



JelMarker™

*Gel Imaging and
Conversion Software*

Operation Manual

version 1.0

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Chapter 1 Summary

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 converts image data into SCF (Standard Chromatogram Format) files that can be uploaded into GeneMarker® for rapid and precise trace analysis of up to 1,000 lanes.

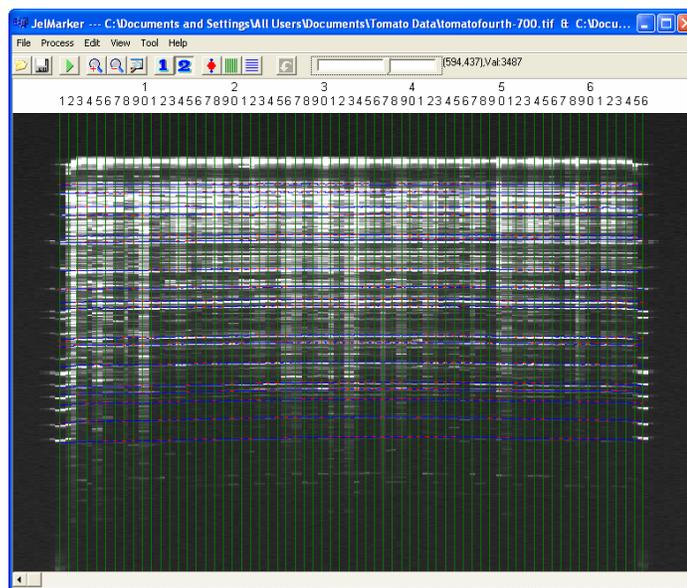
Import up to two TIFF, BIP, JPEG, and TXT files for simultaneous analysis.

Advantages:

- * JelMarker is a simple, stand-alone image reading software.
- * Highly accurate lane and band recognition
- * Readily apparent lanes and bands
- * Easy to modify lane and band positions using a point-and-click methodology
- * Export two color images in SCF format for GeneMarker

Applications: AFLP®, microsatellite, TILLING®, Ecotilling and SNP discovery

JelMarker Main Analysis Window

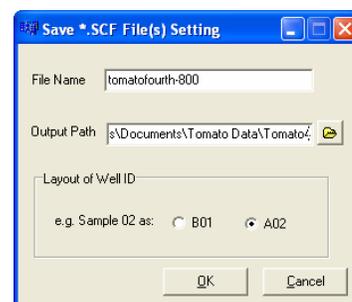
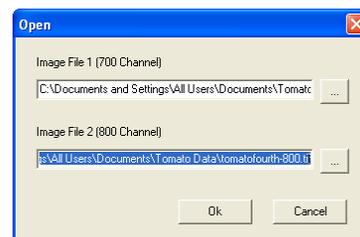


Chapter 2 Procedure

JelMarker automatically identifies lanes and bands using a unique 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 lane identification.

Image Analysis Procedure

1. Click **Open Files** icon
2. Select one or two TIFF, BIP, JPEG, or TXT file(s) to upload
3. Click **Open**
4. The image is uploaded to the *JelMarker* main analysis window
5. Click the **Auto Lane Tracking** icon
6. The image now contains lanes (vertical) and bands (horizontal)
7. Adjust the lanes and bands as necessary (see Chapter 4 Editing)
8. Click the **Save as SCF File** icon
9. The *Save SCF File(s) Setting* box appears
10. Enter a filename in the *Filename* field
11. Select a folder to place the SCF files by clicking the folder icon next to the *Output Path* field
12. Highlight the desired output folder and click **OK**
13. Click **OK** in the *Save SCF File(s) Setting* box
14. The individual SCF files for each lane are saved to the folder you selected.



Fragment Analysis

Once the image has been prepared with JelMarker, the newly created SCF files can be uploaded to GeneMarker. The image will contain fragment peaks in the blue (and green) dye color(s) and Internal Lane Standard (ILS) in the yellow and red dye colors. The yellow ILS trace is derived from the fragments in the identified standard lanes. The red ILS trace is derived from the positions of the bands and calibration dots in JelMarker and is specific to each lane or sample. We recommend using the red ILS trace as the standard for data analysis with GeneMarker (See Chapter 5 Size Calibration).

