

## GeneMarker® Software for Terminal-Restriction Fragment Length Polymorphism (T-RFLP) Data Analysis

David Hulce, PhD, ChangSheng (Jonathan) Liu, PhD, SoftGenetics LLC

### Introduction

One culture-independent molecular method for fingerprinting microbial populations is terminal-restriction fragment length polymorphism (T-RFLP) (1). T-RFLP has been used to demonstrate “species diversity in activated sludge, bioreactor sludge, aquifer sand and termite guts” (2); to distinguish populations of denitrifiers, *Bacteria* and *Archaea* in marine sediments (3); to show “temporal changes in the diversity of the bacterial communities” in polluted marine environments (4); and to study fungal populations in soil (5). This sensitive high-throughput method has the potential to generate large sets of data. Improving speed and efficiency of terminal restriction fragment (T-RF) analysis will further understanding the diversity, richness and dynamics of microbial populations and its relationship to ecological processes.

T-RFLP is a polymerase chain reaction (PCR) based genetic fingerprinting technique. DNA is extracted from a microbial community. One of the primers of a primer pair is labeled with a fluorescent dye and used to amplify a selected region of a gene of interest by PCR. The resulting PCR fragment is digested with one (sometimes two or more) restriction endonuclease and the T-RFs are separated with an automated DNA analyzer. Microbial diversity in a community can be estimated by analyzing the number and peak heights of T-RF patterns (2).

GeneMarker can perform fragment analysis and genotyping on four or five color data sets from any slab gel or capillary electrophoresis system. This software automatically corrects for many common problems—instrument spike, color pull-up, peak pull-up, noisy data, saturated peaks and stutter peaks—saving significant analysis time and cost, efficiently analyzing raw fragment data within seconds. GeneMarker is robust software to analyze DNA fragment data labeled with MegaBACE™ dyes (Amersham), Big Dye® (Applied Biosystems Inc.) or Beckman dyes from a variety of platforms: ABI DNA Analyzer or Genetic Analyzer, Amersham instruments or Beckman instruments. GeneMarker is compatible with files from all major capillary and slab gel electrophoresis systems including ABI files (\*.FSA, \*.AB1, \*.ABI), SCF files, MegaBace® files (\*.RSD, \*.ESD), SpectruMedix files (\*.SMD, \*.SMR), Beckman files and Li-Cor files. The software features high efficiency allele calling, adjustable parameters and a variety of reporting options.

### T\_RFLP Fragment Data Analysis

The software settings shown below are suggested to produce highly sensitive and reproducible results for T-RFLP analysis. To detect low intensity peaks, T-RFLP analyses should include a low peak detection threshold with stutter filter turned off or set to zero. The peak detection threshold intensity value may be estimated based on the peak with the maximum intensity from the negative control in the sample set. All parameters can easily be adjusted to optimize the analysis for your specific requirements.

The following analysis parameters are adjustments made to the default settings for AFLP analysis using GeneMarker® software.

### Suggested Analysis Parameters

#### I. Template Selection

1. Panel: None
2. Analysis Type: AFLP

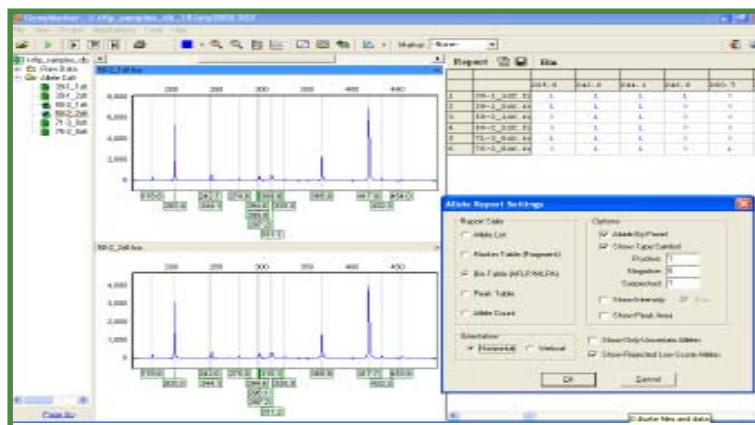
#### II. Data Process

1. Raw Data Analysis: select Smooth
2. Allele Call: deselect Auto Range and set start to 60, end to 500
3. Peak Detection Threshold: Intensity > 40
4. Stutter Peak Filter: deselect or set left and right values to 0

#### III. Additional settings

1. Peak Score: Reject < 0 check 1 < Pass
2. AFLP-Unconfidence at Rightside Score: 1

Following size calling the Analysis Display window will activate (figure 1). The samples listed on the far left are marked with green symbols, indicating that size calling was successful. Sample electropherograms are displayed in the center of the window. An allele/fragment report records the presence (1) or absence (0) of a fragment for the samples (upper right of figure 1). The Allele Report Settings dialog box presents the option to display peak areas and peak intensities in the Report. The report can be saved as a \*.txt file.

