SoftGenetics Application Note

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AFLP Analysis of Gel Electrophoresis Images

Using JelMarker™ Image Reader and GeneMarker®Fragment Analysis Software

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Introduction

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel.

Many DNA fragment analysis techniques use gel electrophoresis to separate fragments based on size. Examples of these techniques include AFLP®, microsatellite, ecotilling, TILLING®, T-RFLP, SNP discovery, fingerprinting, footprinting, etc. **(1-3)**. Often the gel images are overcrowded and tedious to analyze and with the advent of capillary electrophoresis, software development to analyze these gel images has fallen by the wayside. SoftGenetics has recently developed software to fill this gap - JelMarker. JelMarker uses advanced image reading algorithms and specific band scoring techniques to minimize the amount of time a researcher devotes to identifying and sizing fragments. Included is an example of how the AFLP technique analyzed with JelMarker in combination with GeneMarker can make gel image analysis a breeze!

Amplified Fragment Length Polymorphisms – or AFLP - was first introduced in 1995 by KeyGene N.V. as a method for analyzing, comparing, and differentiating organisms using an entire genome approach **(4)**. AFLP has increasingly become the technique of choice for phylogeny analysis and species differentiation **(5)**. To streamline researchers' AFLP procedure, GeneMarker fragment analysis software analyzes AFLP data and applies clustering algorithms for simple differentiation of complex species models. With JelMarker image reading software, researchers using gel separation can also tap the power of GeneMarker's patented size calling algorithms and clustering analyses without expensive capillary electrophoresis equipment.

JelMarker was developed in response to a growing demand for software that can analyze fluorescence, chemiluminescence and autoradiography gel image files – especially those from LI-COR®4300 DNA Analyzer and KODAK®Image Station 4000R. JelMarker can import up to two TIFF, BIP, JPEG, and TXT files for comparison analysis. The software exports SCF files for easy upload to the fragment analysis software - GeneMarker.

Procedure

JelMarker automatically identifies lanes and bands using a highly accurate image reading algorithm. Once the image is processed, the user has full control to manipulate lane/band position and orientation, individual fragment markers and standard identification.

GeneMarker applies a unique size calling algorithm to the data exported from JelMarker. In many instances the resulting sized data is more accurate than standard capillary electrophoresis runs; resolving fragments down to less than one base pair difference. This highly resolved data provides for more accurate clustering analysis results.

JelMarker Image Analysis Procedure

1. Import one or two TIFF, BIP, JPEG, or TXT file(s)
2. Click the **Auto Lane Tracking** icon to process the image
3. After processing, lanes and bands will be defined, adjust as necessary
4. Select **Edit Size Standard** from the main toolbar and import or create a size standard for the data and save
5. Click the **Save as SCF File** icon and save image SCF files
6. The individual data files (.scf) for each lane and two size standard files (.xml) are saved in the file folder selected

GeneMarker AFLP Analysis Procedure

1. Import data files (.scf) created from JelMarker Image Reading software
2. Select the **Run** icon to launch the Run Wizard
3. Recommended Settings:
   Template Selection
   a. Panel – *None* (Created after initial analysis)
   b. Size Standard – **_Band.xml* size standard from JelMarker
   c. Standard Color – *Red*
   d. Analysis Type – *AFLP*
   Data Process
   e. Raw Data Analysis - Select *AutoRange, Smooth, Baseline Subtraction, and Spike Removal* (all others should be deselected)
   f. Size Call – *Local Southern*
   g. Allele Call – User-defined data specific settings
   Additional Settings
   h. Peak Score – *Reject < 1 Check 7 < Pass*
   i. AFLP Unconfidence at Rightside Score - < 30
4. After the data is processed, create a panel in **Tools → Panel Editor** and reprocess the data with the new panel selected in the Template Selection box of Run Wizard
5. After data is compared to the panel, select **Applications → Clustering Analysis** to view dendrogram

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Results

Once the image is processed in JelMarker, launch the GeneMarker application and upload the newly created SCF files for analysis. If two images were analyzed, each SCF file will have fragment traces in two dye colors, blue and green, and the size standards in yellow and red. If just one image was analyzed, the SCF will only contain fragment data in the blue color.

Size standards will be unique for each set of gel images. If LI-COR chemistries were used, a pre-made size standard can be exported from JelMarker and imported into GeneMarker. In addition to the standard LI-COR size standards, JelMarker also exports two size standard files, *_Band.xml and *_SizeStd.xml.

The Band size standard file is created using the actual blue bands in JelMarker and sized according to the LI-COR size standard chosen (or user created size standard). The SizeStd file is size standard created using the fragments of the lanes identified as standards. When the SCF files are imported into GeneMarker, the yellow dye color will match the SizeStd file and the red dye color will represent the Band file size standard.

Figure 1 is the original gel image file displayed in JelMarker with bands identified in blue. In **Figure 2**, we demonstrate how a size standard created on a lane-by-lane basis accurately sizes the data. **Figure 3** shows how the individual lane sizing appears in GeneMarker before and after size calling. Finally, **Figure 4** depicts a clustering analysis in GeneMarker using average linkage and Pearson correlation. Notice the two standard lanes are set apart in a separate block from the sample lanes.

