USER GUIDE

invitrogen™
by life technologies™

GelQuant Express Analysis Software

General information for using the GelQuant Express software for analysis of agarose gels

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life technologies™

Contents

Introduction

About the System

System description

The GelQuant Express software application seamlessly integrates with the E-Gel® Imager hardware to provide the user with all the applications, functions and tools for analyzing gel captured images and data. The friendly GUI lets you perform common actions with a single click of a button or icon.

The GelQuant Express software is designed for two main analysis options, which are:

- The 1 D option that allows the user to analyze 1 D gels.
- The Express mode that allows the user to analyze biological samples for standard applications.

The user should also note that the software can only be used while the safety dongle is inserted in the computer. This maintains software security and ensures its correct and legal use.

GelQuant Express Software Activation Dongle

The GelQuant Express Software Activation Dongle must remain in the computer to use GelQuant Express.

If the software activation dongle is not detected, the following warning will appear: “Sentinel HASP key not found (H007)”.

Intended Use

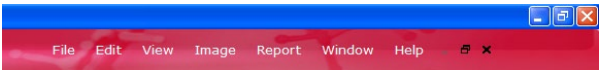
For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Software Overview

GelQuant Express software Main Menu

The main menu for the GelQuant Express software application is located in the red band at the top right of the screen. The name of the experiment, and the most recent save date is displayed in the blue band in the upper left hand corner.

Refer to the following section for a short description of the functions of the Main Menu.



GelQuant Express Main Menu functions

File: Opens a dropdown menu with options to:

- Load/open a file
- Save a file
- Perform invert measurement function to correct an image f which high intensity bands have less grey level amounts than low intensity bands (e.g., dark bands on a light background).
- Exit the GelQuant Express software application

Edit: Opens a dropdown menu with options to:

- Undo an action
- Redo an action

View: Opens a dropdown menu with options to:

- Display the default view of image
- Display the results preview

Continued on next page

Software Overview, Continued

GelQuant Express Main Menu functions, continued

Image: Opens a dropdown menu with options to:

- Crop an image
- Open image tools to change image settings

Report: Opens a dropdown menu with options to:

- Display all lane reports
- Display single lane report

Window: Opens a dropdown menu with options to:

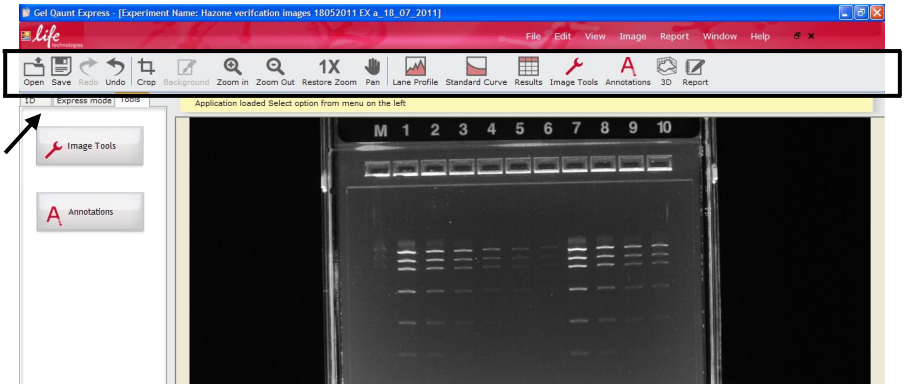
- Navigate through opened experiments as cascade, horizontal tiles, or vertical tiles.

Help: Opens a dropdown menu with options to:

- Display help topics for novice users
- Display software version information

Toolbar and Icons

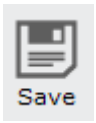
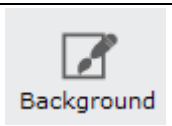
The toolbar of the GelQuant Express software application is located below the Main Menu, and provides easy access to frequently used functions.



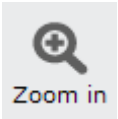
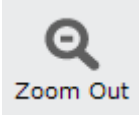
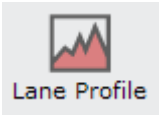
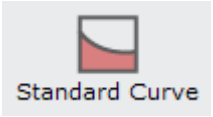
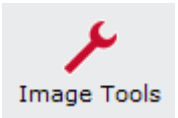
Refer to the **Icons** section (page 6) for a short description of the functions of the icon buttons.

Icons

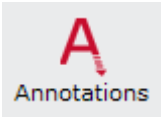
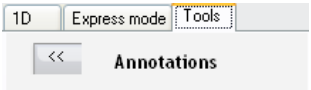
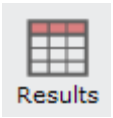
Use the icon buttons to access the most commonly used functions.

Icon	Name	Function
	Open Image	Opens a previously captured and saved .TIFF, JPEG, and BMP files.
	Save	Allows the user to save experiments. By activating this function you save the current, active experiment.
	Undo/Redo	'Undo' / 'Redo' allow the user to go back or forward to correct mistakes. **Note**: The application ONLY allows making a single step back or forward. First, click the 'Undo' icon. This activates the 'Redo' icon. The user can now click 'Redo" for the next step in the work at hand.
	Crop	Allows the user to reduce the image to his personal, specified choice of data.
	Background	Allows the user to designate an area on the image whose pixels values are the noise threshold for band calculations. The background is marked with a highlighted, bright box located at the top left hand corner of the experiment image. **Note**: This function is ONLY available for the Express mode.

Software Overview, Continued

Icon	Name	Function
	Zoom In	Allows the user to resize the image by zooming into the image. **Note**: The zoom process can also be changed by scrolling the mouse wheel.
	Zoom Out	Allows the user to resize the image by zooming out of the image. **Note**: The zoom process can also be changed by pressing and scrolling the mouse wheel.
	Restore	Allows the user to undo the zoom, and fit the image to the screen.
	Pan	Allows the user to move and navigate the image while it's active. Click on this icon and drag the image to lock on to the required area. **Note**: The navigation process can also be changed by scrolling the mouse wheel, as well as by pushing the mouse wheel and dragging it.
	Lane Profile	To display data on current lane and band intensities. Available for 1 D Tool mode only.
	Standard Curve	Gives visual display between current lane and the standard. Provides regression between lane and standard. Available for 1 D Tool mode only.
	Image Tools	Accesses image optimization tools (contrast, rotation etc.).

Software Overview, Continued

Icon	Name	Function
	Annotations	Allows user to add texts and arrow annotations to the image.  **Note**: Annotations can also be activated using the 'Tools' tab.
	3D	Allows the user to transform the gel image into a solid, three-dimensional model with X, Y and Z dimensions. The relative height of data can be decreased or increased by moving the slider on the 3-D image.
	Results	Displays results in the preview table. Note: there are differences between tables displayed using the 1 D Tool the Express Mode.
	Report	Presents final report of the analysis. Available for 1 D Tool mode only.

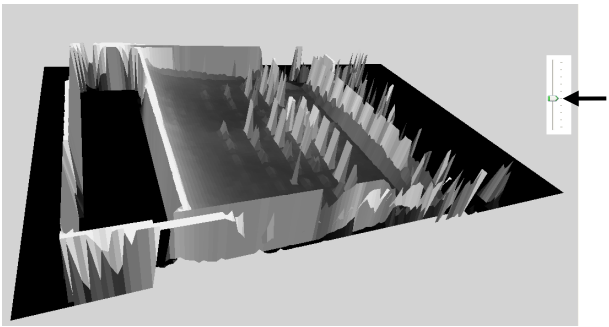
Additional Note on 3 D Mode

The 3 D Mode tool generates a 3-Dimensional image of the bands that can be viewed in solid form with X, Y, and Z dimensions. The Z-dimension (height) refers to relative grey scale intensity of a pixel, while X-, and Y-dimensions refer to coordinates on the 1 D gel image.

The 3 D Mode tool is useful for examining the grey scale profile of the image, and determining whether inverted measurements are required.

The relative height of data can be decreased or increased by moving the slider at right side of the image area up or down.

The image can be freely rotated by left clicking the mouse button, and dragging the image to the desired position.



Software Installation

Getting Started

Software Installation Guidelines

The software is received as a CD or as a file. To install the GelQuant Express software, follow these guidelines:

- Log in as user with Administrator privileges (for installation only). Verify that you are logged in as an Administrator before starting installation. If you do not have Administrator privileges, contact your IT department for help.

  If you do not log in as an administrator, the software is installed for a limited user.
- Change PC power setting to "Never Hibernate".
- The GelQuant Express software installation process requires .NET Framework 4. The .NET Framework installation process is automatically launched if it is not present on your PC.

You do not have to have an active internet connection while performing software installation.

Minimum PC requirements

In order to install and operate GelQuant Express, your PC must meet the following minimum requirements:

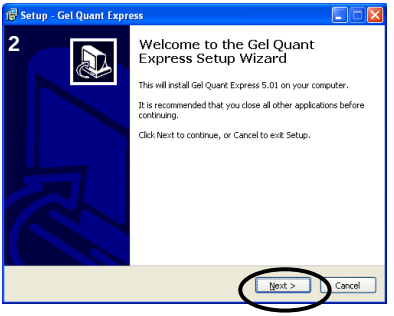
- Intel® Core™ 2 Duo processor, 1.8 GHz.
- Minimum 1024 MB RAM of memory (2 GB recommended).
- 32 bit Windows XP Pro (English version, SP 3) OR Windows 7 Professional (English version) operating system.
- Only PCs with a 32 bit operating system and admission permission at a minimum for the software installation process.
- Minimum monitor resolution of 1024 × 768 pixels.
- A free USB 2.0 port (Not compatible with USB 1.0 or 1.1).
- Adobe Acrobat Reader
- Microsoft Excel

Installing GelQuant Express (Windows XP Pro)

1. To start the GelQuant Express installation process, double click on the GelQuantExpress installation file icon.

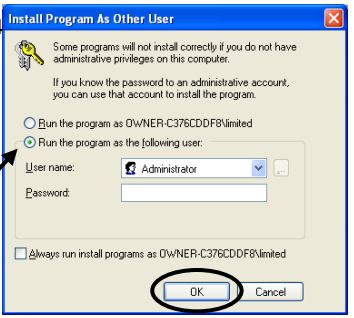
1

2. A welcome screen appears. Click **Next**.



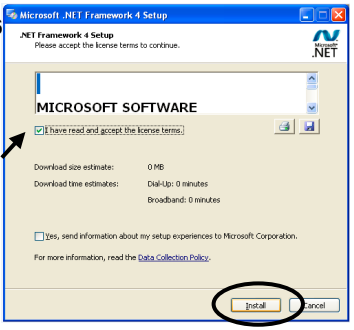
3. Select the "Run the program as the following user" option, and log in as an Administrator.
4. Click **OK**.
   - If your PC already has .NET Framework 4 installed, go to step 9.
   - If your PC does not have.NET Framework 4 installed, go to step 5.

3–4



5. A Microsoft .NET Framework 4 software screen appears. Click the check box: "I have read and accept the license terms".
6. Click **Install**.

