



I N C O G E N

Magelan

“Multiple-Application Gel Analyzer”

INCOGEN, Inc.

263 McLaws Circle, Suite 200
Williamsburg, VA 23185

Phone: 757-221-0550

Fax: 757-221-0117

info@incogen.com

www.incogen.com

1. Introduction

Magelan, the Multiple-Application Gel Analyzer, is a Java-based program designed to efficiently and accurately analyze gel images from restriction digest fingerprinting experiments. The program includes a user-friendly graphical interface (Figure 1) to assist users in quickly completing all steps involved in analyzing the images. The software allows users to operate in three distinct modes: fingerprinting, allele-calling / genotyping, and fragment-sizing. Magelan can process gel images obtained through various scanning technologies and provides an intuitive interface to tasks such as importing an image, placing and adjusting a grid, defining standards, identifying genotypes, viewing data, and verifying and exporting results. The analysis is based on the user's choice of a pre-existing gel template (see Section 3) or a new, custom gel layout and standards definition.

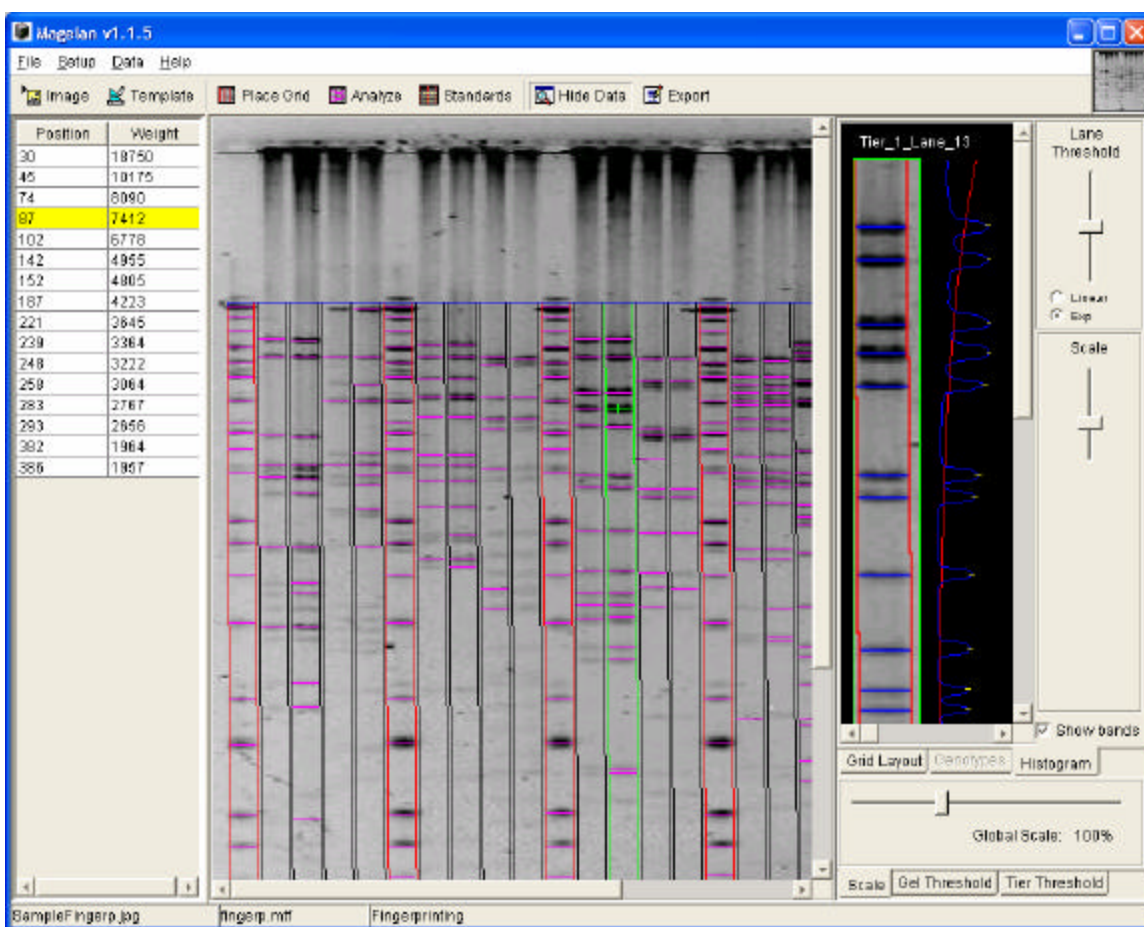


Figure 1: Analysis of a fingerprinting gel using Magelan under the Windows XP operating environment. The exact appearance of the program may vary slightly depending on the operating system and window manager settings.

