Amplified Fragment Length Polymorphism (AFLP®) Analysis on Applied Biosystems Capillary Electrophoresis Systems

Introduction

Amplified fragment length polymorphism (AFLP®) analysis is a genetic mapping technique that uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprint for a particular genome. First developed for plant studies, AFLP analysis is used for a variety of applications, such as:

- Creation of genetic maps for new species
- Determination of relatedness among cultivars
- Establishment of linkage groups in crosses
- Genetic diversity and molecular phylogeny studies

The power of AFLP analysis derives from its ability to quickly generate large numbers of marker fragments for any organism, without prior knowledge of the genomic sequence. In addition, AFLP analysis requires only small amounts of starting template and can be used for a variety of genomic DNA samples.

The AFLP procedure consists of two amplification steps: a low-level or pre-selective amplification, followed by a more selective amplification, which generates a set of fragments that can be used as the discriminatory marker set for a particular sample (Figure 1).

Polymorphisms in band patterns map to specific loci, allowing individuals to be genotyped or differentiated based on the alleles they carry.

Performing Successful AFLP Analysis

The success of an AFLP assay depends on three factors:

1. Optimized reagents
2. A robust and reliable electrophoresis platform
3. Effective analysis software

In this Application Note, we will use the Applied Biosystems Plant Mapping

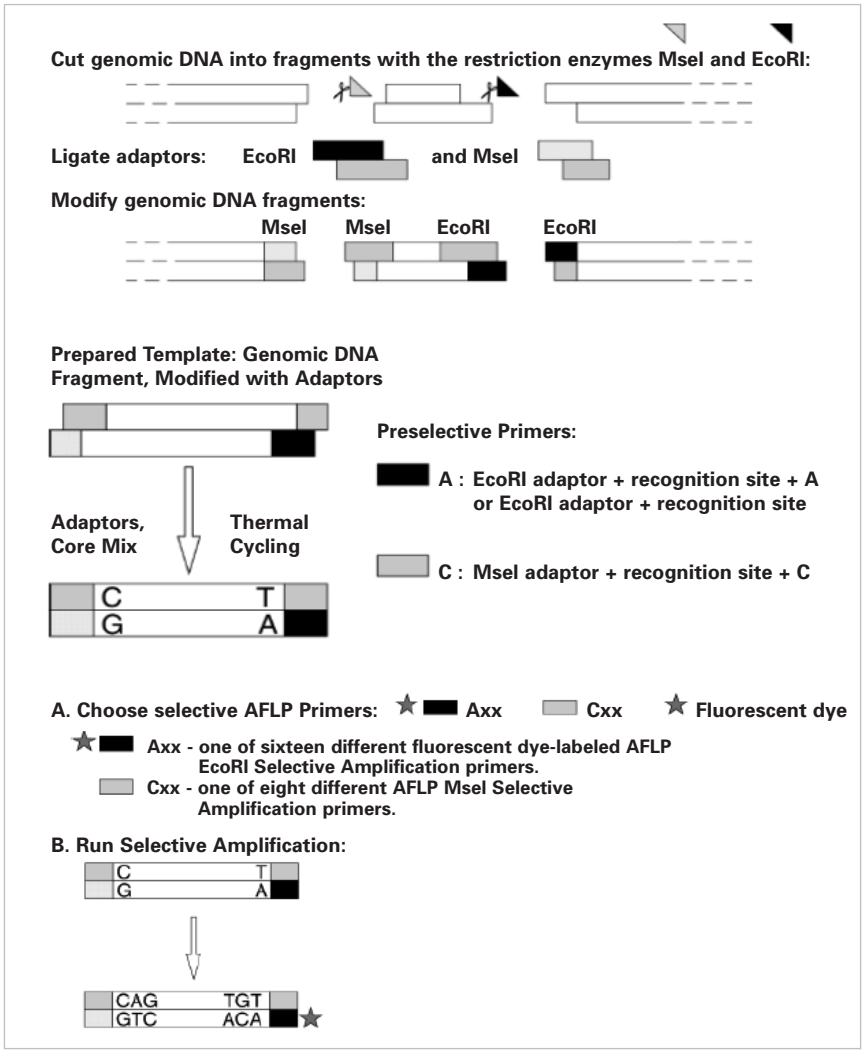

Figure 1. Flowchart of steps required for an AFLP assay.