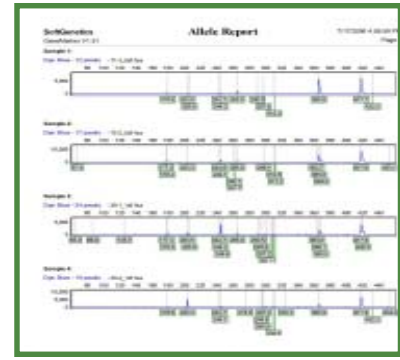


**Figure 1.** GeneMarker analysis window and Allele Report Setting dialog box: The samples are listed on the far left. Two sample file electropherograms are displayed in the center. The allele report table is displayed on the right. The Allele Report Settings dialog box is shown below the Report

A peak table summarizing fragment size, peak height and peak area, along with a comments field, can be activated (clicking the third icon from right in the toolbar) and used to record comments, edit alleles and confirm fragments. The peak table can be saved as a \*.txt file (clicking second icon from right in the toolbar). Text files can be imported into other applications for further analysis.



**Figure 2.** Peak table displayed below a sample electropherogram showing the option menu for editing items in the peak table (clicking the right mouse button with the cursor on a highlighted cell will activate the editing menu).



**Figure 3.** Print Report: Comparison of electropherograms from four samples (only one sample from a duplicate pair is shown).

## Results

Samples 50-2\_2df and 50-1\_1df are replicates (Figure 1). The electropherograms for these duplicate samples display a nearly identical pattern of fragment sizes, all within 0.4 bp. The peak heights for both samples display comparable intensities.

The pattern of fragment sizes and peak heights displayed in the electropherograms presents a genetic fingerprint for each of the sample populations (figure 3). Sample 39-1\_df contains two unique fragments, 86.8 and 125.7, with fragment 244.2 displaying greatest intensity among these samples. Fragment 203.4 +/- 0.1 is common among the four samples, but shows greatest intensity in sample 50-2\_1. The differences among these genetic fingerprints may reflect the relative abundance and diversity of organisms that are contained in these populations.

## Discussion

There are many molecular methods for investigating microbial ecology: denaturing gradient gel electrophoresis (DGGE), microarrays, quantitative polymerase chain reaction (Q-PCR), restriction fragment length polymorphism (RFLP) and T-RFLP, to name a few. Changing a few parameters for AFLP analysis quickly and efficiently adjusts fragment detection for T-RFLP analyses. T-RF fragment size and abundance is easily visualized on the electropherograms, quickly showing differences and similarities among the samples. Saving the fragment size and peak information as a \*.txt file provides the opportunity to export the analytical results to a data base.

T-RFLP analyses provide genetic fingerprints of microbial communities. GeneMarker® is an accurate, sensitive and easy to use software tool which can simplify DNA fragment analysis.

### References:

1. Christopher B. Blackwood, Terry Marsh, Sang-Hoon Kim and Eldor A. Paul. 2003. Terminal restriction fragment length polymorphism data analysis for quantitative comparison of microbial communities. *Appl. Environ. Microbiol.* 69:926-932.
2. Wen-Tso Liu, Terence L. Marsh, Hans Cheng and Larry J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 63:4516-4522.
3. Gesche Braker, Héctor L. Ayala-del-Rio, Allan H. Devol, Andreas Fesefeldt and James M. Tiedje. 2001. Community structure of denitrifiers, Bacteria and Archaea along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. *Appl. Environ. Microbiol.* 67:1893-1901.
4. R. Denaro, G. D'Auria, G. Di Marco, M. Genovese, M. Troussellier, M. M. Yakimov and L. Giuliano. 2005. Assessing terminal restriction fragment length polymorphism suitability for the description of bacterial community structure and dynamics in hydrocarbon-polluted marine environments. *Environ. Microbiol.* 7:78-87.
5. Ian C. Anderson and John W. G. Cairney. 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environ. Microbiol.* 6:769-779.

### Acknowledgement:

We would like to thank David Burke, Ph.D., The Holden Arboretum, for collaborating with developing the analysis parameters for this application.

**SoftGenetics LLC 200 Innovation Blvd. Suite 235 State College, PA 16803 USA**

Phone: 814/237/9340 Fax 814/237/9343

[www.softgenetics.com](http://www.softgenetics.com) email: [info@softgenetics.com](mailto:info@softgenetics.com)