5–6

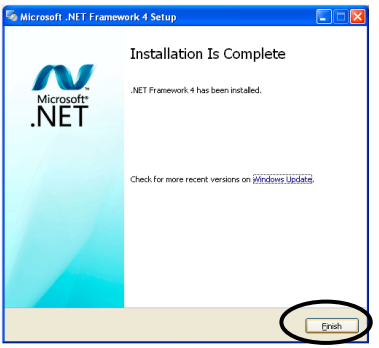


Continued on next page

Installing GelQuant Express (Windows XP Pro), Continued

7. A notification screen appears, stating that installation is complete. Click **Finish**.

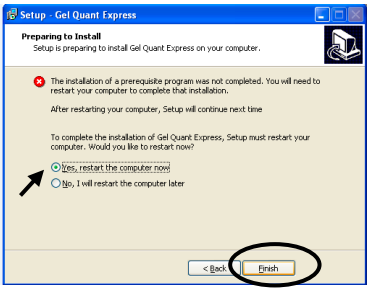
7



8. The "Completing Setup Wizard" screen appears. Click the checkbox "Yes, restart the computer". Click **Finish**. The computer will then restart.

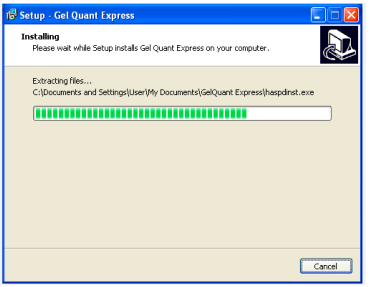
   After the PC restarts, the GelQuant installation process automatically continues.

8



9. A progress bar indicates the status of the GelQuant installation process.

9

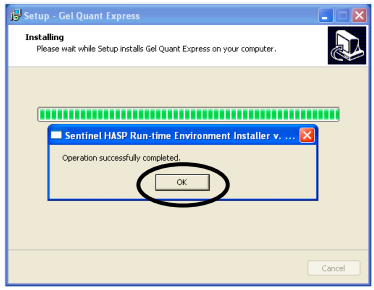


Continued on next page

Installing GelQuant Express (Windows XP Pro), Continued

10. Upon completion of the installation process, a notification message appears, stating that installation was successful. Click **OK** to finish.
11. Launch the GelQuant Express software either by using the software icon located on the desktop, or the start menu.

10

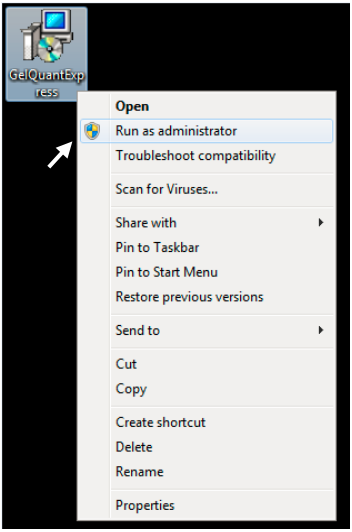


Note: The GelQuant Express Software Activation Dongle must remain in the computer to use GelQuant Express. If the software activation dongle is not detected, the following warning will appear: "Sentinel HASP key not found (H007)".

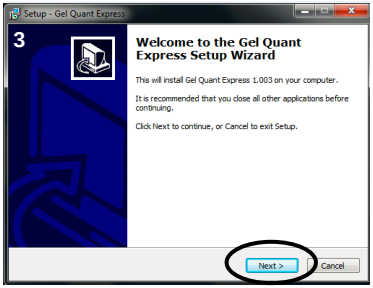
Continued on next page

Installing GelQuant Express (Windows 7)

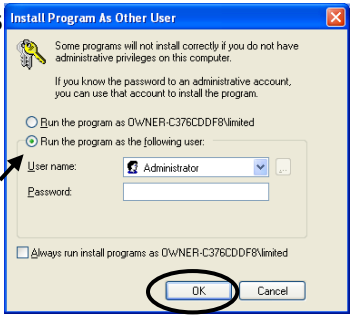
1. To start the GelQuant Express installation process, double click on the GelQuantExpress installation file icon.
2. Left click on “Run as administrator”.



3. A welcome screen appears. Click **Next**.



4. Select the “Run the program as the following user” option, and log in as an Administrator.
5. Click **OK**.
   - If your PC already has .NET Framework 4 installed, go to step 10.
   - If your PC does not have.NET Framework 4 installed, go to step 6.

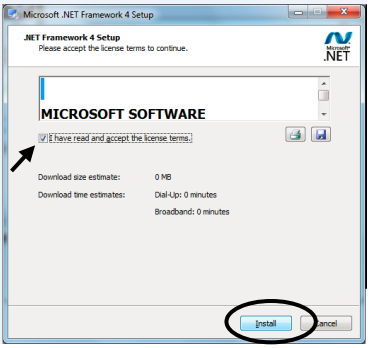


Continued on next page

Installing GelQuant Express (Windows 7), Continued

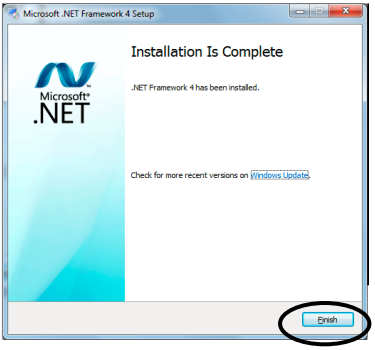
6. A Microsoft .NET Framework 4 software screen appears. Click the check box: “I have read and accept the license terms”.
7. Click **Install**.

6–7



8. A notification screen appears, stating that installation is complete. Click **Finish**.

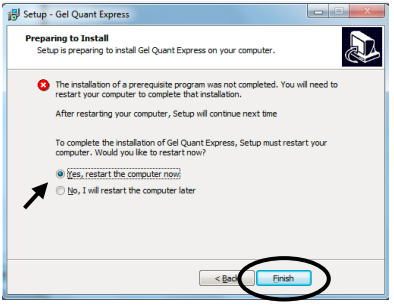
8



9. The “Completing Setup Wizard” screen appears. Click the checkbox “Yes, restart the computer”. Click **Finish**. The computer will then restart.

   After the PC restarts, the GelQuant installation process automatically continues.

9

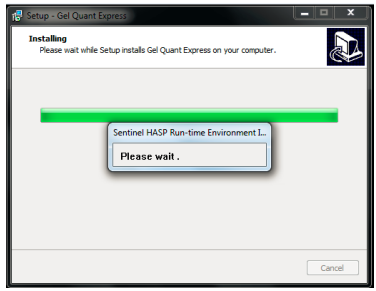


Continued on next page

Installing GelQuant Express(Windows 7), Continued

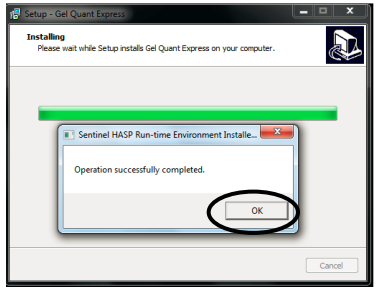
10. A progress bar indicates the status of the GelQuant installation process.

10



11. Upon completion of the installation process, a notification message appears, stating that installation was successful. Click **OK** to finish.
12. Launch the GelQuant Express software either by using the software icon located on the desktop, or the start menu.

11



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Continued on next page

Software Operation

Getting Started

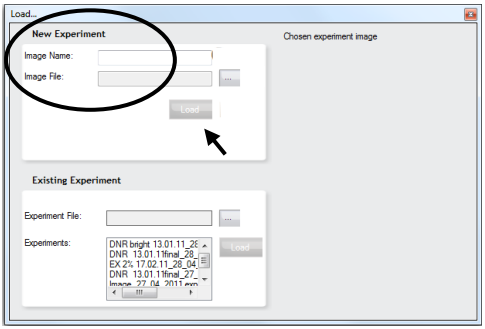
Launching GelQuant Express

Launch the GelQuant Express software application through the 'Start' menu or by double clicking the desktop icon.

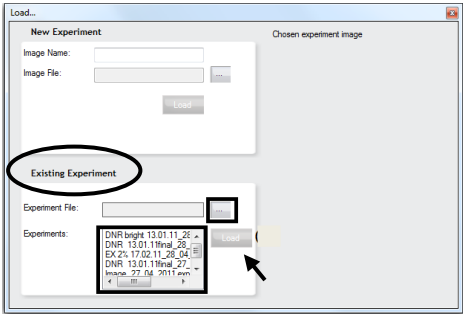
Loading images

To load an image file into GelQuant Express, select **File** > **Load…** from the Main Menu, or use the **Open** icon from the toolbar to display the load screen.

1. To load a New Experiment, use the "New Experiment" section of the load screen.
2. Click on the ... button to browse for the image you wish to load.
3. Enter an image name for the new experiment.
4. Click **Load**.



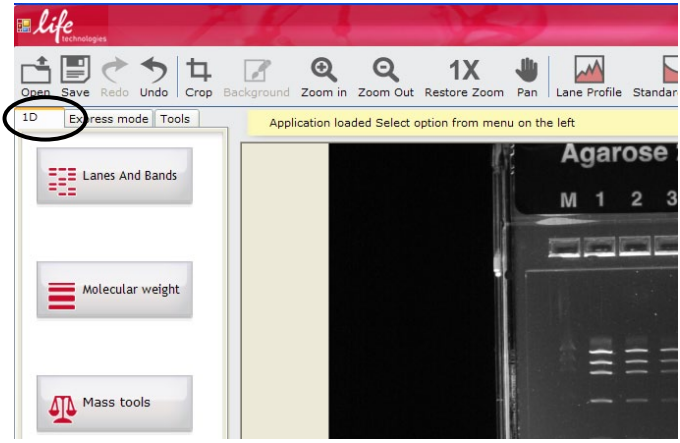
1. To load an Existing Experiment, use the "Existing Experiment" section of the load screen.
2. Click on the ... button to browse for the image you wish to load, **OR**
3. Upload a recent experiment by choosing from the Experiment list.
4. Click **Load**.



Quantitive Analysis of Gels Using the 1 D Tool

Using the 1 D Tool

The 1 D Tool is accessed using the 1 D tab in the left panel of the main screen.

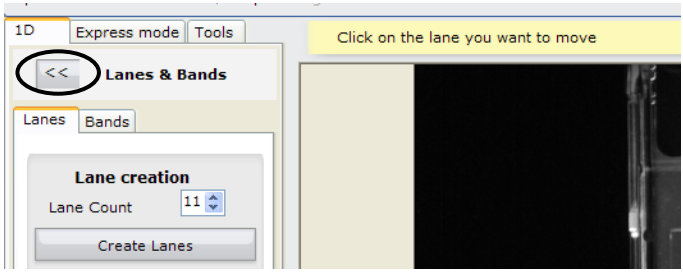


The 1 D Tool has three main functions:

- **Lanes and Bands**: To identify gel lanes and bands (page 19–30).
- **Molecular Weight**: To compare gel bands to a standard in order to calculate molecular weight (page 31–37).
- **Mass Tools**: To calculate additional information gel related to band mass (page 38–40).

After clicking on the 1 D tab, the functions can be accessed by clicking respective buttons on the left side of the main screen.

To return to the main 1 D Tool menu, press the **<<** button.



Continued on next page

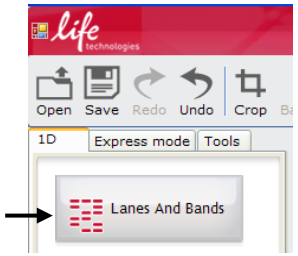
Lanes and Bands

Creating lanes

The Lanes and Bands tool is used to set the Area of Interest for the 1 D tool, and define lanes in the gel image.

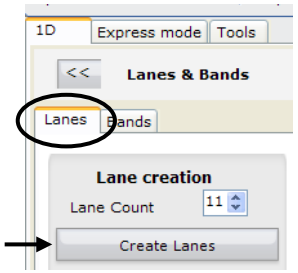
1. Open the image file, and click the **Lanes and Bands** button to create Lanes.