Kit with genomic DNA samples from maize to demonstrate that the Applied Biosystems capillary electrophoresis platforms, the Applied Biosystems 3130 series Genetic Analyzers or the Applied Biosystems 3730 series DNA analyzers in conjunction with GeneMapper® v.3.7 software or higher provides a complete solution that enables researchers to perform accurate and reproducible AFLP projects in a routine fashion.

The AFLP Assay

When setting up an AFLP assay, an optimization step may be necessary to identify those primer combinations that will generate sufficient unique marker fragments for a study. The Applied Biosystems Plant Mapping Kit contains eight selective EcoRI primers for regular plant genomes, eight selective EcoRI primers for small plant genomes and eight MseI primers. The primers are labeled with either 5-FAM™, NED™, or JOE™ fluorophores (dye set F). This kit allows researchers to choose from 128 possible primer combinations that have been tested across several crop genomes, enabling them to identify the optimal pair(s) for a given organism without having to design, synthesize, or perform quality control tests of their primers.

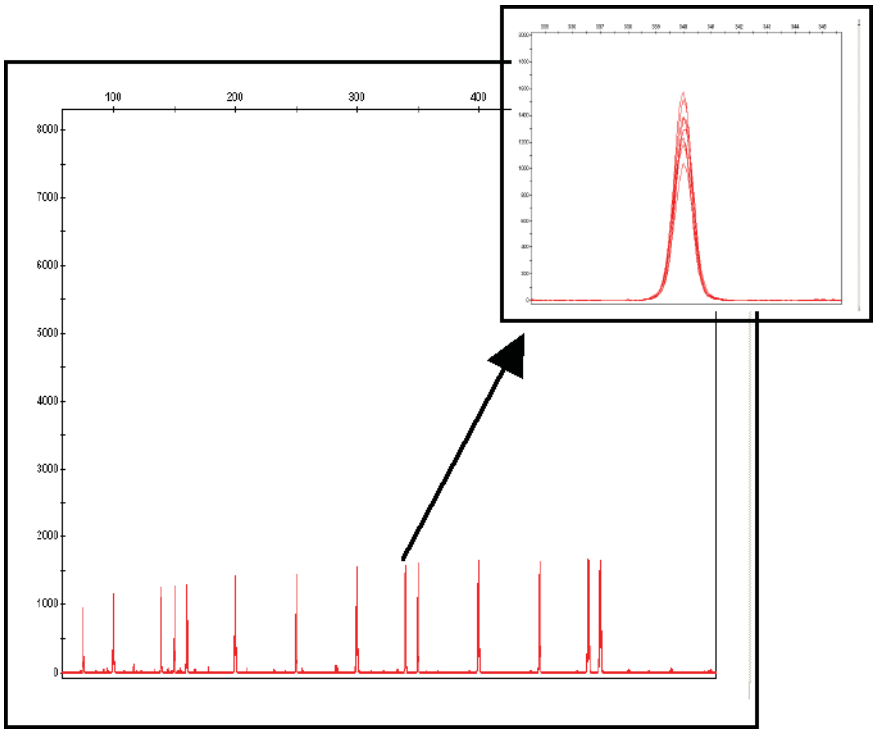

Figure 2. Overlay of size-standard electropherograms generated from 16 runs of the same sample containing the GeneScan 500 ROX size standard. A magnified view of one set of peaks highlights the sizing precision of the Applied Biosystems capillary electrophoresis platforms.

Capillary Electrophoresis

The Applied Biosystems 3130 series Genetic Analyzers and 3730 series DNA Analyzers are fully automated, high-performance, fluorescence-based, multi-capillary systems that can run 4 (3130 Genetic Analyzer), 16 (3130*xl* Genetic Analyzer), 48 (3730 DNA Analyzer) or 96 (3730*xl* DNA Analyzer) samples. Sample analysis on these instruments is fully automated, from the moment each 96- or 384-well plate is placed on the instrument and the run is initiated. Each of the systems provide continuous, unattended operation, from automated polymer loading and sample injection to separation, detection, and data generation.