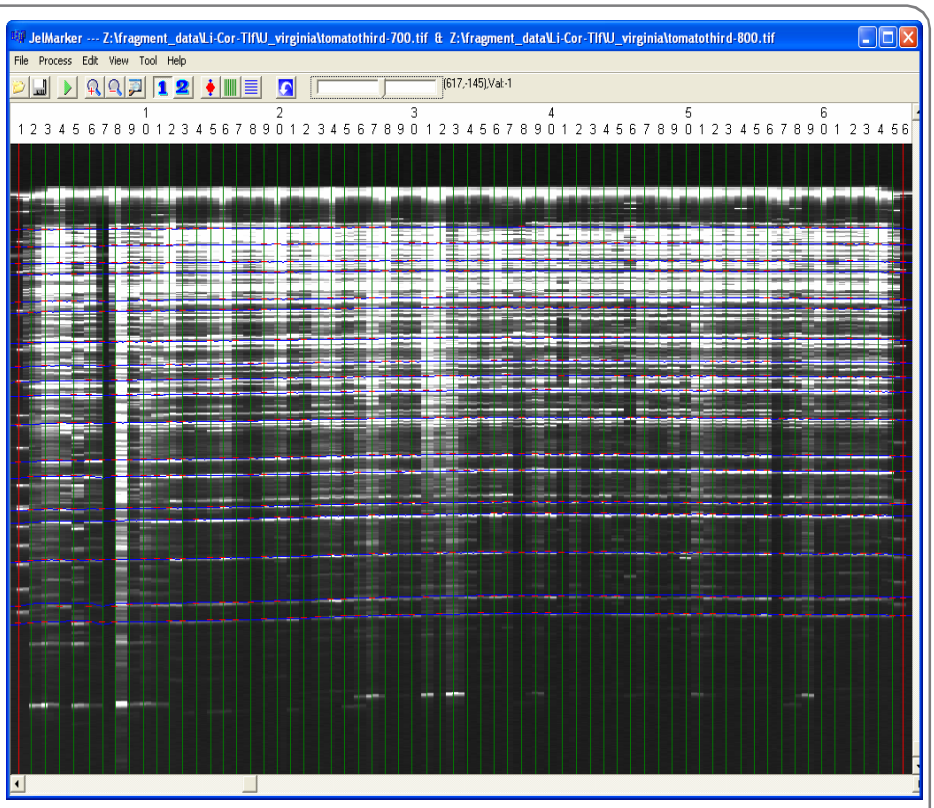

Figure 1. JelMarker – Bands are blue horizontal lines. Lanes are green vertical lines. Standard lanes are red vertical lines.

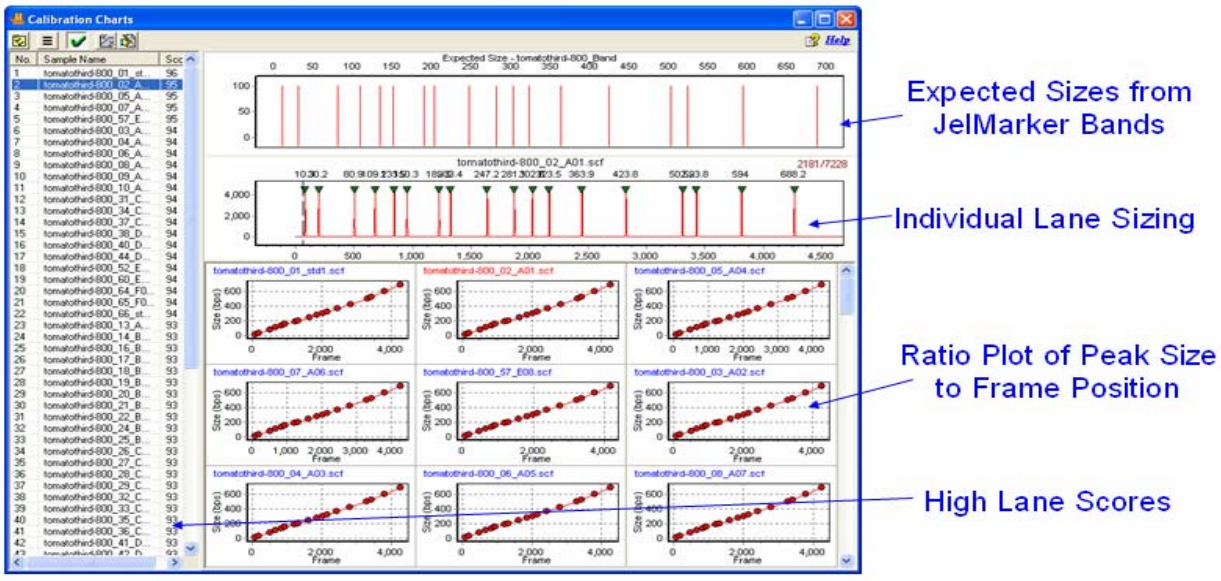

Figure 2. Automatically generated Size Standard based on band positions.

