

## Software for Amplified Fragment Length Polymorphism Analysis

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### Introduction

**Amplified Fragment Length Polymorphism (AFLP)** is a polymerase chain reaction (PCR) based genetic fingerprinting technique developed in the early 1990's by Keygene. AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of complementary double stranded adaptors to the ends of the restriction fragments. A subset of the restriction fragments are then amplified using 2 primers complementary to the adaptor and restriction site fragments. The fragments are visualized on denaturing polyacrylamide gels through either autoradiographic or fluorescence methodologies<sup>1</sup>.

The AFLP technology has the capability to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As a result, AFLP has become widely used for the identification of genetic variation in strains or closely related species of plants, fungi, animals, and bacteria. The AFLP technology has been used in criminal and paternity tests, in population genetics to determine slight differences within populations, and in linkage studies to generate maps for QTL analysis<sup>2</sup>.

GeneMarker<sup>®</sup> is an efficient, user-friendly software tool designed for the analysis of data generated by AFLP technology. The software is compatible with electrophoresis systems worldwide, including **ABI** (Applied BioSystems) files, **MegaBACE** files, and **SpectruMedix** files, as well as slab gel output. The software features high efficiency allele calling, adjustable parameters and various reporting options including a trace comparison report.

### Procedure

The software settings shown below are recommended to produce the best results for AFLP analysis. The correct settings for AFLP should include a low peak detection threshold and stutter filter, in order to detect small peaks as well as large peaks. The stutter peak filter is designed to remove stutter peaks within 2.5 bp of each detected allele peak. If the user would like to decrease the amount of false positives, then the stutter peak filter percentages can be increased. If the user would like to see all peaks, even those with minimal intensities, then the peak detection thresholds can be set to zero and the stutter peak filter can be turned off.

#### Suggested Analysis Parameters:

1. **Analysis Type:** AFLP
2. **Peak Detection Threshold:** Intensity >100; Percentage >1 Max; Local Region % > 1 Local Max.
3. **Stutter Peak Filter:** (%) Left: 5 Right: 5
4. **Plus-A Filter:** selected
5. **Allele Evaluation Score:** Reject < 1 Check 7 < Pass; **Unconfidence at Rightside Score** < 30

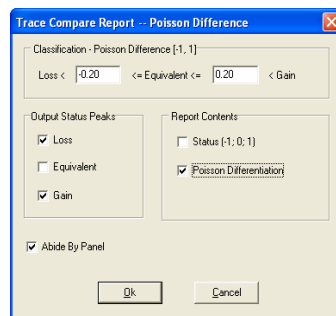
### Trace Comparison

After running the data with a size standard and panel, there are a few different reports and tools available to identify the presence and/or absence of alleles within sample traces. The Trace Comparison tool is designed primarily for AFLP data to identify length polymorphisms between closely related species.

The Trace Comparison tool is accessible through the Applications menu in the main toolbar. A window will open displaying two quantification methods: Poisson Difference and MLPA (Multiplex Ligation-dependent Probe Amplification) ratio. Please choose Poisson Difference unless working with MLPA data.

The trace comparison function uses Poisson distribution to calculate significant allelic differences between samples or closely related species. The software calculates and displays the allelic differences between a user defined reference and the sample traces in a histogram below each sample electropherogram.

The trace comparison report, which presents the loss, equivalent and gain of allele peaks, can be saved as a text file to be imported into Excel for printing. The Poisson Difference thresholds have default settings of Loss < -0.20 <= Equivalent <= 0.20 < Gain, but may be altered by the user for a narrower or wider range. It is recommended that the user leave the "Abide By Panel" and "Poisson Differentiation" options checked in order to view the allelic probabilities



### Results

The allele report designed for AFLP analysis outputs the numbers 1 and 0 to represent the presence and absence of peaks. The symbol,?, is used to represent questionable peaks, which the user is advised to check in the electropherogram. The allele report can also output the peak intensity for each detected allele. Both of these reports can be saved as text files to be imported into Excel.

The allele report displaying the presence, absence, and questionable presence of alleles is shown in Figure 1 and the allele report displaying the peak intensities is shown in Figure 2. The peaks with a green symbol are high confidence and those with a yellow symbol are of lower confidence.