1



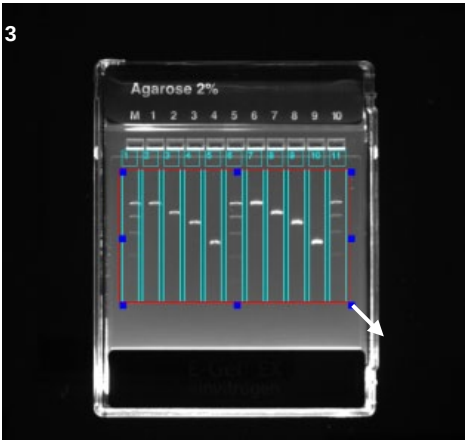
2. Click the Lanes tab, and enter the number of lanes contained in the gel image. Click the **Create Lanes** button.

2



3. Left click the mouse button, and drag the cursor from the upper left corner to lower right corner to create a Delimiter Box that defines the region of the gel image where the bands are located. Make sure that all of the lanes that are to be analyzed are included in the Delimiter Box.
4. Proceed to **Adjusting lanes** (page 20).

3



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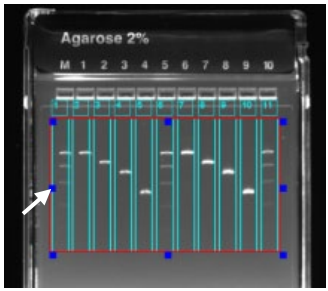
Lanes and Bands, Continued

Adjusting lanes

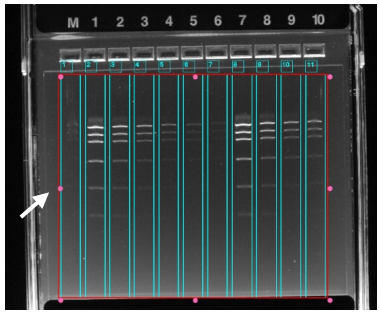
The lanes created within the Delimiter Box are framed with vertical green lines, and identified by a number at the top of the lane. Adjust the Delimiter Box as necessary as follows:

1. Adjust the size of the Delimiter Box by left clicking on one of the blue squares on the red frame of the Delimiter Box, and dragging the frame to achieve the desired size.
2. Rotate the Delimiter Box by placing the cursor over any of the blue squares on the red frame of the Delimiter Box.
3. Double click the blue square, and the squares become replaced with pink circles.
4. Left click on the cursor on any of the pink circles and rotate the Delimiter Box to the desired angle.
5. After achieving the desired angle, double click on one of the pink circles to return to the blue rectangles.

1

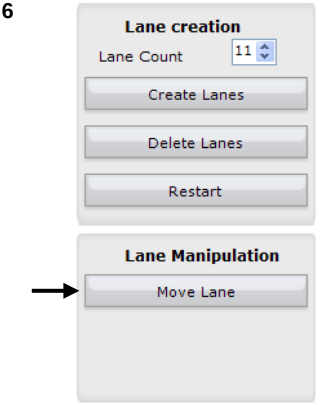


3–5

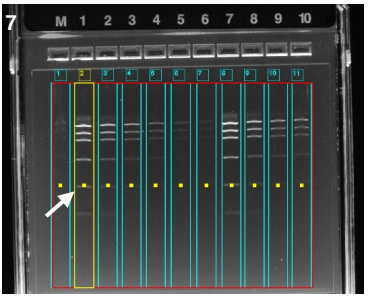


Continued on next page

6. Adjust the position of individual lanes by clicking on the **Move Lane** button.



7. Yellow rectangles appear in the center of each lane. Left click on a lane, and the blue frame turns yellow, allowing it to be moved to the left or right.
8. Once all of the lanes are properly identified, proceed to **Creating bands** (page 22).



Continued on next page

Lanes and Bands, Continued

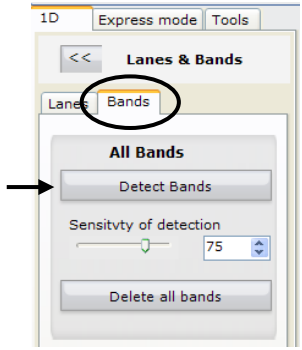
Creating bands

After all of the lanes in the gel have been properly identified, use the Create Bands function to detect bands within each lane. Options that exist for working with bands include:

- **All Bands**: Used to detect bands, and set band detection sensitivity.
- **Single Bands**: Used to resize, add, or delete individual bands.

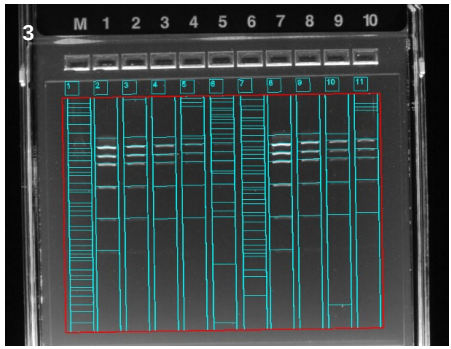
1. Click the Bands tab.
2. Click the **Detect Bands** button to detect bands within the lanes defined within the Delimiter Box.

1–2



3. The detected bands are marked with blue lines.
4. If necessary, proceed to **Adjusting band sensitivity** (page 23), **Adding bands** (page 24), **Resizing bands** (page 25), or **Deleting bands** (page 26).

3



Continued on next page

Adjusting band sensitivity

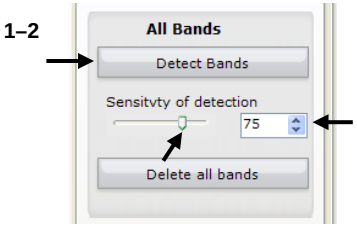
Examine the gel image on the screen, and if necessary, adjust the detection sensitivity.

Raising band sensitivity allows detection of high and low intensity bands. Lowering band sensitivity results in detection of only high intensity bands.

1. Set the band detection sensitivity (from 2–100) using the sliding scale or by using the up/down arrows.

   Note: Raising band sensitivity increases the number of detected bands, can potentially increase the number of false positives.

2. Click the **Detect Bands** button to detect bands using the new setting.



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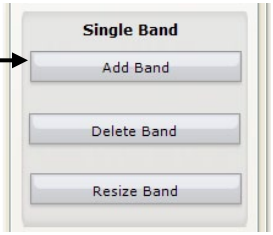
Lanes and Bands, Continued

Adding bands

Occasionally, bands that are visible to the eye on the gel image are not detected. Bands can be added manually using either the Add Band function from the left panel of the main screen as described in the following section, or by selecting an individual lane and using the Lane Profile tool (page 28).

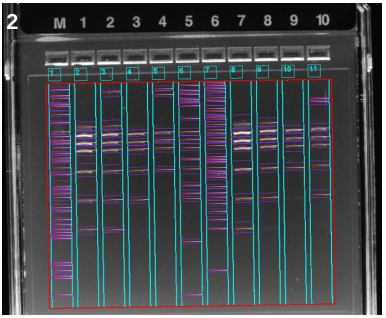
1. Click the **Add Band** button.

1



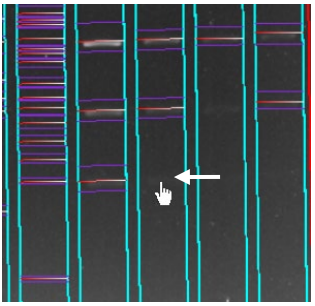
2. The margins of the detected bands are marked with purple lines.

2



3. Place the cursor at the location where you want to add a band, and left click the mouse button.
4. If necessary, proceed to **Resizing bands** (page 25).

3



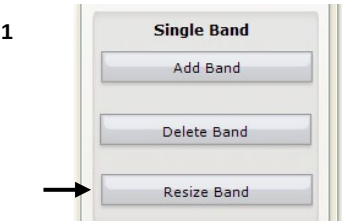
Continued on next page

Lanes and Bands, Continued

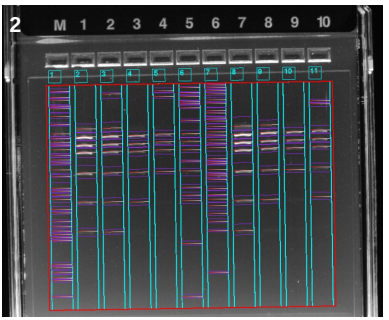
Resizing bands

Bands that are detected on a gel can vary in width, and may require adjustment to provide the most accurate data when performing analysis. Band size can be adjusted using either the Resize Band function from the left panel of the main screen as described in the following section, or by selecting an individual lane and using the Lane Profile tool (page 28).

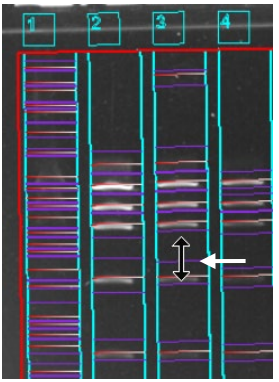
1. Click the **Resize Band** button.



2. The margins of the detected bands are marked with purple lines.



3. Set the cursor on the purple line at the upper or lower border of the band, and a two-headed arrow appears.
4. Left click the mouse button and drag the purple line up or down to resize the band.



Continued on next page

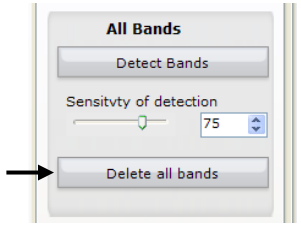
Lanes and Bands, Continued

Deleting bands

Bands can be deleted using either the Delete Band function from the left panel of the main screen as described in the following section, or by selecting an individual lane and using the Lane Profile tool (page 28).

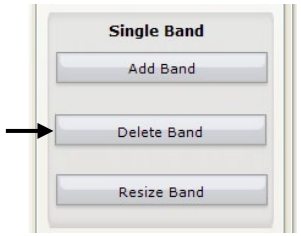
1. Click on the **Delete all bands** button to delete **all** detected bands in the gel image, OR

1



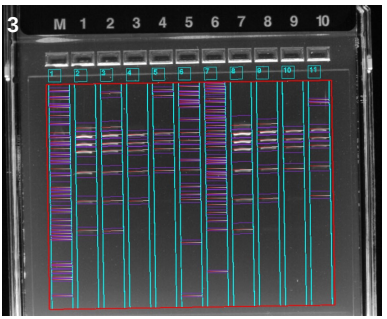
2. Click the **Delete Band** button to delete individual bands.

2



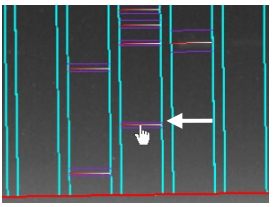
3. The margins of the detected bands are marked with purple lines.

3



4. Set the cursor in the middle of the band, and left click the mouse button to delete the band.

4



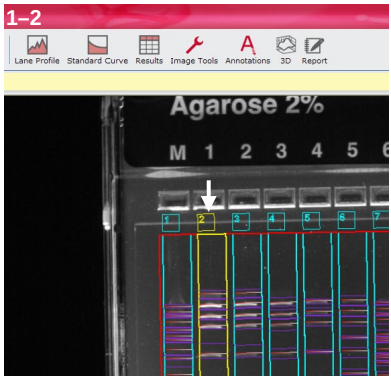
Continued on next page

Lanes and Bands, Continued

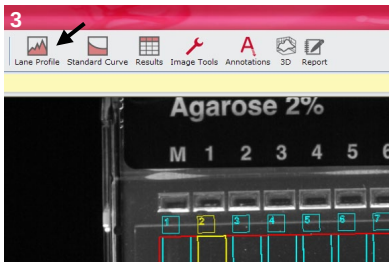
Using the Lane Profile tool

Selecting the Lane Profile tool allows Single Band functions to be used with more precision. The function provides a view of the lane, and a histogram showing individual band peak values.