2. Program Architecture / System Requirements

Magelan was written in Java 2 and is available on a variety of computing platforms. Table 1 illustrates the minimum and recommended system requirements for running the software on various platforms.

Operating System	Minimum Requirements	Recommended
Windows 95, 98, 2000, NT, XP	Pentium 300 MHz processor 64 MB of memory SVGA Resolution 10 MB free disk space	Pentium III processor 128 MB of memory 1024x768 resolution
Solaris	Sparc 5 64 MB of memory X-Windows 10 MB free disk space	Ultra 5 128 MB of memory X-Windows
Apple	iMac 300 MHz processor 128 MB of memory Mac OS X	G4 processor 128 MB of memory Mac OS X

Table 1: Minimum system requirements and recommended system specifications for Magelan.

3. Description of Operation

Magelan provides a user-friendly interface to analyze images of 1-D electrophoresis gels. The program accepts and reads images from a variety of common image formats, such as tiff, jpeg and gif. After loading an image, the user is required to position a grid onto the gel image to delineate the tiers and lanes in the gel. The positioning can be done directly on the image using the mouse or through user-friendly controls located at the right of the screen (Figure 2). The grid is flexible and can be adjusted for distortions in the gel and lanes that run irregularly, such as with an extreme curve or slant.

The program allows users to define standards that provide a method to compare data from different gels as well as from different tiers on the same gel. Magelan offers a simple interface through which the user can swiftly define sets of standards and save them for future use (Figure 3).

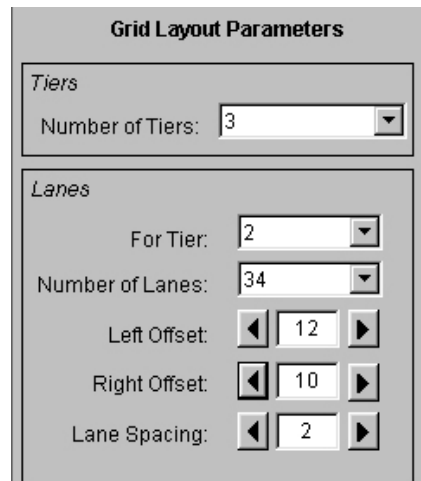


Figure 2: Grid Layout Parameters for positioning the grid.

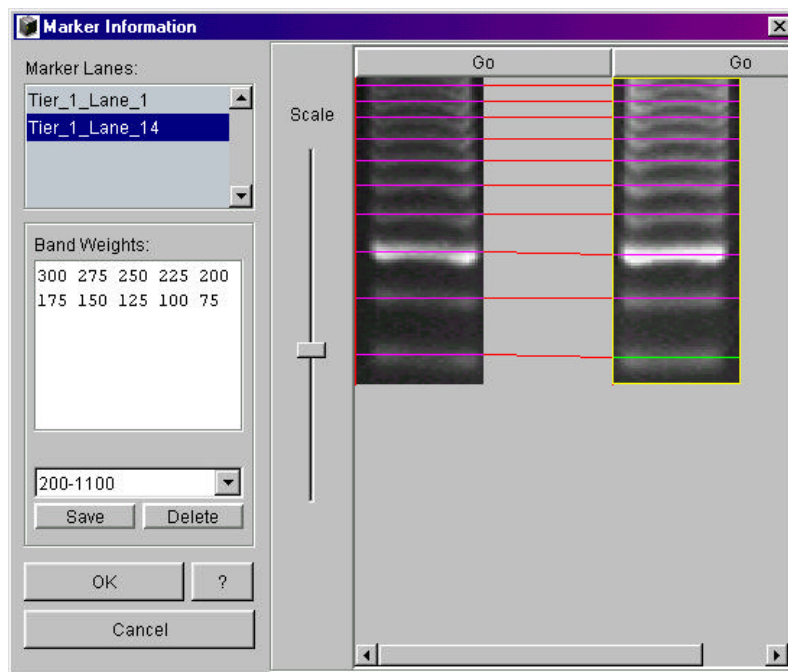


Figure 3: Defining Standards via the Marker Information interface.

To increase efficiency in preparing the analysis, Magelan can store information relevant to a particular protocol in a gel *template*. The template records the image format, the number and location of tiers and lanes, the number and location of standards lanes, and the number and location of bands within the standards lanes. Templates can be loaded for use with multiple images that follow the same protocol; i.e. gels that have the same number of tiers and lanes, that use the same standards in the same locations on the gel.

Magelan utilizes an efficient algorithm to perform a rapid analysis of the gel image. Based on the grid layout, the gel image is divided into tiers and lanes and the intensities of all possible bands are calculated. Background noise is eliminated by a local weighted average and a smoothing function. The resulting values represent the intensities of each band candidate. Users can further fine-tune the analyses through the provided thresholding functions. An analysis of a typical gel image lasts less than 5 seconds on a Pentium III 400 MHz processor.

