BAC Fingerprinting Application on Applied Biosystems 3730/3730xl DNA Analyzers

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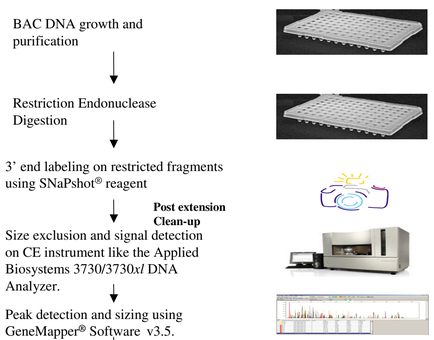
Abstract

Many researchers today in the field of genomic research have found the use of large genomic fragment libraries, such as bacterial artificial chromosomes (BACs), to be crucial. New BAC libraries require a rapid and efficient method for characterization and assembly of clones into contigs that are then arrayed into physical maps of the chromosome. One widely used technique to generate these physical maps reflecting the sequence of chromosomes from contigs is by fingerprinting fragments of BACs generated from restriction enzymatic digests with fluorescently labeled dideoxynucleotides ([F]ddNTPs). The ABI PRISM® SNaPshot® Multiplex System, a kit originally developed for SNP validation and screening, offers a readily available solution for BAC Fingerprinting application. Although the SNaPshot® system currently does not have supported run modules on the Applied Biosystems 3730/3730*xl* DNA Analyzers, the high throughput nature of BAC Fingerprinting has led many researchers to inquire about the possibility of running this application on a high throughput instrument like the 3730/3730xl platforms. Here, we will demonstrate how researchers had utilized the Any5Dye dye set feature in Data Collection Software v2.0 to allow them to run SNaPshot® System samples on their 3730/3730xl instruments.

Process Setup of BAC Fingerprinting



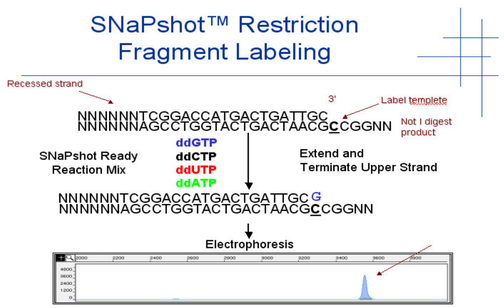
SNaPshot System Components for BAC Fingerprinting

•1. **The SNaPshot Multiplex Kit**: The kit offers a one-tube, single-base extension/termination reagent for end labeling of unlabeled, sequence specific oligonucleotide primers using fluorescently labeled dideoxynucleotides. 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**: A fifth dye internal standard is used in all samples as an internal size ladder for automated peak detection and size interpolation. Fragments up to 500 base pairs (bp) can be sized within an hour, far less time than other methods require, making it fast and economically feasible.

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

Use of SNaPshot Reagent in BAC Fingerprinting



Possible Restriction Endonucleases In BAC Fingerprinting

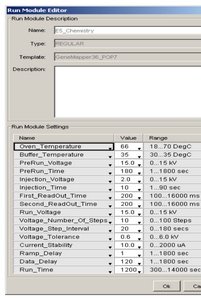
Restriction Endonuclease	Restriction Site	ddNTP	Fluorescent Dye Label	Restriction Fragment Color
*Eco*RI	G↓AATTC	A	dR6G	Green
*Bam*HI	G↓GATCC	G	dR110	Blue
*Xba*I	T↓CTAGA	C	dTAMRA	Yellow
*Xho*I	C↓TCGAG	T	dROX	Red
*Hae*III	GG↓CC	None		

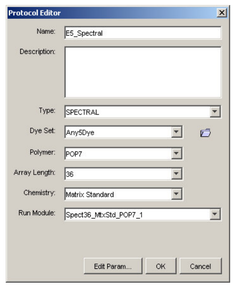
Data courtesy of Dr. Luo, Department of Plant Sciences, University of California, Davis

Set up procedure of 3730/3730*xl* Analyzer to run BAC Fingerprinting samples

- New Run Module w/ GeneMapper36_POP7.
- New Spectral and Regular Run protocols need to be created with Any5Dye dye set.
  - Spectrals are run using 3700 Matrix Standards DS-02.
  - Spectrals are stored under Any5Dye dye set.
- GeneMapper® Software v3.5 installed on 3730/3730*xl* Analyzer.