1. Select the lane to be examined by left clicking on the numbered box at the head of the lane.
2. The borders of the selected lane changes from blue to yellow.



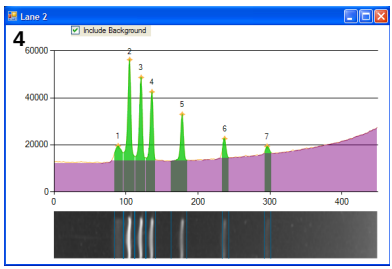
3. Click the **Lane Profile** icon on the tool bar to open the 'Lane Profile' window.



4. The Lane Profile histogram displays data on its X- and Y-axis, along with a gel image of the bands in the lane.

   Band peaks are displayed in green, while background is shown in purple (if the Include Background box is checked).

   The borders of the bands appear as blue lines in the gel image underneath the histogram.



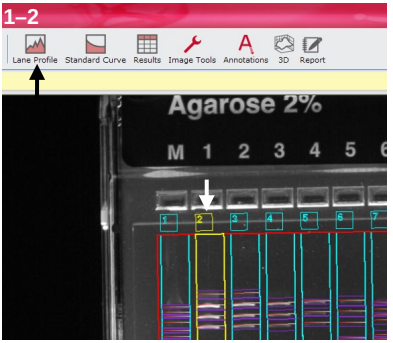
Continued on next page

Lanes and Bands, Continued

Single Band functions using the Lane Profile tool

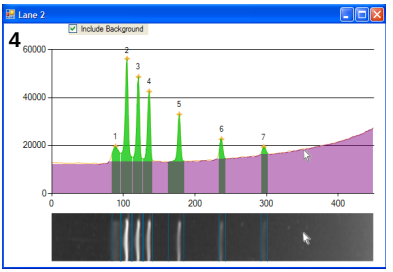
Bands can be added, deleted, or resized from the Lane Profile window using the Single Band functions located in the left panel of the main screen.

1. Select the lane to be examined by left clicking on the numbered box at the head of the lane.
2. Click the **Lane Profile** icon on the tool bar to open the 'Lane Profile' window.

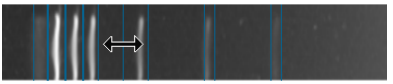


3. Click the **Add Band**, **Delete Band**, or **Resize Band**, button on the left panel of the main screen.

4. To add or delete bands, left click the mouse button in either the histogram or gel image at the location where you wish to add or delete a band.



5. To resize bands, set the cursor on the blue line at the left or right border of the band, and a two-headed arrow appears.
6. Left click the mouse button and drag the blue line left or right to resize the band.



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Lanes and Bands, Continued

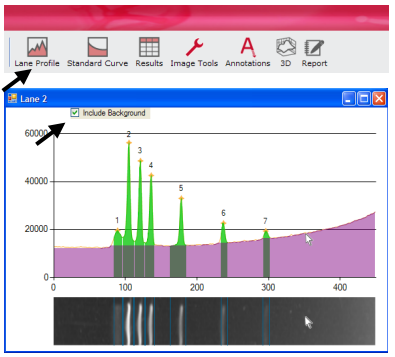
Background

Adjust the background of the gel image as required. Background signals can be reduced or even eliminated prior to performing analysis.

Background can be increased, or decrease for all lanes.

1. Click the **Lane Profile** icon on the toolbar to open the 'Lane Profile' window.
2. Click on "Include Background" check box to display background (shown in purple).

1–2

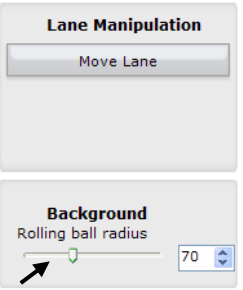


3. Adjust the rolling ball radius for background subtraction.

   The rolling ball radius is a range from 2–200.

   A large rolling ball radius indicates that less background is removed, while a small rolling ball radius indicates that more background is removed.

3



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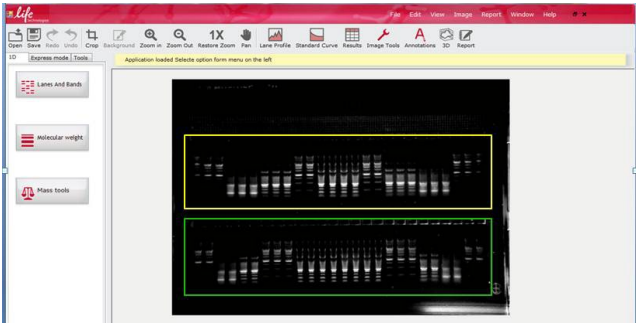
Multi-tier

The multi-tier function is used for gels which have more than one sample section. An example of a gel with multi-tiers is shown below, with the two sections marked in yellow and in green for the purposes of illustration only.



Since there are multiple sample sections, independent analyses must be performed for each tier.

To perform these individual analyses, the same step-by-step process for single samples should be applied to multi-tiered gels. The order of actions is as follows:

1. Create Lanes
2. Adjust Delimiter Box
3. Rotate Delimiter Box
4. Adjust Single Lane
5. Whole Lane Movement
6. Detect Bands/Delete All Bands
7. Single Band (Delete Band, Resize Band and Lane Profile)

Similarly, multi-tiers must receive individual attention to each separate section when dealing with the molecular weight and mass (see page 31).

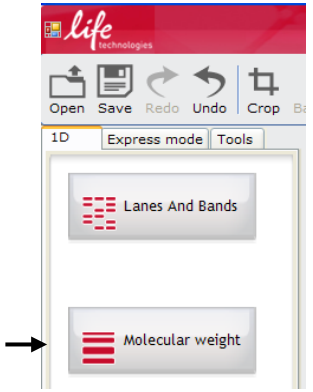
Molecular Weight

Determining molecular weights

The Molecular Weight function matches bands of known molecular weight from a standard run on the gel, to bands of unknown molecular weight.

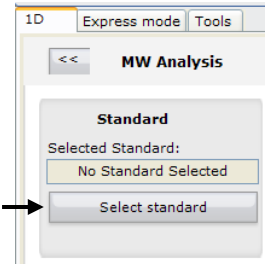
1. Open the image file, and click the **Molecular weight** button to display the MW Analysis screen.

1



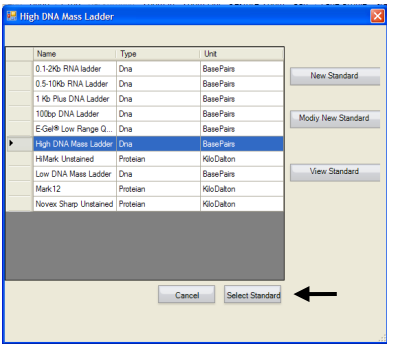
2. Click on the **Select standard** button to upload a new standard or choose an existing standard.

2



3. To select an existing standard from the default list, left click the row containing the standard you want to use.
4. Click the **Select Standard** button.

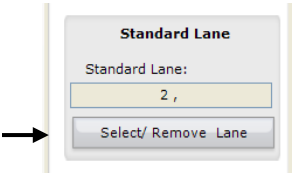
3–4



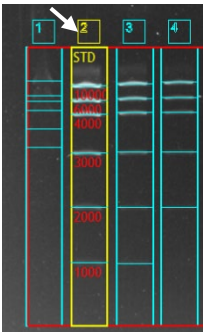
Continued on next page

Molecular Weight, Continued

5. Click the **Select/Remove Lane** button.

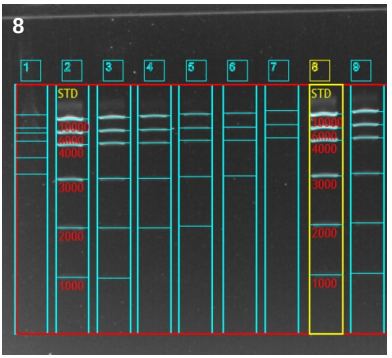


6. Select the lane containing the molecular weight standard by left clicking on the numbered box at the head of the lane.
7. The borders of the standard lane changes from blue to yellow, and is labeled “STD” at the top. The molecular weight values are displayed next to the bands.



8. Setting more than one standard lane can improve analysis results for a gel containing multiple lanes with the same standard.

   Additional lanes can be designated as standards by clicking on the numbered box at the head of the lane.
9. To remove a standard, click on the numbered box at the head of the standard lane a second time.



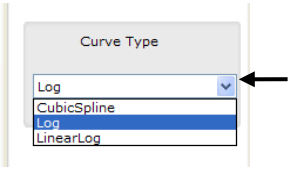
Continued on next page

10. Open the dropdown menu for and select the Curve Type for creating the standard curve.

    There are three types of curve:

    - **Cubic Spline**: Generates curves that are third order polynoms which pass through every data point.
    - **Log**: Generates a linear curve fitting of molecular weight and log scale of RF (Relative Front).
    - **Linear Log**: Generates a linear curve fitting of log scale of molecular weight and linear scale of RF.

10

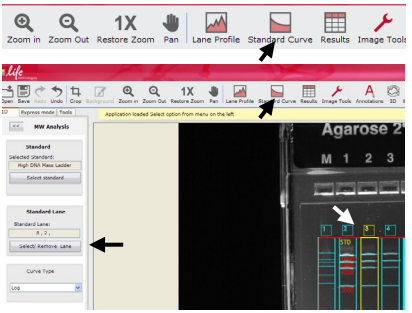


11. Select a lane for analysis by left clicking on the numbered box at the head of the lane.

    Note: Make sure the **Select/ Remove Lane** button is deselected when you are finished setting standards to avoid accidental designation of sample lanes as standard lanes. You can check the message in the yellow bar above the main image screen to see the status of the button.

12. Click the **Standard Curve** icon to perform the regression between the bands of the standard and those of the selected lane.

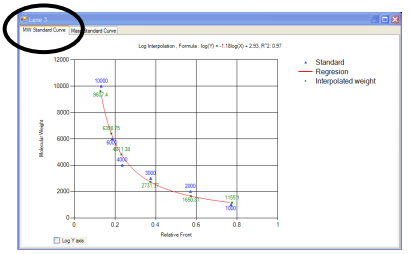
11–12



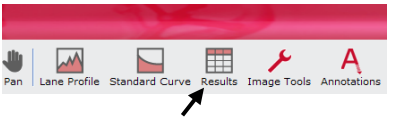
Continued on next page

Molecular Weight, Continued

13. A new window appears. The **MW Standard Curve** tab shows the values for the molecular weight standard (in blue), and the interpolated weight of the bands in the selected lane (in green).



