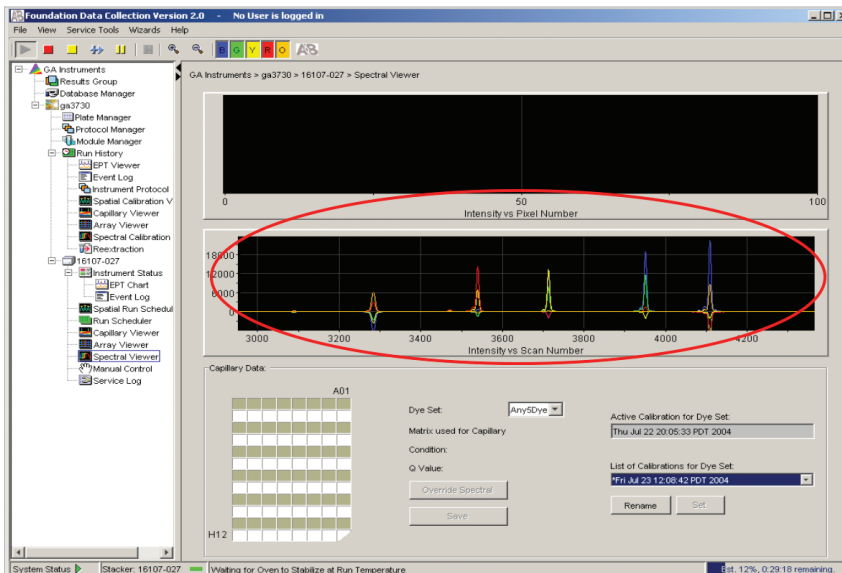
**Figure 5.** When setting up a spectral calibration file for SNaPshot kit chemistry, the parameters in the Edit Spectral Params window may need to be adjusted.



**Figure 8.** New Regular Run protocol created for running SNaPshot kit samples.



**Figure 6.** Spectral run of DS-02 Matrix Standard, using the Any5Dye dye set.



**Figure 7.** Failed spectral, showing pull-up and pull down.

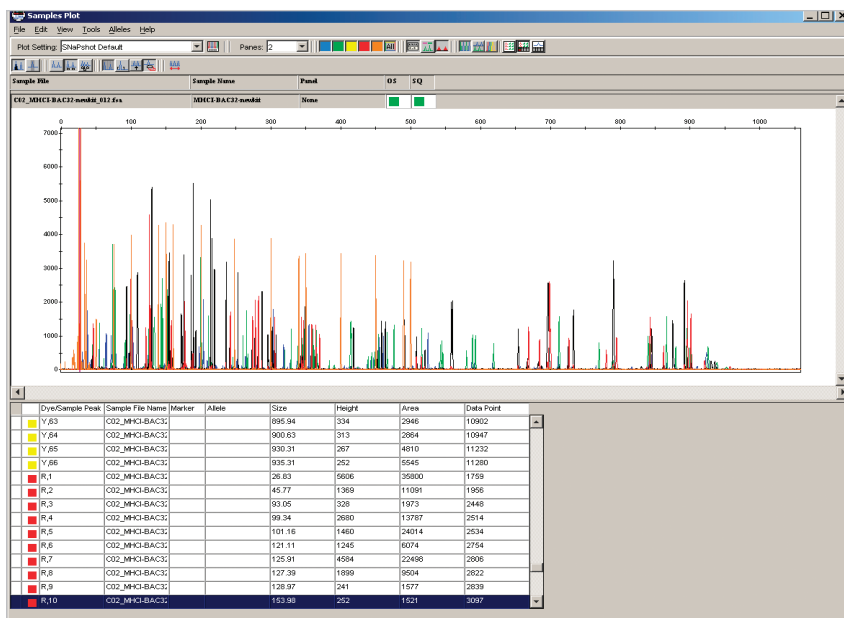
## Sizing Differences

In fragment analysis, a fragment is sized by its relative mobility to size standards as it passes through the polymer. Mobility of these fragments is affected by any change in run conditions, such as capillary length or polymer type. Sizing differences resulting from mobility changes between various types of polymer are more apparent for sequences < 50 bp. Due to the nature of the polymer, smaller fragments (< 50 bp) run on POP-7™ Polymer on the 3730/3730xl Analyzer may have slightly lower sizing accuracy.

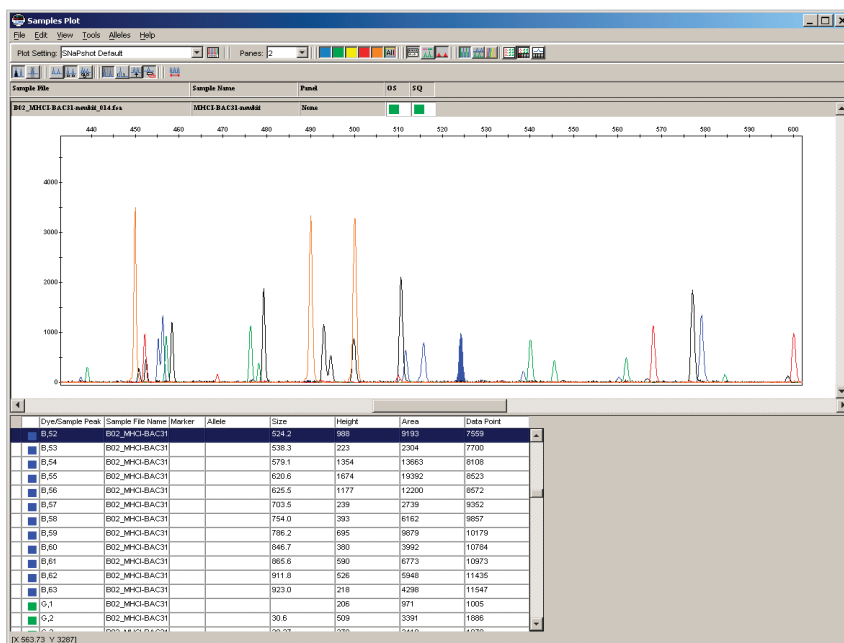
BAC fingerprinting is based upon pattern recognition; therefore, data analysis is focused on relative size and distribution precision. This is best achieved under well controlled conditions with standard operating procedures. To that end, Applied Biosystems recommends using a dedicated instrument platform for the sole purpose of ensuring low random error due to sizing imprecision.