Chapter 3 Icons and Functions

File



Open Image – Select TIFF, BIP, JPEG, or TXT files to upload



Save as SCF – Save processed image file lanes as SCF files

Open/Save Project – Opens/Saves edited image files in .ltp format

Exit – Closes JelMarker

Process



Run – Analyzes the image for Lane and Band positions

Options – Set the number of samples and change viewing options

Edit



Dot Edit – Reposition individual fragment band markers (red dots)



Lane Edit – Reposition individual Lanes (vertical green lines)



Band Edit – Reposition individual Bands (horizontal blue lines)

View



Zoom In – Increase magnification with respect to center of image



Zoom Out – Decrease magnification with respect to center of image



View OnePic – Restores view to original global view of one image



View TwoPic - Restores view to original global view of two images

Tool

Edit Size – Launches the Size Standard creation window

Help

Lane Tracking Help – Launches JelMarker software manual

About – JelMarker software copyright information

Additional Icons



Zoom to Window – Restores view to global view after zooming



Undo – Restore the last editing operation

Grayscale – Slide scale right/left to increase/decrease grayscale

Additional Functions

Zoom In – Hold left-click and drag a box from upper left to lower right in the main analysis window

Zoom Out – Hold left-click and drag a box from lower right to upper left in main analysis window

Scroll – Hold right-click and drag cursor across image

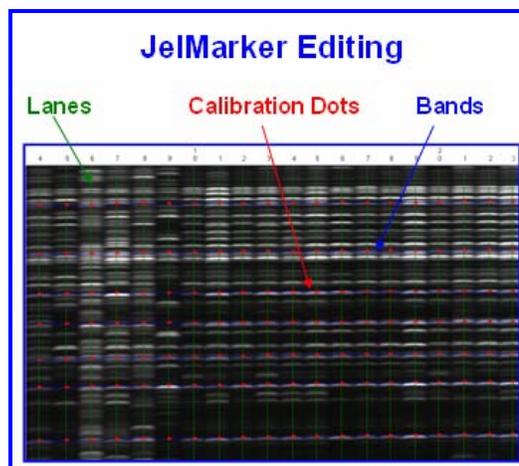
NOTE: Ensure no edit icons are selected when attempting to zoom or scroll

Chapter 4 Editing

Lanes

Lanes are the green and red vertical lines in the main analysis window. They indicate where the Lane Tracking software has identified an individual lane or sample.

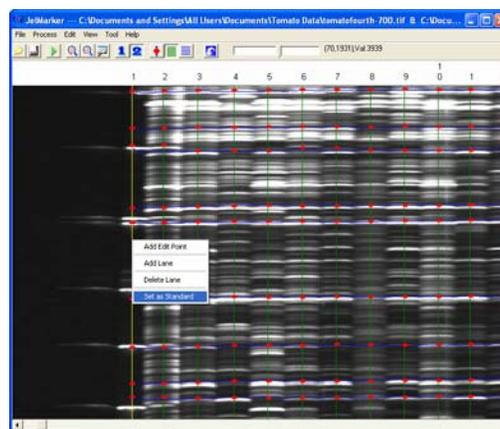
Whenever editing a lane, ensure the *Edit Lane* icon in the main toolbar is selected (except when *Rotate Lanes* is selected).



Move: In the main analysis window, click and hold the left mouse button to drag a lane right or left. Release the left mouse button to leave it in the new position.

Angle: Select the lane of interest in the main analysis window with a single left mouse click. The lane indicator will turn yellow and pink boxes will appear on either end. Hold the left mouse button and drag the pink box right or left to adjust the lane angle.

Curve: Select the lane of interest in the main analysis window with a single left mouse click. The selected lane will turn yellow. Right mouse click on the selected lane and a menu appears. From the menu, click *Add Edit Point*. A pink box will appear in the location of the right mouse click. Hold the left mouse button and drag the pink box right or left to adjust the lane curve.



Add/Delete: In the main analysis window, select the lane or position of interest with a single left mouse click. The selected lane will turn yellow. Right mouse click on the selected lane and a menu appears. From the menu, click *Add a Lane* or *Delete a Lane*. When adding a lane, the position of the mouse cursor when the

right-click occurred is the position where the new lane will be added.