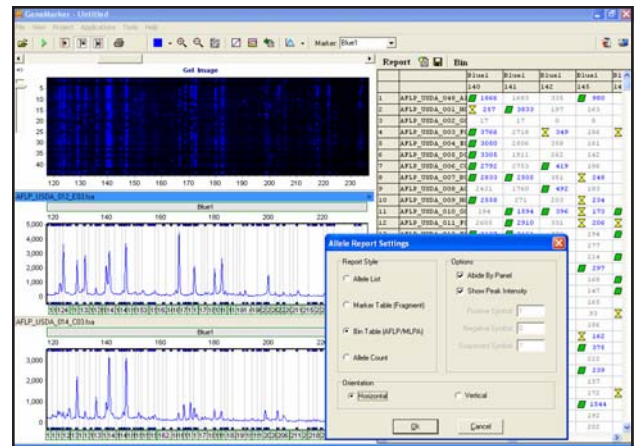


Figure 1. Report displaying presence and absence of alleles

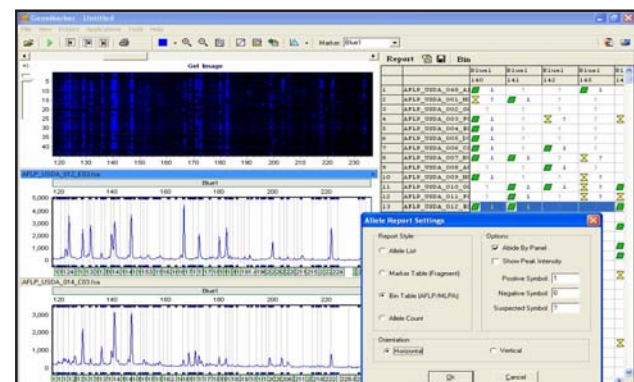
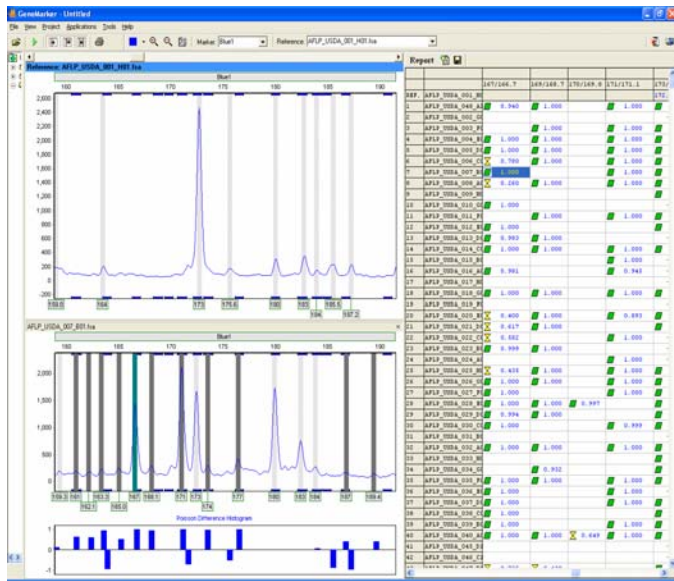


Figure 2. Allele Report displaying peak intensities

The Trace Comparison report shown in Figure 3 portrays the allelic differences between sample 007\_B01 and reference 001\_H01. Sample 007 contains alleles at positions 167 and 171 that are not in the reference trace. Accordingly, the Poisson distribution value for both alleles is 1.000 as shown in the allele report, because they exist in the sample but not in the reference trace. When the reference trace contains an allele, which the sample trace does not contain, the Poisson distribution value is -1.



**Figure 3. Trace Comparison showing allelic differences between sample and reference at positions 167 and 171.**

### Discussion

There are many advantages to AFLP when compared to other marker technologies including randomly amplified polymorphic DNA (RAPD), restriction fragment-length polymorphism (RFLP), and micro satellites. AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level than other techniques, but it has the capability to amplify between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification. As a result, AFLP has become extremely beneficial in the study of taxa including bacteria, fungi and plants, where much is still unknown about the genomic makeup of various organisms<sup>2</sup>.

AFLP is widely accepted as an effective tool for identifying genomic differences among closely related species, and GeneMarker has the ability to quantify and report these differences. Similar to AFLP, GeneMarker is highly accurate, sensitive and easy to use, making it a successful complement to the genotyping technique.

### References

1. AFLP: a new technique for DNA fingerprinting. Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Lee, Th. van der; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M. & Zabeau, M. *Nucleic Acids Research*. 1995. 23(21): 4407-4414
2. AFLP Genotyping and Fingerprinting. Ulrich G. Mueller and L. LaReesa WolfenBarger. *Tree*. October 1999. Vol.14 no.10.

\*AFLP is a registered trade mark of KeyGene, N.V.