When the Autoanalysis feature of GeneMapper® software is used for data analysis, the systems offer true one-button operation from sample loading to data analysis. The instruments also contain several features, such as:

- Enhanced sizing precision (Figure 2) from a detection cell heater that facilitates better thermal control
- An automated polymer delivery system that significantly reduces set-up time and cost
- Easy to use wizards for instrument operation

Automated Data Analysis with GeneMapper® Software v3.7 or higher

Crucial to the successful completion of an AFLP assay is the availability

Table 1. Advanced data analysis capabilities of GeneMapper Software.

Samples | Genotypes

	Sample File	Dye	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	Allele 12	Allele 13	Allele 14	Allele 15	Allele 16	Allele 17	Allele 18	Allele
1	004_B02_aflp_20	B	1	0	0	1	0	1	1	0	1	0	1	1	0	1	1	1	1	1	0
2	008_D02_aflp_20	B	0	0	1	0	0	1	0	0	0	0	1	1	1	1	0	1	0	1	1
3	010_E02_aflp_20	B	0	1	1	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1

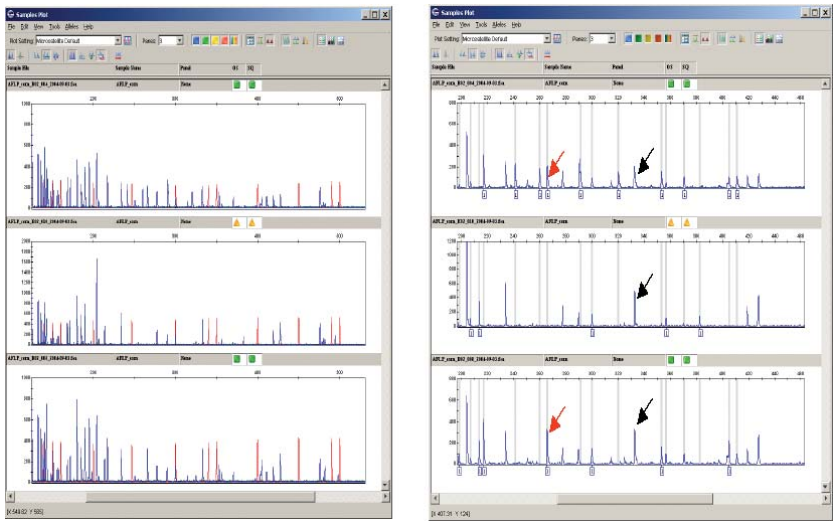

Figure 3. Analysis of AFLP assay data from the P1, P2, and F1 samples. Plots on the left contain the AFLP peaks (blue) and size standard peaks (red) for the three samples. The plot on the right side shows the bins (gray bars) and allele calls. Data from the 3130*xl* Genetic Analyzer are presented here.

of analysis software that can accurately and reproducibly score AFLP samples. GeneMapper Software v3.7 or higher offers a new AFLP analysis method and several new features for accurate and reproducible AFLP analysis. These features include the ability to use the sample files to generate the panels which are the collection of markers for that sample set. In addition, the software also contains a custom plot feature that allows multiple plots, each with a unique color, to be overlaid.

The software uses advanced analysis algorithms that can rapidly and accurately identify common and polymorphic peaks among large numbers of samples. Genotyping quality (GQ) scores flag sample data with lower quality for manual review.

When analysis is complete, the software automatically compiles the marker genotype results in a standard binary format (Table 1), which can be exported for further downstream analysis. The software also contains a convenient new Report Manager feature that generates reports containing final results that can be printed or exported for further analysis. Researchers can customize the format of these reports to meet the needs of their individual projects.