14. Click on the **Results** icon to generate a table containing all of the molecular weight data for all lanes.



15. A new window appears, showing all the values for the bands for each lane of the gel image.

Results Preview

Switch Orientation | Export Image | Export Table

Lane 1

Number	Base Pairs (bp)	RF	Volume plus	Net Volume
1	11250.00	0.12	6715392.00	759818.53
2	7093.10	0.17	7694592.00	547903.84
3	5809.11	0.19	3090112.00	112325.27
4	4378.85	0.23	8667840.00	330037.15
5	3167.76	0.29	4921920.00	103046.84

Lane 2

Number	Base Pairs (bp)	RF	Volume plus	Net Volume
1	10000.00	0.14	18521792.00	8999189.39
2	6000.00	0.19	15204608.00	6802311.58
3	4000.00	0.24	12840896.00	5558905.77
4	3000.00	0.38	13174784.00	3654950.59
5	2000.00	0.57	10889600.00	1747072.53
6	1000.00	0.77	8307904.00	706118.60

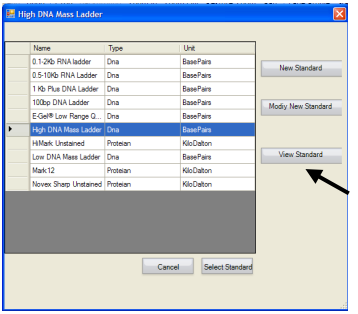
Molecular Weight, Continued

Viewing molecular weights

To view the molecular weight values for each band in a standard, perform the following steps:

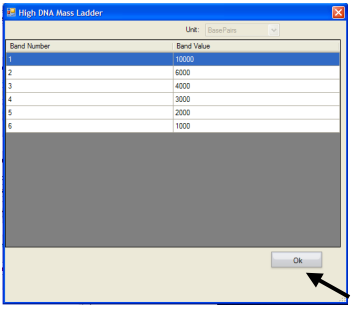
1. Left click the row containing the standard.
2. Click the **View Standard** button.

1–2



3. The values for each band of the selected standard are displayed in a new window.
4. Click **OK** to exits the window.

3



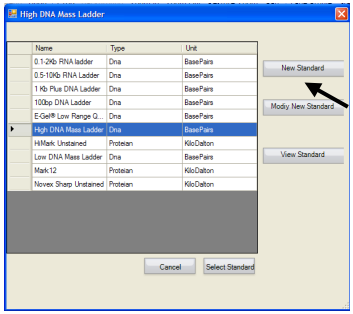
Molecular Weight, Continued

Creating molecular weight standards

New standards can be manually added to the list of molecular weight standards. These standards can also be edited as required. To add a molecular weight standard to the list, perform the following steps:

1. To create a new standard, click the **New Standard** button.

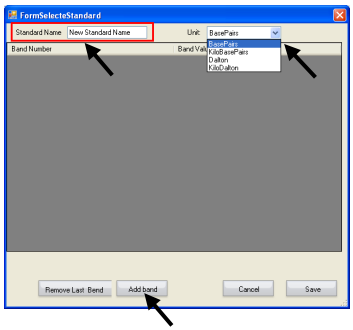
1



2. A dialog window is displayed.
3. Enter a name for your standard in the "Standard Name" text box.
4. Select unit value for the bands from the "Unit" dropdown menu.
5. Click the **Add band** button, and enter a molecular weight value for the band.

   Molecular weight values should be entered in order of decreasing weight, starting from the band with the highest molecular weight value.
6. Repeat step 5 until molecular weight values for all the bands in the standard have been added.

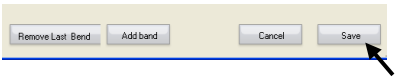
2–6



7. Click the **Save** button to save the standard.

   Note: The new standard can be canceled by clicking the **Cancel** button at any stage in the process.

7



Molecular Weight, Continued

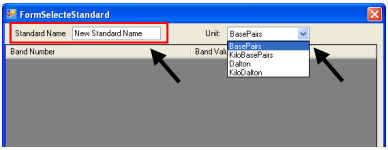
Changing molecular weight standards

Manually entered standards can be edited as required. The default standards, however, are protected from editing.

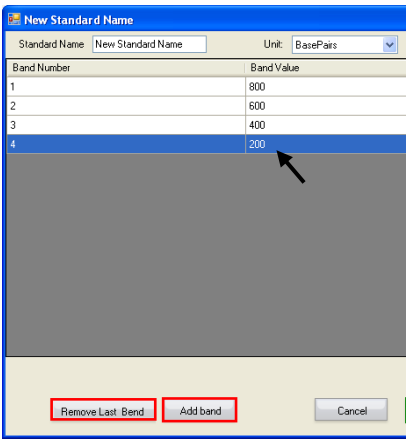
To change a molecular weight standard to the list, perform the following steps:

1. To change a new standard, click the **Modify New Standard** button.
2. To change the name of the standard, enter a new name in the "Standard Name" text box.
3. Change the units for the molecular weight if necessary using the "Unit"dropdown menu.
4. A dialog window is displayed.
5. To change the band value, click the left cursor button on the numeric value of the band, and enter a new value.
6. Band values can be added, by clicking on the **Add band** button.
7. Band values can be deleted from the bottom up, by clicking on the **Remove Last Band** button.

1–3



4–5



Mass Tools

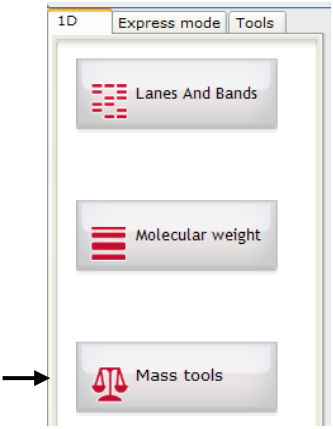
Mass analysis

Mass analysis should only be performed after the bands and lanes have been set. Mass is defined according to volume of the band without the background. Calculations should not be performed when the image has over-exposed pixel mass, because it will result in inaccurate values. The Mass Analysis tool can be used to perform:

- **Relative Mass Analysis**: compares sample band intensities to those of a selected reference band.
- **Absolute Mass Analysis**: Calculates band mass according to known masses of detected bands.

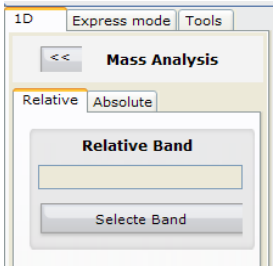
1. Click the **Mass Tools** button to display the Mass Analysis screen.

1



2. Select the **Relative** tab to perform relative mass analysis (page 39), OR
3. Select the **Absolute** tab to perform absolute mass analysis (page 40).

2–3



Continued on next page

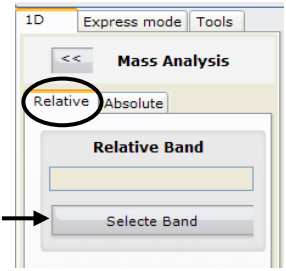
Mass Tools, Continued

Relative mass analysis

To perform relative mass analysis on the bands in a gel image, perform the following steps:

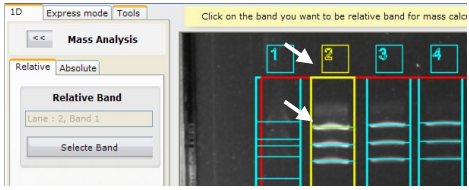
1. Select the **Relative** tab.
2. Click the **Select Band** button.

1–2

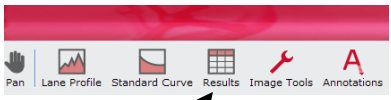


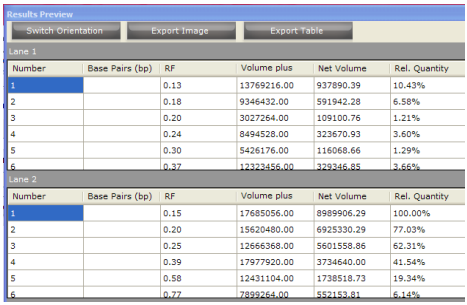
3. Select a lane containing the reference band (the band against which all other bands will be compared) by left clicking on the numbered box at the head of the lane.
4. The selected lane turns from blue to yellow.
5. Click on the reference band.
6. The blue line marking the selected band turns yellow.
7. Click the **Results** icon.
8. The "Results Preview" window appears, showing the values for the bands for each lane of the gel image in the **Rel. Quantity** column.

3–6



7–8





Mass Tools, Continued

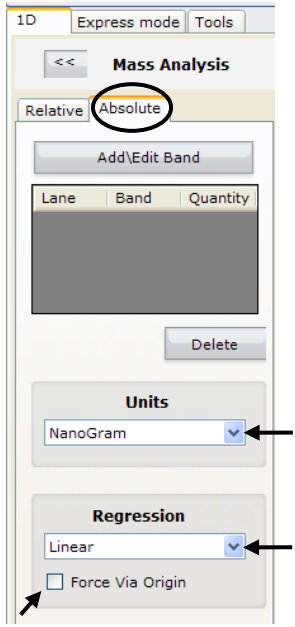
Absolute mass analysis

To perform absolute mass analysis on the bands in a gel image, perform the following steps:

1. Select the **Absolute** tab.
2. Select a unit of weight for the mass using the "Units" dropdown menu.
3. Select the regression method using the "Regression" dropdown menu. The regression method is used to generate the curve for fitting the band volume.

   There are two types of curve used for regression:

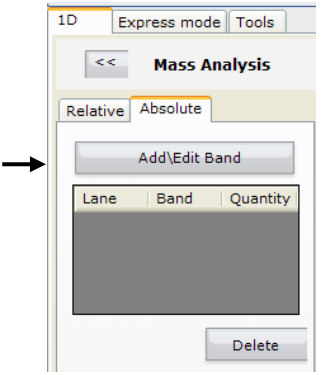
   - **Cubic Spline**: Generates curves that are third order polynoms which pass through every data point and, as such cannot be represented by a single equation.
   - **Linear**: Generates a linear curve fitting of band volume of mass.
4. To force the curve arc to pass through the point of origin for the selected regression, click the checkbox to "Force Via Origin".



5. Click the **Add/Edit Band** button to add reference bands.

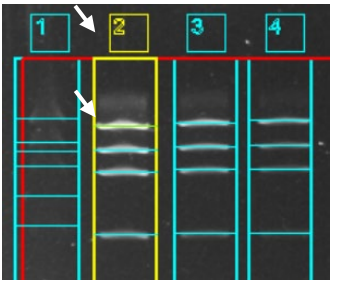
   Note: A minimum of two bands must be selected in order to create a curve.

5



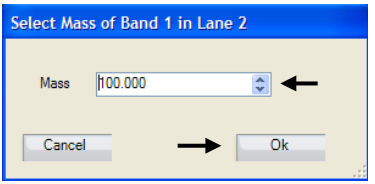
6. Select a lane containing a reference band by left clicking on the numbered box at the head of the lane.
7. The selected lane turns from blue to yellow.
8. Click on the blue band marking the band you want to use as a reference.

6–8



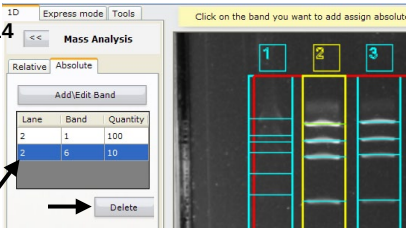
9. A pop-up window appears.
10. Enter the band mass in the text box.
11. Click **OK** to confirm the value.

9



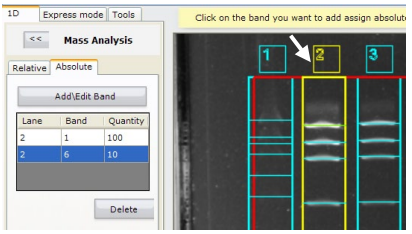
12. Repeat steps 8–11 for the desired number of reference bands.
13. To edit band mass values, re-enter the band mass data on a previously selected reference band, and click **OK**.
14. To delete a band mass value, select the band you wish to delete in the "Add/Edit Band" table, and click Delete.