Standard/Sample: In the main analysis window, select the lane of interest with a single left mouse click. Right mouse click on the selected lane and a menu appears. From the menu, click *Set as Standard* to mark a sample lane as a standard or click *Set as Sample* to mark a standard lane as a sample. Standard lanes are red and sample lanes are green.

Bands

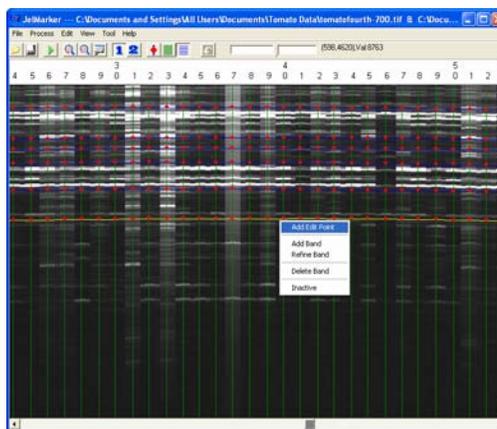
Bands are the blue, horizontal lines in the main analysis window and indicate where the Lane Tracking software has detected a fragment band in most of the lanes. Bands mark the positions of expected base pair sizes. This is analogous to the positions of peaks in a fragment analysis software's Internal Lane Standard (ILS) viewer.

Whenever editing a band, ensure the *Edit Band* icon in the main toolbar is selected.

Move: In the main analysis window, hold the left mouse button and drag a band up or down. Release the left mouse button to leave it in the new position.

Angle: Select the band of interest in the main analysis window with a single left mouse click. The selected band indicator will turn yellow and pink boxes will appear on either end. Hold the left mouse button and drag the pink box up or down to adjust the band angle.

Curve: Select the band of interest in the main analysis window with a single left mouse click. The selected band will turn yellow. Right mouse click on the selected band and a menu appears. From the menu, click *Add Edit Point*. A pink box will appear in the location of the right mouse click. Hold the left mouse button and drag the pink box up or down to adjust the band curve.



Refine: Select the band of interest in the main analysis window with a single left mouse click. The selected band will turn yellow. Right mouse click on the selected band and a menu appears. From the menu, click *Refine Band*. The calibration point will adjust to more closely match fragment positions.

Add/Delete: In the main analysis window, select the band or position of interest with a single left mouse click. The selected band will turn yellow. Right mouse click on the selected band and a menu appears. From the menu, click *Add Band* or *Delete Band*. When adding a band, the position of the mouse cursor when the right-click occurred is the position where the new band will be added.

Active/Inactive: In the main analysis window, select the band of interest with a single left mouse click. Right mouse click on the selected band and a menu appears. From the menu, click *Inactive* to mark the selected band as inactive or click *Active* to mark an inactive band as active. Active bands are blue and will be used to create a size standard. Inactive bands are grey and will not be used in the size standard calculation.

