Sizing of Large DNA Fragments

Generated by BAC Fingerprinting on Capillary Electrophoresis System

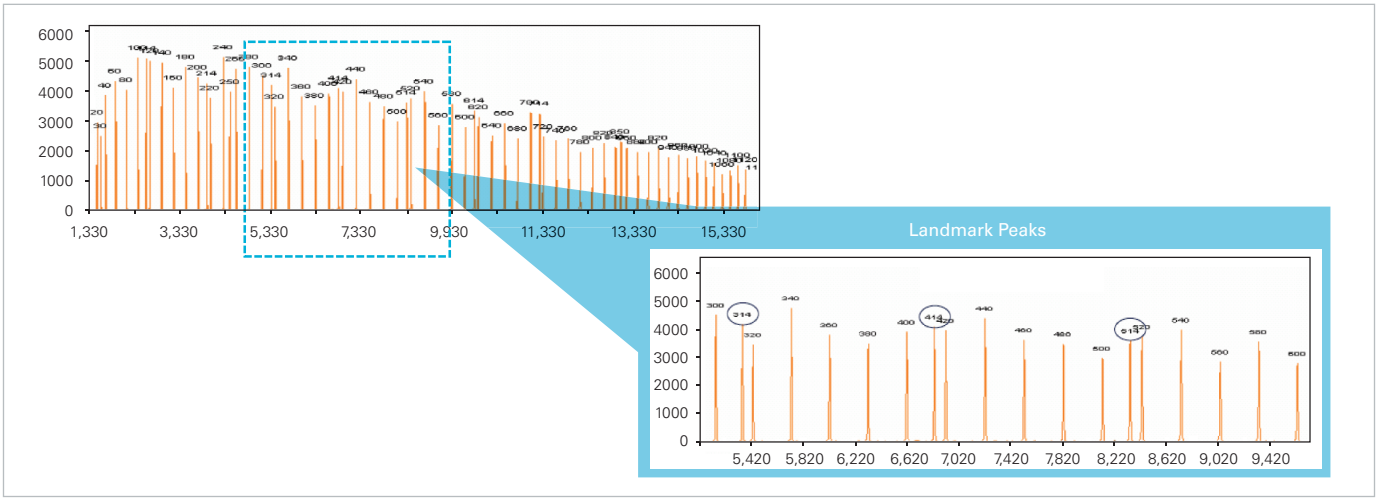

Figure 1. Electropherogram of the GeneScan™ 1200 LIZ® Size Standard. Expanded range shown between 300 - 600 bp provides a clear illustration of the landmark fragments.

Introduction

BAC fingerprinting provides an efficient and cost-effective method of characterizing large genomic fragment libraries for genome sequencing, positional cloning, and physical mapping efforts.

Restriction endonuclease digestion of BAC clones followed by fluorescent dye labeling can be used to generate a profile or fingerprint. Overlap between fingerprints are subsequently used to assemble contiguous sequences (contigs) in the construction of whole-genome physical maps. Physical maps are important resources for genome sequencing efforts, positional cloning, comparative genomics, and to determine the size and structure of genomes.

In this application note, the GeneScan 1200 LIZ Size Standard (a high-density size standard for sizing DNA fragments up to 1200 bp with a high degree of precision) is used along with the SNaPshot® Multiplex System for BAC fingerprinting of wheat DNA fragments analyzed by capillary electrophoresis on an Applied Biosystems 3730*xl* DNA Analyzer.

Overview of the GeneScan 1200 LIZ Size Standard

The GeneScan 1200 LIZ Size Standard is designed for larger DNA fragment sizing applications, and leverages Applied Biosystems proprietary 5-dye technology.

- Fragments from 20—1200 bp enable analysis of a wide range of DNA fragment lengths
- High fragment density (68 fragments) yields greater sizing precision
- Landmark fragments included for easy peak pattern identification during data analysis
- Size standard fragments labeled with LIZ dye for 5-dye fragment analysis applications, allowing 33% greater multiplexing throughput than 4-dye applications
- Ideal for BAC fingerprinting, AFLP, T-RFLP, VNTR, STR, and many other DNA fragment analysis applications

Overview of the SNaPshot Multiplex System:

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 oligonuceotide primer or primers. For BAC fingerprinting, the chemistry extends from the 3′ recessed end of restriction fragments, with the 5′ overhang serving as template. Four fluorescent dyes are used to distinguish each nucleotide.