## Creating FPC-Ready Files with GenoProfiler Software

Currently, the most popular contig assembly program is the FPC (FingerPrint Contig) program developed by Soderlund et al. (1997). FPC was initially developed for fingerprinting on polyacrylamide gel in which it compares the size of a set of restriction fragments (fingerprint) of two or more clones to determine



**Figure 9.** Electropherogram of BAC fingerprinting data analyzed on an Applied Biosystems 3730xL DNA Analyzer with POP-7 polymer, 36-cm capillary array and a LIZ500-3730 Std setting on GeneMapper Software v3.5. Data was analyzed by sizing the restriction enzyme digested fragments with GeneMapper Software v3.5.



**Figure 10.** Expanded view of BAC FP with LIZ500-3730 Std setting on GeneMapper Software v3.5.

the amount of similarity between these clones. The FPC algorithm then automatically clusters clones into contigs based on their probability of coincidence. Finally, the software builds a consensus band (CB) map that is similar to a restriction map.

For fingerprinting samples run on CE instruments, the graphical representation of restriction fragments have

changed from a gel image containing bands to one that contains different colored peaks with corresponding sizing information. Thus, to input such data to FPC, sample files from CE instruments must be converted from the multicolored and sized peaks to one that resembles a gel file. Currently, GeneMapper software does not provide a file conversion tool from .fsa files (output from GeneMapper

software) to .fpc files (input to FPC). Many customers have found the GenoProfiler Software to be useful for this and other applications. Developed at the University of California, Davis, GenoProfiler software supplies the cross-platform functionality required for more efficient downstream FPC analysis.

GenoProfiler software provides high-throughput, automated analysis from the size data created by GeneMapper Software v3.5. Software algorithms extract fragment size data from GeneMapper Software v3.5, automatically remove background noise and “false” bands, and create edited size files in FPC format (.fpc) for direct input to FPC for contig assembly.

GenoProfiler software is available at <http://wheat.pw.usda.gov/Physical Mapping> FPC is available at [www.genome.arizona.edu/software/fpc](http://www.genome.arizona.edu/software/fpc) A detailed description of GenoProfiler Software and a protocol are available at <http://wheat.pw.usda.gov/Physical Mapping/tools/genoprofiler>

### Increased Productivity with the 3730/3730xL DNA Analyzer

BAC fingerprinting techniques have become increasingly automatable. For performing multiple restriction endonuclease digestions, or both digestions and labeling in one step, these protocols are increasingly efficient and cost-effective. With the use of robotics, processing thousands of BAC clones per day is now feasible.

Although the 16-capillary ABI PRISM® 3100 Genetic Analyzer has the ability to perform the same separations, researchers increasingly want to leverage the high-throughput capacity of the Applied Biosystems 3730/3730xL Analyzer for BAC fingerprinting (Table 2).

Instrument	Samples processed/run	Samples processed/8 hr
3100	16	320
3730	48	960
3730 <i>xl</i>	96	1920

**Table 2.** Throughput for BAC Fingerprinting on three Applied Biosystems instruments.

### Acknowledgement

We would like to acknowledge Dr. Jan Dvorak, Dr. Ming-Chen Luo, and their staff at the University of California, Davis, and Dr. Cari Soderlund at the Arizona Genome Computational Laboratory, University of Arizona, Tucson, for the protocols and software that they developed for BAC Fingerprinting and for the data that they supplied for this Application Note. We believe that their contributions will be valuable to other researchers as well.

### References

1. M.C. Luo et al., "High-throughput fingerprinting of bacterial artificial chromosomes using the SNaPshot labeling kit and sizing of restriction fragments by capillary electrophoresis," *Genomics* 82 (2003) 378–389.

2. Frank You at <http://wheat.pw.usda.gov/PhysicalMapping/tools/genoprofiler/genoprofiler.html>

3. Engler, F., J. Hatfield, W. Nelson, and C. Soderlund (2003). "Locating sequence on FPC maps and selecting a minimal tiling path." *Genome Research* 13:2152:2163.

4. Soderlund, C., S. Humphrey, A. Dunhum, and L. French (2000). "Contigs built with fingerprints, markers and FPC V4.7." *Genome Research* 10:1772-1787.

5. FPC home page: [www.genome.arizona.edu/software/fpc/](http://www.genome.arizona.edu/software/fpc/)

### Ordering Information

Description	Quantity/Instrument	P/N
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
3730/3730 <i>xl</i> DNA Analyzer User's Guide v2.0		4347118
GeneMapper® Software v3.5 (5 User Kit)		4346648
GeneScan™ 120 LIZ® Size Standard		4324282
48 capillary x 36 cm Capillary Array	3730 DNA Analyzer	4331247
96 capillary x 36 cm Capillary Array	3730 <i>xl</i> DNA Analyzer	4331244
Hi-Di™ Formamide		4311320
Matrix Standard Set DS-02 (dR110, dR6G, dTAMRA™, dROX™ LIZ® Dyes)	3700 DNA Analyzer	4322849
FG POP-7™ Polymer	15 bottles/box, 375 mL	4335612



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