Parentage Testing with AFLP Analysis

To highlight the use of the 3130 and 3730 series platforms for AFLP analysis with a specific application, a parentage assay was performed on maize DNA samples isolated from three individuals:

1. Parent one (P1)
2. Parent two (P2)
3. The first-generation offspring (F1) from a cross between P1 and P2

Primers labeled with the 5-FAM™ dye from the Applied Biosystems AFLP® starter kit were used for selective amplification, and the reaction products were analyzed using a 36-cm capillary array and POP-7™ polymer. The reaction products were mixed with the GeneScan™-500 ROX™ dye-labeled size standard and electrophoresed on the 3130*xl* Genetic Analyzer and the 3730*xl* DNA Analyzer using the following protocols:

- FA_36_POP-7 run module and F dye set (3130*xl* Genetic Analyzer)
- GeneMapper_36_POP-7 run module and Any4Dye set (3730*xl* DNA Analyzer)

For the 3730 series systems the user should review all the data from the spectral calibration when using the AnyDye feature to verify that no poor quality data was used. Condition Numbers and Quality Score boundaries should be optimized for each dye set. Refer to the "BAC Fingerprinting on the Applied Biosystems 3730/3730*xl* DNA Analyzer" application note (S/N 107AP04-01) for more detail on this procedure.

The peak patterns in the F1 sample are consistent with those of a cross between P1 and P2 (Figure 3). In addition to several non-polymorphic peaks in both

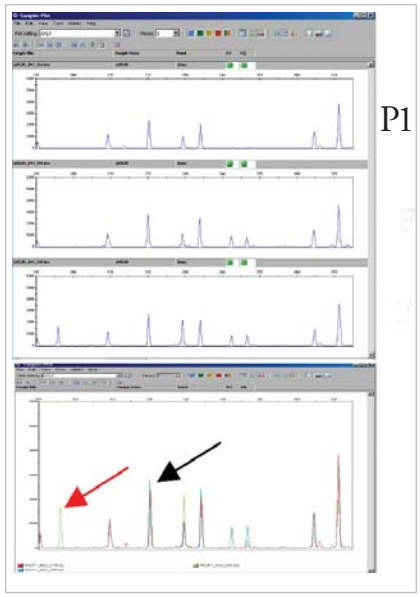

Figure 4. Electropherograms overlaid with custom plot colors. The top three panels show a section of plots for the P1, P2, and F1 samples, while the lower panel shows the same three plots overlaid using the "custom plot color" option. The P1 peaks are red, P2 peaks are green and F1 peaks are in blue. The black arrow indicates a common peak and the red arrow indicates a polymorphic peak. Data from 3730*xl* DNA Analyzer are shown here.

P1 and P2, which are also present in F1 (black arrow in Figure 3), unique peaks present in the F1 sample that were inherited from one parent (red arrow in Figure 3) can also be identified.

Researchers can use the "custom plot colors" option in GeneMapper software for easy identification of polymorphic peaks (red arrow) and common peaks (black arrow). For example, in the data shown in Figure 4, a polymorphic peak can be easily identified in the P1 sample (red arrow). The use of the POP-7 polymer offers advantages such as high resolution, especially for large fragment sizes, which should result in more accurate allele calling by GeneMapper software, and superior sizing precision (Figure 2) with reduced run times.

Conclusion

The Applied Biosystems capillary electrophoresis platforms, 3130/3130*xl* Genetic Analyzers and the 3730/3730*xl* DNA Analyzers, in conjunction with optimized AFLP® primer kits and GeneMapper® Software v3.7 or higher, provide a complete solution for performing AFLP assays. System features include high resolution, superior sizing precision, low turnaround times, and ease of use. The integration of GeneMapper software with these instruments provides one-button operation for sample loading, data collection, fragment size-calling, and accurate marker scoring in both graphical and standard binary formats. All of these features enable the generation of ample high-quality data with minimal hands-on time required and little need for manual data review.

Ordering Information

Description	P/N
3130*xl* and 3100 Capillary Array (36 cm)	**4319531**
3130 and 3100-Avant Capillary Array (36 cm)	**4315931**
3730 Capillary Array (36 cm)	**4331247**
3730*xl* Capillary Array (36 cm)	**4331244**
3130 POP-7™ Polymer	**4352759**
3730 POP-7™ Polymer (5 pack)	**4335615**
10X 3130 Genetic Analyzer Buffer with EDTA	**402824**
10X 3730 Running Buffer with EDTA	**4335613**
Hi-Di™ Formamide	**4311320**
AFLP Ligation/Preselective Amplification Module	**402004**
AFLP® I Selective Primer Kit	**4303050**
AFLP® II Selective Primer Kit	**4303051**
Matrix Standard Set DS-32	**4345831**
GeneScan™-500 ROX™ dye-labeled Size Standard	**401734**

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