14



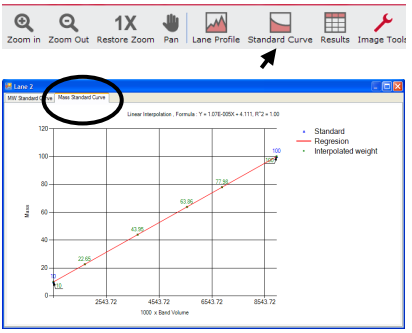
15. Select a lane for analysis by left clicking on the numbered box at the head of the lane.

15



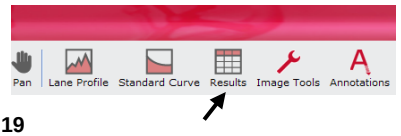
16. Click the **Standard Curve** icon to perform the regression between the bands of the standard and those of the selected lane.
17. A new window appears. The **Mass Standard Curve** tab shows the values for the mass standard (in blue), and the interpolated weight of the bands in the selected lane (in green).

16–17



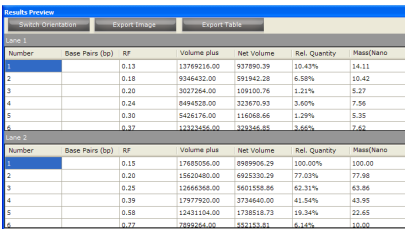
18. Click the **Results** icon to generate a table containing all of the mass data for all lanes.

18



19. The “Results Preview” window appears, showing the values for the bands for each lane of the gel image in the **Mass** column.

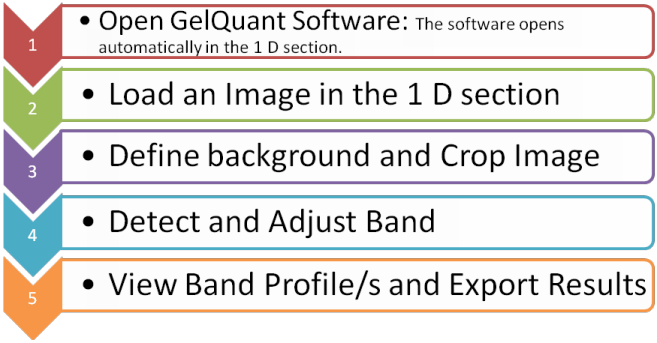
19



Express Mode

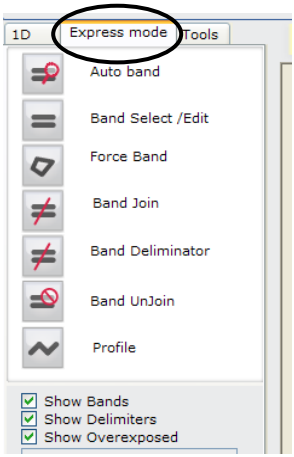
Overview

The Express Mode allows the user to perform relative calculations for gel images. The Express mode is recommended for quickly generating quality results.
To perform analysis using the Express mode, perform the following steps:



Load an image

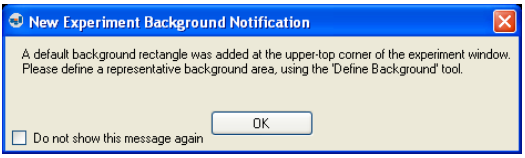
1. Launch the GelQuant Express software application. The left panel of the main screen is set to the 1 D Tool by default until an image is loaded.
2. Load an image as described on page 17.
3. After the image is loaded, click the **Express Mode** tab.



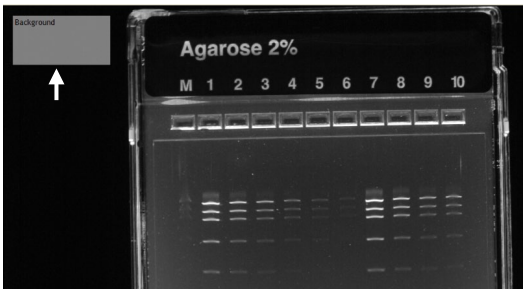
Continued on next page

Default background

4. When the Express Mode tab is selected, a dialog window is displayed.

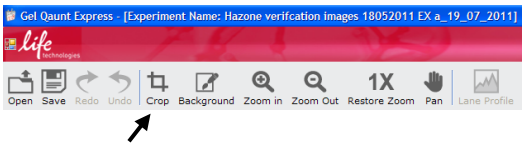


5. Click **OK**. An image background field is created in the image.



Crop image

6. Click the **Crop** icon on the toolbar.

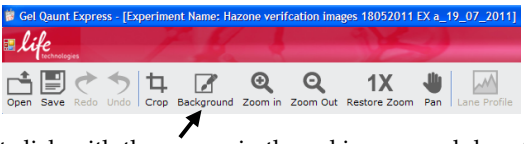


7. Left click with the mouse in the gel image, and drag the cursor, to define the region of the gel that you are interested in analyzing.

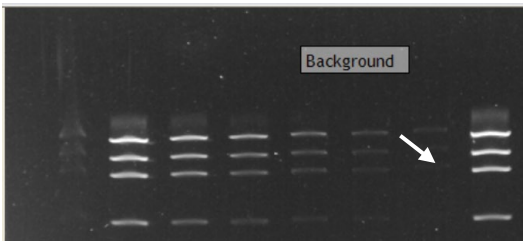
Continued on next page

Specify background area

8. Click the **Background** icon on the toolbar.



9. Left click with the mouse in the gel image, and drag the cursor, to define the exact background area for use in analysis.



10. Release the mouse button to define the area. The text "Background" appears in the defined area.

Detect and adjust bands

Band detection is performed after defining the background and adjusting the image analysis area by cropping.

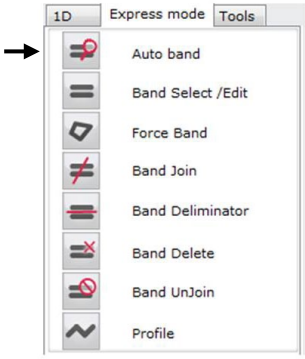
There are three band detection tools:

- **Auto Band**: Allows the user to detect bands in the gel image automatically. Automatic detection may not be able to detect every band, therefore, there are two other tools are available for manual addition of bands (see page 46).
- **Band Select/Edit**: If Auto band detection fails to find a band, the band can be manually defined using this tool (see page 48).
- **Force Band**: If both Auto band detection and the Band Select/Edit functions fail to find a band, the band can be manually defined using this tool (see page 49).

Each of these three tools can be used for detecting and adjusting bands. Read the following section carefully to understand how and when they are used.

Auto band

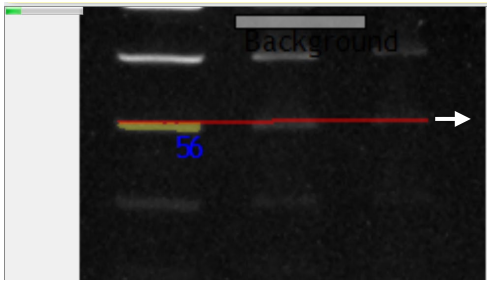
1. Left click the **Auto band** icon.



2. Place the cursor over a band in the image, and drag the cursor horizontally across a row of band, or vertically down a column of bands.

   The software automatically detects bands designated by the cursor. A green progress indicator is displayed in the upper left image screen during the automatic detection process.

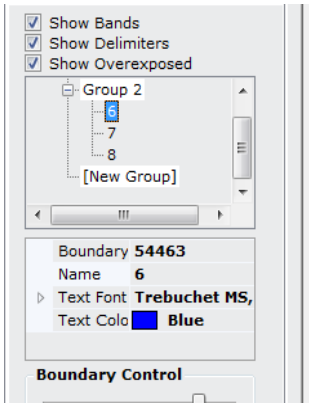
   Each band that is detected is assigned a unique number to facilitate identification.



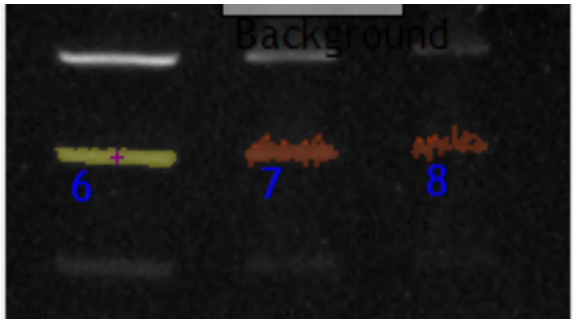
Band group table

When the Auto band function is used, a table showing the detected bands in a given group is displayed in the lower half of the left panel of the main screen.

Each group represents a lane that has been specified by the user. For example, there will be two groups if two lanes have been caught by the dragging action.

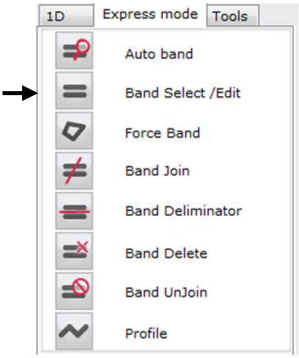


The example displayed here shows Group 2 with bands 6, 7 and 8. The individual band colors and corresponding band numbers appear on-screen. In this case, band 6 is colored yellow because it has been selected in the table, while bands 7 and 8 are colored orange and not selected.

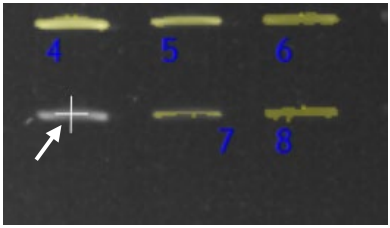


Manual band addition using Band Select/Edit

1. Click the **Band Select/Edit** icon.

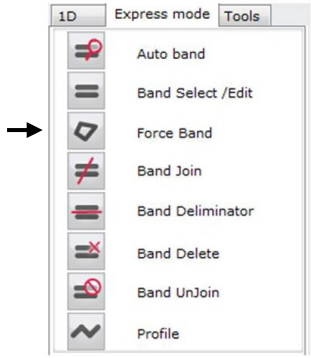


2. Place the cursor over the center of the band to be added, and left click the mouse button.

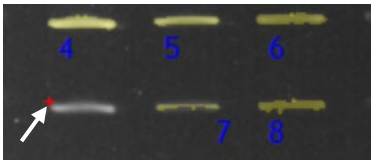


Manual band addition using Force Band

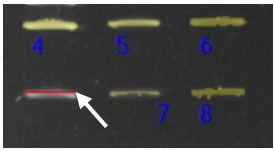
1. Click the **Force Band** icon.

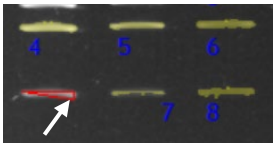


2. Place the cursor at the upper left hand corner of the band to be added, and left click the mouse button.

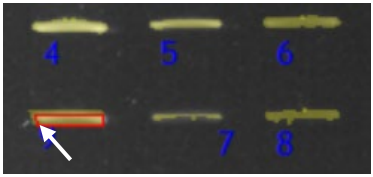


3. Left click at the upper right hand corner, and the lower right hand corner of the band.





4. Double click at the lower left hand corner of the band, enclosing it completely with the red band frame.



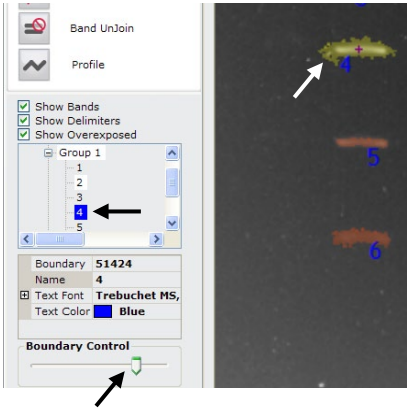
5. The band turns yellow, and is displayed as a new band in the band group table.