The BAC Fingerprinting Workflow

After the desired BACs are obtained, sample preparation begins with the following steps:

1. Selective growth of BAC containing bacteria
2. BAC DNA purification
3. Restriction endonuclease digestion
4. Fragment end-labeling using the SNaPshot® Multiplex Kit
5. Post-extension clean-up of the labeled fragments

6. Separation of fragments on a capillary electrophoresis instrument

The workflow begins with the growth and isolation of the BAC DNA. This is followed by restriction endonuclease digestion of the BAC clones with several different enzymes. The fragments are then labeled with fluorescent dye-labeled primers included in the SNaPshot kit. The dye-labeled primers are bound to the BAC fragments based on the overhangs left by the restriction enzymes (see Table 1).

The pool of dye-labeled DNA fragments are analyzed on a capillary electrophoresis system such as the Applied Biosystems 3730*xl*. Because the technique can generate large DNA fragments, the GeneScan 1200 LIZ Size Standard is chosen for analysis with the dye labeled BAC fragments. When analyzed by electrophoresis, the dye-labeled BAC fragments generate a fragment "fingerprint." Overlap between fingerprints are used to assemble contigs for the construction of whole-genome physical maps. These physical maps serve as important resources for genome sequencing efforts, positional cloning, comparative genomics, and to determine the size and structure of genomes.

Precision Sizing of Large Wheat DNA Fragments

In this study, control wheat BAC samples were digested, labeled, and analyzed on the Applied Biosystems 3730*xl* Genetic Analyzer using POP-7™ polymer, a 50-cm capillary array and using GeneMapper® v4.0 Software. Sizing precision (allele size standard deviation) per run was calculated from 276 samples. (See Figures 2 and 3 for analysis results)

TABLE 1. RESTRICTION ENDONUCLEASES IN BAC FINGERPRINTING

Enzyme	Site	ddNTP	Dye	Color
*Eco*RI	G^AATTC	A	dR6G	Green
*Bam*HI	G^GATTC	G	dR110	Blue
*Xba*I	T^CTAGA	C	dTAMRA™	Yellow
*Xho*I	C^TCGAG	T	dROX™	Red
*Hae*III	GG^C	None		

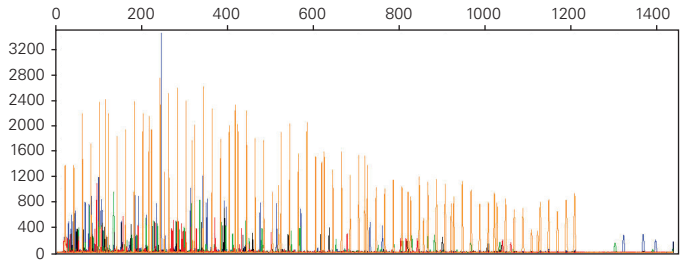

Figure 2. GeneMapper analysis results of BAC fingerprinting data using the new GeneScan™1200LIZ® Size Standard. DNA fragments from 40 to 1000 base pairs were analyzed with GeneScan 1200 LIZ Size Standard.

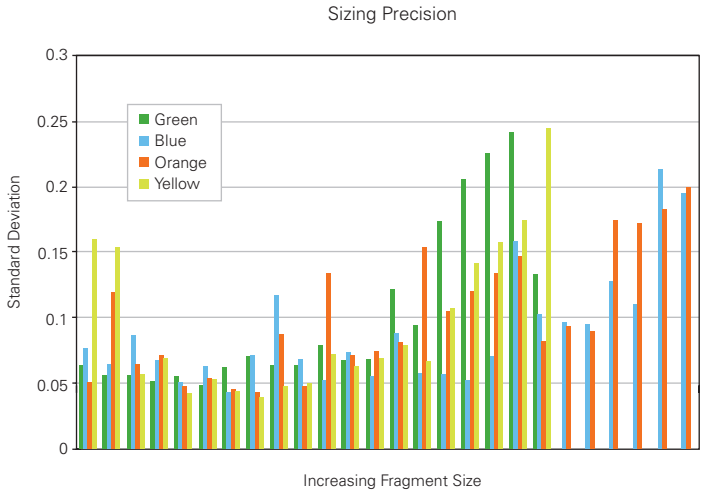

Figure 3. Sizing precision Comparison per Fragment
Sizing precision of various labeled control wheat BAC fragments labeled using the SNaPshot® Kit along with GeneScan 1200 LIZ Size Standard.

Figure 2 & 3 Data courtesy of Drs. M.-C. Luo and Y. Ma, Department of Plant Sciences, University of California at Davis

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