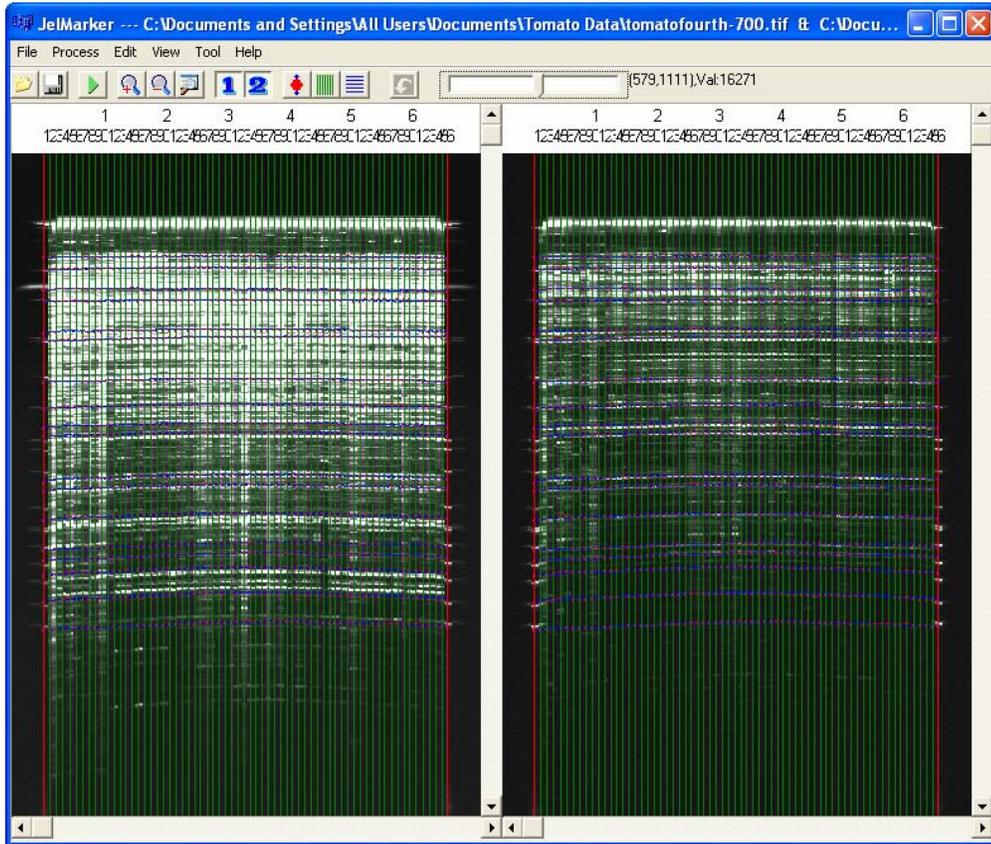
Calibration Dots

Calibration Dots are the red ovals along the bands. They mark the position of a detected fragment band within a lane. Typically, calibration dots will be placed at the apex pixel of the most intense fragment image in the band/lane.

Whenever editing a dot, ensure the *Edit Calibration* icon in the main toolbar is selected.

Move: In the main analysis window, hold the left mouse button and drag a dot to a new position. Release the left mouse button to leave it in the new position.

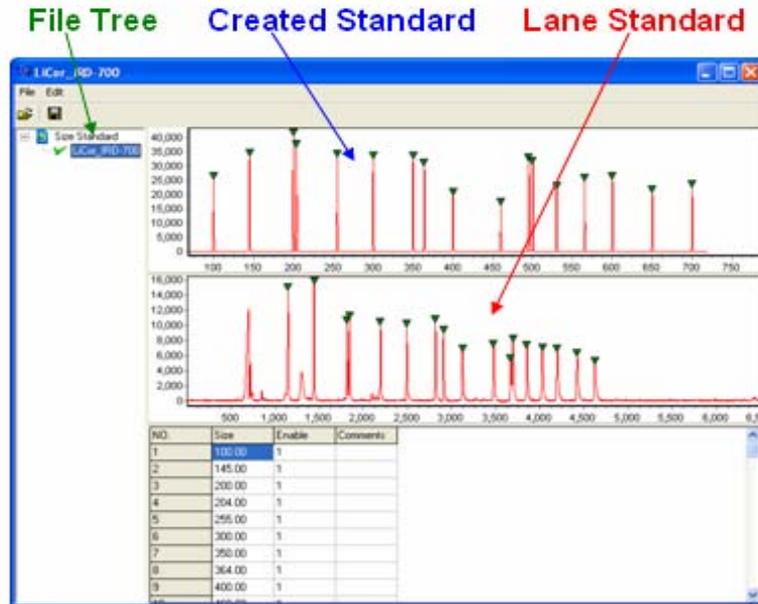
Two Color Data in JelMarker



Chapter 5 Size Calibration

Once the lanes and bands are edited, open the *Size Calibration* tool to create a size standard for import into GeneMarker.

Size Calibration Feature



LI-COR Size Standard Procedure

1. Select **Tool** menu from main toolbar and click **Edit Size**
2. The *Edit Size* box will appear
3. In the *Index* field, enter the number of one size standard lane
4. Click **OK**
5. The *Size Calibration* window will appear
6. Select **File** menu from toolbar and click **Open**
7. Select a pre-made LI-COR size standard from the *Open* box
8. Click **Open**
9. The size standard you selected will now appear in the file tree on the left
10. Select the size standard and a green check mark will appear indicating it is selected.
11. The selected LI-COR size standard now appears as the top trace, the lane size standard is the bottom trace
12. Select **Edit** in the toolbar and click **Auto-Match**