Boundary control

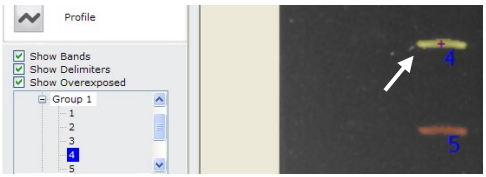
The Boundary control tool allows the user to control the size of the band area. If the software has not detected the entire band, or has included areas of background in the band area (indicated by a yellow band area that is either smaller, or larger than the band area), the boundary control tool can be used to adjust the size of the band by changing detection sensitivity.

The Boundary Control data appears at the lower corner of the left panel of the main screen, and is used as follows:

1. Click on a band number in the band group table, and the selected band change color to yellow.
2. The Boundary Control slider appears below the band group table.



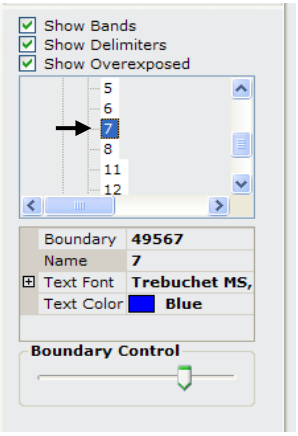
3. Slide the 'Boundary Control' indicator to the left or right to change the size of the band area.
4. Adjust the yellow area until it is the same size as the area of the band.



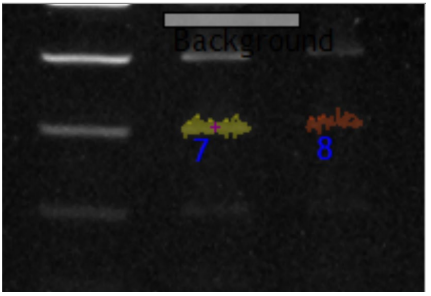
Express Mode, Continued

Deleting a detected band

1. To delete a band, select the band from the band group table in the left panel of the main screen.



2. The selected band (targeted for deletion) is shown in yellow. Bands that are not selected are colored orange.



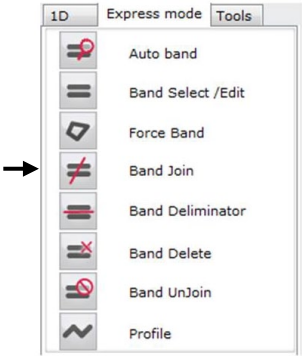
3. Press the **Delete** button on your computer keyboard to delete the band.

Joining broken bands

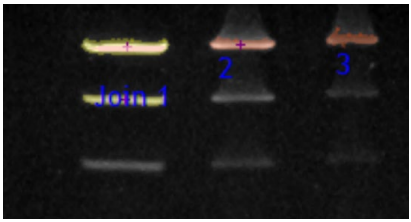
If a band is separated into two or more sections by gel artifacts (e.g., air bubbles) in the image, the software may detect the band as two or more individual bands.

These broken bands can be formed back into a single band using the Band Join function as follows:

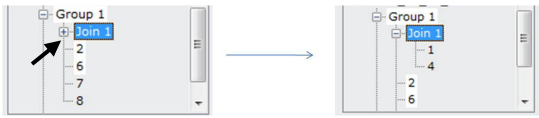
1. Identify the elements of the broken band.
2. Click the **Band Join** icon.



3. Click on each element of the broken band to join them together. The joined bands are designated as "Join 1".



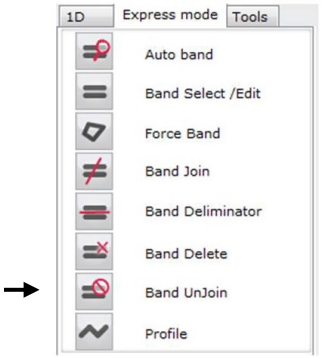
4. Deselect the Band Join icon after all the elements of the broken band have been selected.
5. This joined band is displayed in the band group list at the lower corner of the left panel of the main screen as "Join 1". Clicking on "Join 1" allows the individual bands that make up the joined band to be viewed.



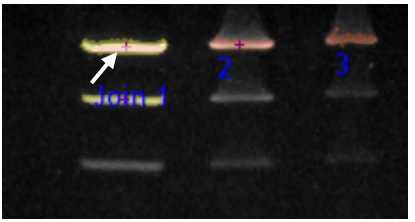
Unjoining joined bands

To separate joined bands back to their original state, use the Band UnJoin function as follows:

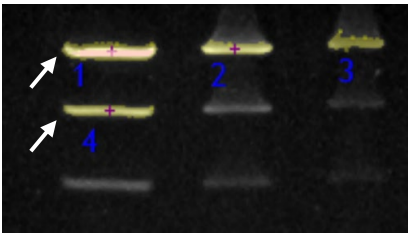
1. Click the **Band UnJoin** con.



2. Place the cursor over any of the joined bands, and left click the mouse button.



3. The selected band(s) are separated into individual bands.

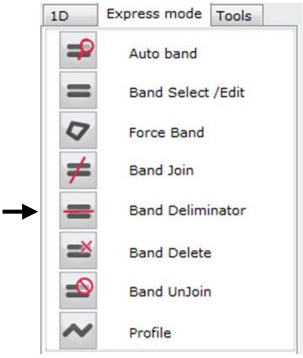


Removing band section artifacts

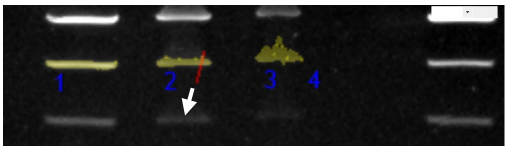
If the GelQuant Express software detects a band area containing the actual band in addition to a gel artifact, the Band Deliminator tool can be activated used to eliminate the parts not intended for analysis.

Any detected band part closest to the band edge will be eliminated.

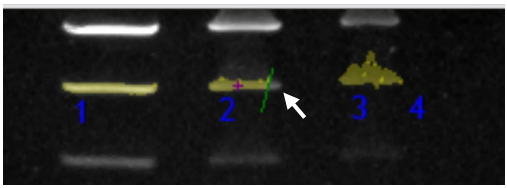
1. Identify the part of the band that needs to be removed.
2. Click the **Band Deliminator** icon.



3. Left click the mouse button, and drag the cursor across the band at the juncture between the actual band, and the artifact to be removed.



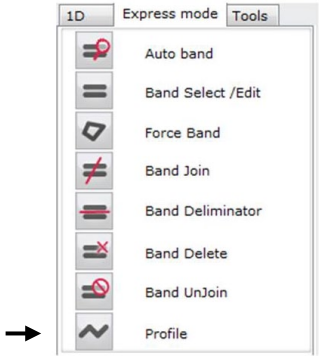
4. Release the mouse button, and the red line turns green. The part of the band with the shortest distance between its center and its edge is eliminated, and the eliminated area changes color from yellow to the basic image color.



Profiling

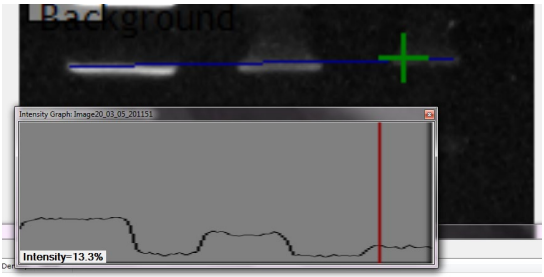
The Profile tool is used to compare the intensity of a given band with that of other bands. To use the Profile tool, perform the following steps:

1. Click the **Profile** icon.



2. Left click the mouse button, and drag the cursor horizontally across a row of bands, or vertically down a column of bands to view bands intensities.

3. An active intensity graph image with a vertical red line is displayed. The red line corresponds to the green "+" displayed on the image in the main screen.



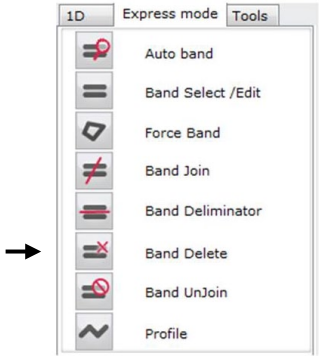
4. Place the cursor on the red line and move it left or right to compare band intensities expressed as percentages of the camera grey level. The camera discriminates between 65535 grey scales from black (0) to white (65535).

Express Mode, Continued

Deleting bands

The Delete function can be used to exclude bands from analysis. To use the Band Delete tool, perform the following steps:

1. Click the **Band Delete** icon.



2. Set the cursor in the middle of the band, and left click the mouse button to delete the band.

Overexposure

Overexposure occurs when the camera receives too many photons of light from the sample, and defines a state in which part of, or the whole image contains saturated areas.

Overexposure usually results in white or bright areas that yield inaccurate data when analysis is performed.

If there are over exposed area in the image, the GelQuant Express software automatically indicates these areas in pink, and launches the following pop up message:

Click **OK** to dismiss the message.

To display overexposed pixels in the image, make sure that the "Show Overexposure" checkbox is selected in the Preview window (see page 65).

Important: Analyzing images with overexposed areas will result in inaccurate analysis results.

Express Mode, Continued

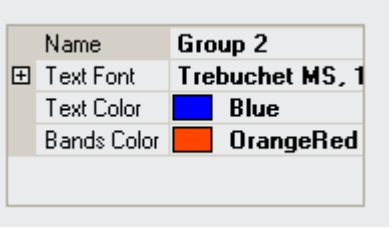
Express Mode check boxes

Express mode check boxes allow the user to show or hide various marks set in the image.

- **Show Bands**: Used to show detected bands if checked. If the box is unchecked, the color overlay for detected bands are not shown.
- **Show Delimiters**: Delimiters are displayed if checked, or hidden if not checked.
- **Show Overexposed**: Overexposed areas are displayed if checked, or hidden if not checked.

Font and colors

To view an image and use analyis functions in the clearest possible manner, change the text font and colors used for annotation as desired.

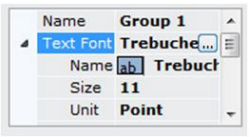


- **Text Font**: Left click on Text Font, or expand the row to make changes in font style.
- **Text Color**: Left click on Text Color to choose different colors for text.
- **Bands Color**: Left click on Bands Color to choose different colors for detected bands.

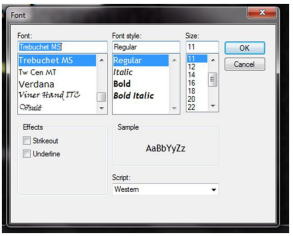
Express Mode, Continued

Font selection

1. Left click on Text Font, or expand the row by clicking the "+" icon.
2. Click on the ... button.



3. A new window appears.



4. Choose the desired font, style, size, and effects.
5. Click **OK**.

Color selection

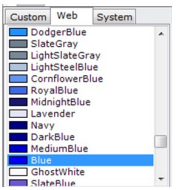
1. Left click on Text Color, or Bands Color.
2. A dropdown menu arrow appears at the right side of the Text Color area.

3. Click the arrow to open the color option window.

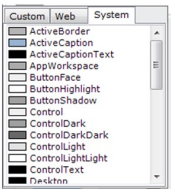
   The color window option allows the user to choose colors from three menus:

Custom

Web



System



Express Mode, Continued

Results preview

The Results Preview window summarizes the following data for detected bands in tabular form:

- **Area**: Derived from pixel count.
- **Density**: Band density (related to band intensity).
- **Value**: The agglomerated band value. The band value parameter takes into consideration the area, density and the background and eliminates the background calculation.