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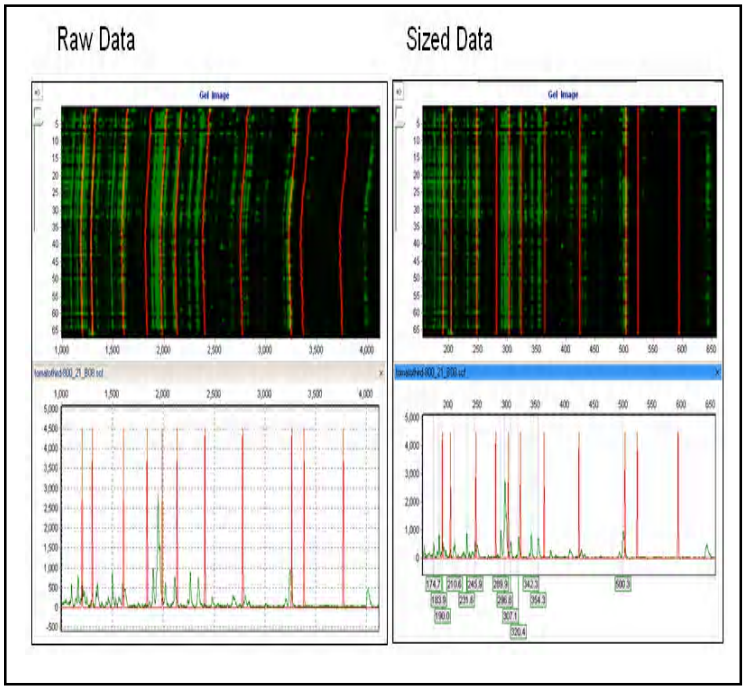

Figure 3. Highly resolved size calling in GeneMarker.

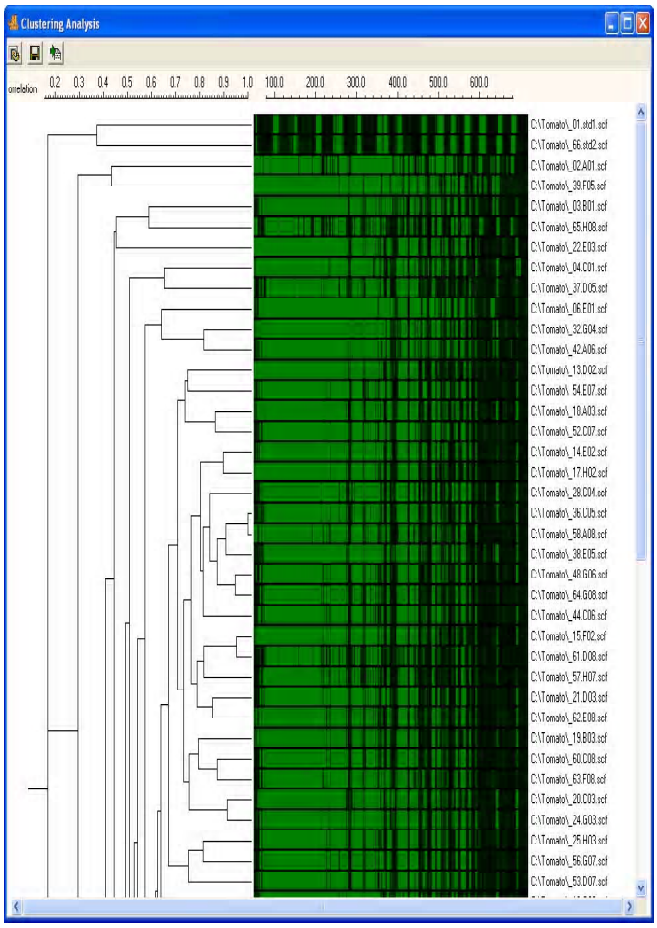

Figure 4. Clustering Analysis of Tomato data in GeneMarker

Discussion

AFLP is just one of the many applications researchers can use to identify genetic differences between individuals or species. Gel electrophoresis is an inexpensive way to resolve DNA fragments with high resolution results. The disadvantage to gel electrophoresis is that differential mobility shift from lane-to-lane makes it difficult to identify the size bands. Often bands can be curved and irregular especially toward the end of the run. Resolving these inconsistencies is difficult and can lead to inaccurate results if not taken into account.

SoftGenetics development of JelMarker for reading gel electrophoresis images makes analyzing fragments easy by removing the worry of inaccurate sizing. JelMarker identifies the band positions for each lane individually. To do this, the software firsts subtracts the background and filters out noise. Second, JelMarker identifies lanes and bands based on peak centers determined from the mean intensity of the local region. Next a slope correction for DNA migration time is applied and the bands are refined. From these refined bands, a size standard is created. Base pair sizes for the size standard are determined by the user and are automatically interpolated after the first two sizes are entered. Finally, the user can export each lane's individual fragment data which is translated into trace format. Two size standards are also exported with the fragment data; one standard correlates to band positions and the second correlates to the specified standard lane. In this way the user can choose to use the lane standard for sizing or the JelMarker generated bands. We recommend choosing the JelMarker generated band size standard due to its precise match for each lane.

Acknowledgements

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