In allele-calling mode (Figure 4), users can further define genotype standards, against which Magelan will compare all samples to determine the expressed genotype. These standards can be defined anywhere on the gel image, regardless of whether specific lanes were used as controls, and they can be applied to every lane on the image or any subset of lanes. During analysis, the software will perform the band-finding algorithm described above and will determine the best possible fit of the defined genotypes for each lane.

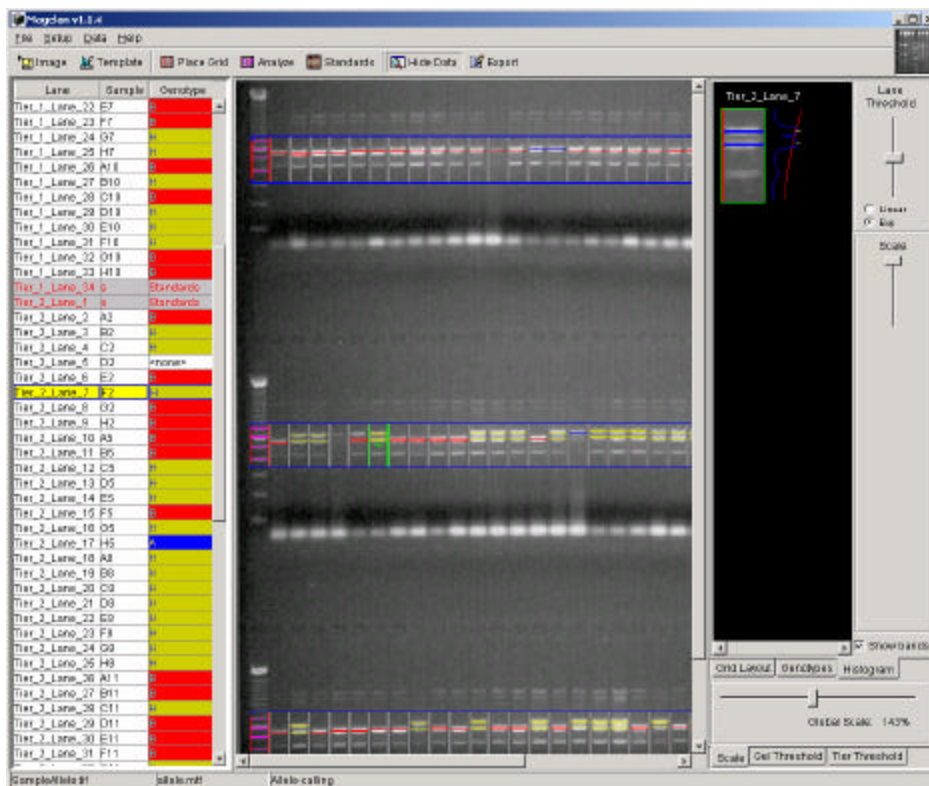


Figure 4: Genotyping analysis with Magelan.

After the analysis has completed, the results are visually presented by superimposing a digital fingerprint over the image as well as being presented in a tabular format at the left of the screen. Highlighting a row in the data table will highlight the corresponding band on the image for a pinpoint view of a particular result. In fingerprinting and fragment-sizing modes, the data table presents two values for each detected band: a distance from the top of the tier and the corresponding molecular weight, calculated relative to the standards previously defined. In allele-calling mode, the table presents the detected genotype.

A histogram and lane image are displayed for the active lane at the right side of the window for a close-up view. The histogram conveys the intensities of each row of the lane image by a blue curve, the current threshold value by a red curve, and any detected bands by yellow ticks on the intensity curve (Figures 1 and 4). The threshold can be applied only to the active lane, to an entire tier or to the gel as a whole. Additional tools, such as zooming controls and navigational shortcuts allow users to quickly and precisely garner useful data from the image.

The results can be exported for use in spreadsheet or database applications. Fingerprinting and fragment-sizing data can be exported to a tab-delimited file. Allele-calling data can be exported in the same format with options for additional information (lane names, sample names, etc), or in a "Plate" format if a plate-to-well mapping was defined through a simple Magelan utility. The plate option exports a grid representing the specified plate format where each cell of the grid contains the name of the genotype detected in that sample.

4. Summary

Magelan is a tool that encompasses broad functionality and a flexible interface for specific research and analysis needs. It provides scientists with a powerful and user-friendly interface to process gel images from restriction digest fingerprinting experiments. This software allows researchers to considerably improve their efficiency and quality of data for fingerprinting, fragment-sizing and allele-calling analyses.