The data can be exported as a Microsoft Excel compatible file by clicking on the **Export Table** button.

The image can be exported by clicking on the **Export Image** button. The image is copied onto a clipboard, and can be copied to any other PC location.

Toolbar icons in Express mode

Toolbar icons are identical to the ones used in 1 D with the following four exceptions:

- There is no Lane Profile tool.
- There is no Standard Curve tool.
- The report refers only to data related to the 1 D mode. The report will contain only the image basic information without presenting the Results Preview table.
- The Background icon on the toolbar is only usable in Express mode.

Tools

Tools mode

The Tools mode contains the following two functions:

- **Image Tools**: Allows optimization of the image adjustment prior analysis, to display faint details. All adjustments made using the Image Tool only affect the way the image is displayed, and do not alter the original image file.

  The Image Tools can only be used prior to setting lanes and band detection.

- **Annotations**: Allows adding and editing the image with text and arrows.

Image tools

1. Load a new image.
2. Launch the Image Tools by clicking on the button in the left panel of the main screen, or from the toolbar.

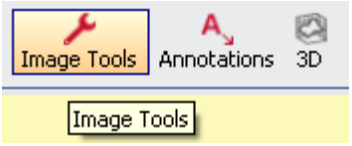


Image tools, continued

3. The Preview window opens.

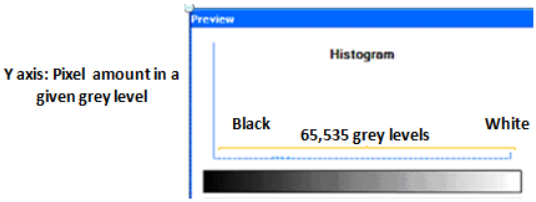
Note: The Preview window cannot be activated if lanes or bands were already set.

4. Make the desired changes to the image (see pages 63–65).
5. Click the **Apply** button to confirm the adjustments to the image, and close the Preview window,
6. Click the **Restore** button to reset all the settings to the default, OR
7. Click the **Cancel** button to reset all the settings to the default, and close the Preview window.

Histogram

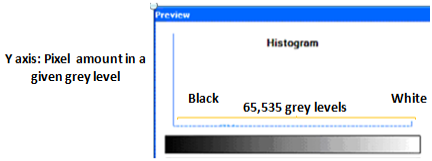
The histogram shows the distribution, and amount of grey levels for the image. It provides a quality reflection of the image black and white colors kind and amount (as the camera can discriminate up to 65,535 levels of black and white).

X-axis indicate the grey level kind and the Y-axis indicates the grey level amount.



Contrast sliders

Every image has its own information based on grey levels. In order to reveal faint bands, or to make an image clearer, the grey level range can be changed. The Contrast sliders allows the Min and Max grey level limits to be changed, which results in a different image contrast.



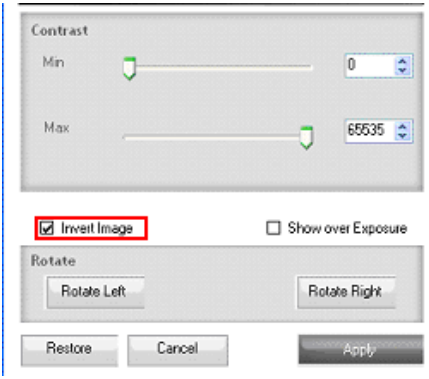
Use the Min slider to set the minimum grey level value for your gel image.

Use the Min slider to set the minimum grey level value for your gel image.

Note: You can set the min/max numerical values in the edit boxes next to the sliders as well.

Invert image

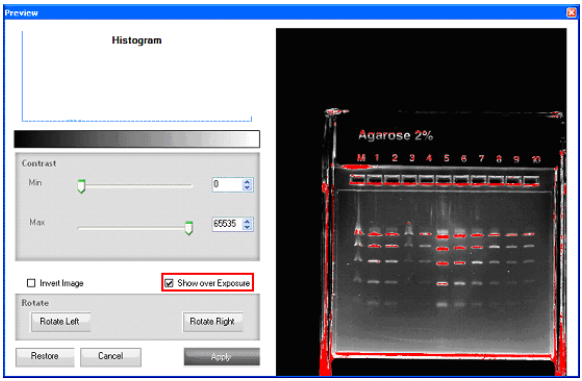
To view the image in opposite colors (white becomes black and black becomes white), click the check box that performs this function.



Show overexposure

Image (pixel) saturation can result in faulty analysis results. In order to avoid overexposure, click the check box that performs this function.

Saturated areas are marked in red, and their presence indicates that a new image should be captured for accurate analysis to be performed.

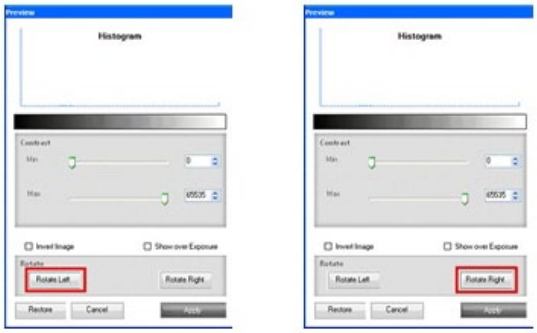


Note: The overexposed areas marked with red are shown in the Preview window only, and are not displayed in the main screen.

Rotate

The rotate function allows the image orientation to be changed.

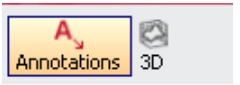
The **Rotate Left** and **Rotate Right** buttons turn the image 90° to the left or right accordingly.



Tools, Continued

Annotations tool

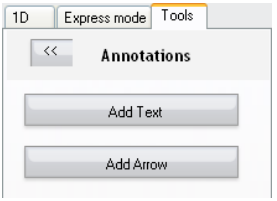
The Annotation tool allows the user to annotate the image with either text, or arrows. The tool is accessed through the 'Tools' tab in the left panel of the main screen, or the 'Annotations' button on the tool bar.



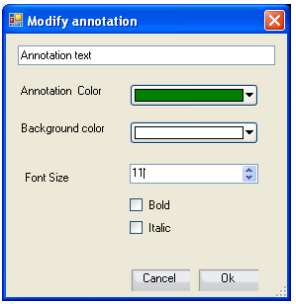
Adding text annotations to an image

To create and place text in an image, click the 'Add Text' button, and left click the mouse button on the image in the location where you want to place the text.

To change the placement of text, left click on the text to be moved with the mouse, and drag the text to a new location.



The 'Modify annotation' dialog box appears. Enter text into the text field, and adjust text options as needed.

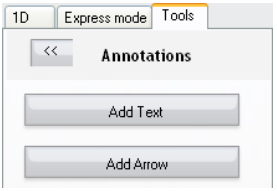


Deleting text annotations in an image

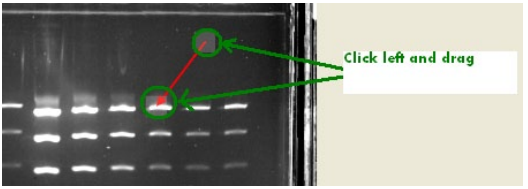
To delete annotations, place the cursor over the text in the image, and right click the mouse button.

Adding arrows to an image

To add an arrow to an image, click the 'Add Arrow' button.



Set the cursor to the point of origin for your arrow in the image to be annotated. Left click the mouse button and drag the cursor (while still pressing the left mouse button) to set the end point of the arrow.

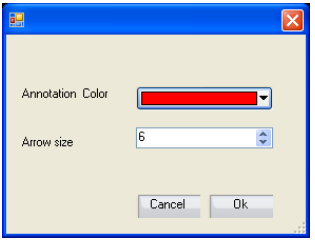


To change the placement of an arrow, left click on the arrow with the mouse, and drag it to a new location.

Changing arrow direction

To change the direction of the arrow, left click the mouse button, and drag one end of the arrow (boxed region) to reposition it in a new direction.

To modify the appearance of an arrow, double left click on the arrow. The Arrow Modification dialog box appears, adjust arrow options as needed.



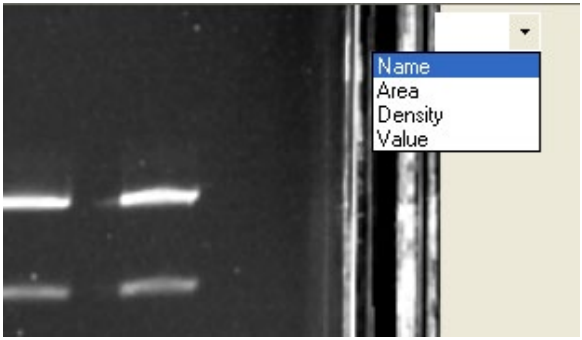
Deleting text annotations in an image

To delete an arrow, place the cursor over the arrow, and right click the mouse button.

Band annotation

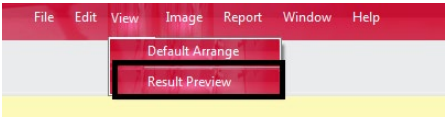
The band annotations dropdown box allows specific annotations to be noted beside a detected band. The dropdown box is located at the top right corner of the main screen. The following options are available:

- Name
- Area
- Density
- Value



1D Lane Results and Data

Results are generated as lane analysis data below the image on various screens. These results are also accessible by clicking View >> Result Preview.

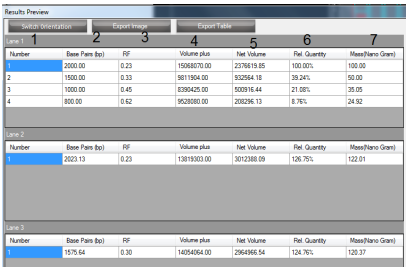


The following actions can be performed in the Result Preview window.

- **Switch Table Orientation**: Switch orientation from vertical to horizontal and vice versa.
- **Export Image**: Export the image displayed on the main screen.
- **Export Table**: Export the table as a .CSV formatted file compatible with Microsoft Excel.

Results data

Each lane of the Results Preview window has the following data:



1. Band number.
2. Band molecular weight: Displayed if a molecular weight standard is assigned.
3. Band relative front (RF): The band measurement based on the location of the band relative to the standard.
4. Band Volume with background.
5. Band volume after background has been subtracted out.
6. Band Relative Quantity: Displayed if a reference band for relative quantity is assigned.
7. Band Mass: Displayed if reference bands for absolute quantity are assigned.

Continued on next page

1D Lane Results and Data, Continued

Report

After the image has been analyzed, a report can be generated in a PDF format. It displays results and image data, with print capabilities either for a specific lane or the entire image with every lane.

To generate a report, click on in the drop-down menu and select either All Lanes or Single Lane Report.

Image statistics graph

Quantity	Gray level amount
Gray Level	Gray level type (0–65,535).
Count	The number of gray levels in the image.
Edge	Refers to image sharpness.
Peak	The grayscale value with the highest number of pixels.
Min	Indicates the lowest reading in the statistical curve.
Max	Indicates the highest reading in the statistical curve. A maximum of 65,535 indicates the highest rating.
Median	The median grayscale is the value in the exact middle between the maximum grayscale and the minimum grayscale of the image.

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.invitrogen.com** for:

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@invitrogen.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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Continued on next page

Purchaser Notification, Continued

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