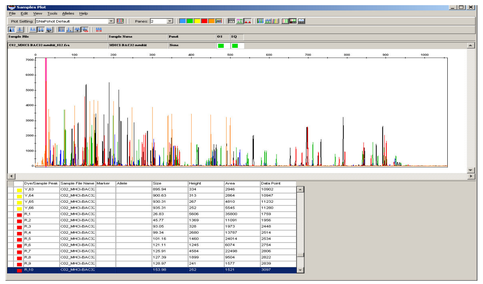
Create a new run module, using the GeneMapper36_POP7 setting as a template.





Create a new spectral protocol in Protocol Manager, using the Spect36_MtxStd_POP7_1 setting.

GeneMapper Software analyzed results



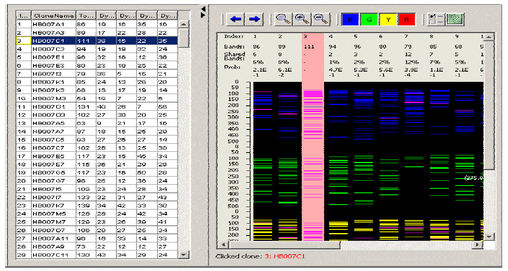
Electropherogram of BAC fingerprinting data analyzed on an Applied Biosystems 3730*xl* DNA Analyzer with a POP-7™ Polymer, 36-cm capillary array and a "LIZ500-3730 Std" setting on GeneMapper® Software v3.5.

Data courtesy of Dr. Luo, Department of Plant Sciences, University of California, Davis

Post GeneMapper Software Data Analysis: GeneMapper Software data output to GenoProfiler.

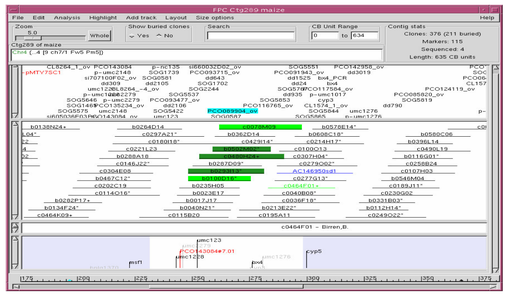
- Software algorithms extract fragment size data from GeneMapper® Software v3.5.
- Automatically removes background noise and "false" bands.
- Creates edited size files in FPC format (.fpc) for direct input to FPC for contig assembly.

Sizing data conversion to FPC readable format



Data courtesy of Dr. Luo, Department of Plant Sciences, University of California, Davis.

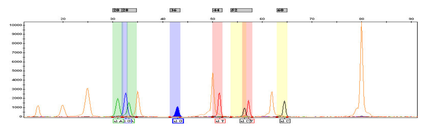
FPC created contigs



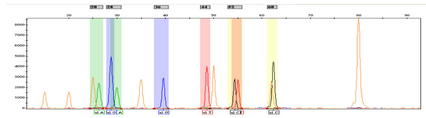
Data courtesy of Dr. Cari Soderlund, Arizona Genome Computational Laboratory, University of Arizona, Tucson

Sizing differences in small fragments

- BAC fingerprinting is based upon pattern recognition; therefore, data analysis is focused on relative size and distribution. This is best achieved under well controlled conditions w/ 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.
- Sizing differences between various types of polymer are more apparent for sequences < 50 base pairs (bp). Smaller fragments (< 50 bp) run on POP-7™ Polymer on 3730/3730xl Analyzer may have slightly lower sizing precision.



SNaPshot® Control Template on 3730xl POP-7™ Polymer



SNaPshot® Control Template on 3100 POP-4™ Polymer

Conclusion

BAC fingerprinting techniques have become increasingly efficient and cost-effective due to enhancements in reagent chemistry and automated sample handling and analysis. Processing thousands of BAC clones per day is now feasible. The Any5Dye feature in Data Collection Software v2.0, in conjunction with commercially available fluorescent labeled, dideoxynucleotides, enables researchers to leverage the high-throughput capacity of the Applied Biosystems 3730/3730*xl* platforms for BAC fingerprinting. Although currently this is not a fully supported application, we are continually working towards developing a more integrated and complete system for BAC fingerprinting. We welcome your comments and suggestions to help guide the future direction of this project.

References

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- FPC home page: www.genome.arizona.edu/software/fpc/

Disclaimer

Certain customers of Applied Biosystems have demonstrated that the Applied Biosystems 3730, 3730xl DNA Analyzer 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.

PLEASE REFER TO THE SNaPshot Kit USER'S MANUAL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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 data that they supplied for this poster. We believe that their contributions will be valuable to other researchers as well.

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