13. JelMarker will identify peaks in the lane trace that match the LICOR size standard – identified peaks are indicated with a green triangle
14. Select **File** from the toolbar and click **Save**
15. Enter a new name for the size standard
16. Click **Save**
17. The newly created size standard is ready to be imported into *GeneMarker*

Customized Size Standard Procedure

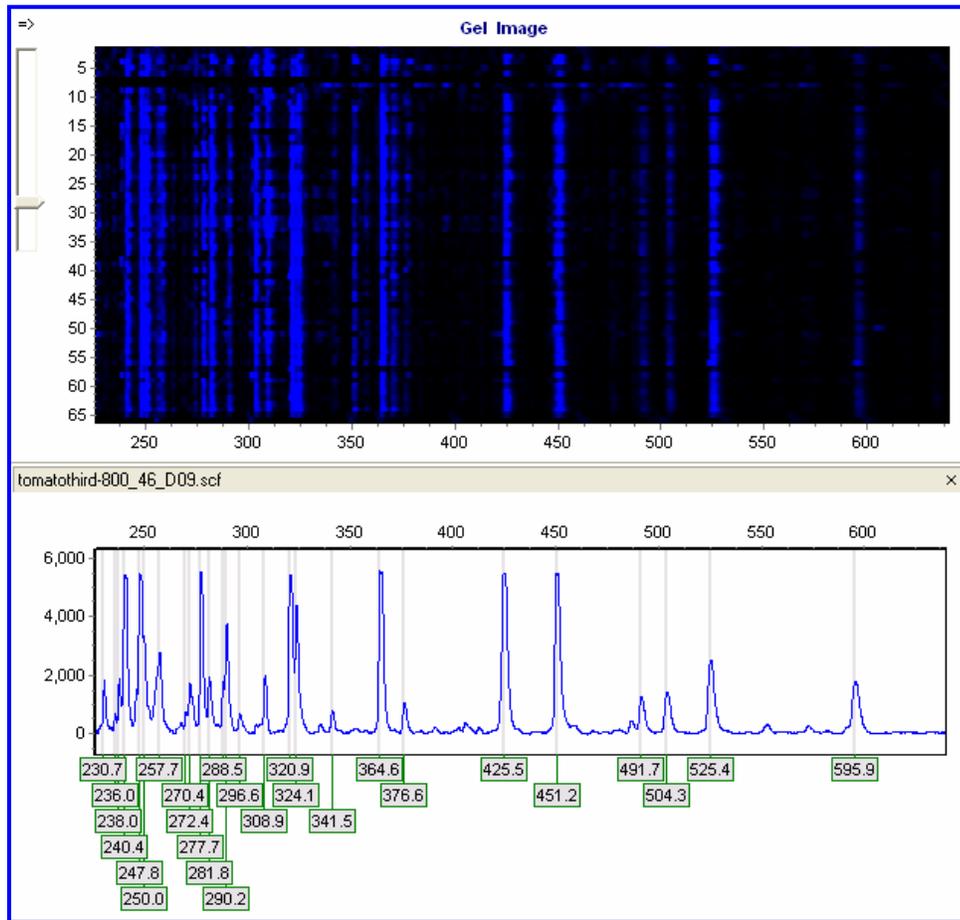
1. Follow steps 1-5 above
2. To begin creating a size standard, **right-click** at the top of the first standard peak in the red lane trace
3. Select **Insert Size**
4. The *Insert Size* box appears
5. Enter the correct expected size for the first peak
6. *Insert Size* for the second peak and enter the correct expected size
7. *Insert Size* for the third peak and *JelMarker* interpolates what the correct expected size should be automatically
8. Continue *Insert Size* for the remaining peaks
9. When complete, select **File** from the toolbar and **Save**
10. Enter a new name for the size standard
11. Click **Save**
12. The newly created size standard is ready to be imported into *GeneMarker*

Automatic Size Standard

In addition to creating a custom size standard in JelMarker, two automatic size standards will be exported with the SCF files. The first automatic size standard is labeled as *_Band.xml and the second is labeled *_SizeStd.xml. The Band file is created using the actual blue bands in JelMarker. The SizeStd file is 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.

NOTE: The Band file size standard is the recommended size standard for data analysis.

AFLP Analysis in GeneMarker



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