MAGELAN

TUTORIAL

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1 INTRODUCTION

1.1 About Magelan

Welcome to Magelan. This program is designed to automate analysis of electrophoresis gels. Through a user-friendly, graphical interface, Magelan allows you to quickly and accurately identify the positions of DNA fragments for three analysis modes: fingerprinting, allele-calling and fragment-sizing. We welcome your feedback and comments regarding all aspects of this program.

1.2 About this Tutorial

This tutorial will lead you through a step-by-step analysis of three sample gel images, one for each mode of the software. The role of the tutorial is to provide you with information about the main functions of the software and to assist you in analyzing your own gel images using Magelan.

Sections preceded with "**Note:**" offer additional information that may not be necessary for the analysis of the sample image.

Sections 2-5 of this tutorial are steps that are common to all three modes of operation of Magelan. Section 7 explains the processes and features associated with fingerprinting mode. Section 8 deals with fragment-sizing mode, and section 9 covers allele-calling.

If you have already installed Magelan on your computer, please skip to Section 3, **STARTING MAGELAN**; otherwise please continue to Section 2, **SETUP AND INSTALLATION**.

2 SETUP AND INSTALLATION

2.1 Microsoft Windows

To install Magelan on a computer running a Microsoft Windows operating system, place the Magelan Installation CD in your CD-ROM drive. If the *Autorun* feature is enabled, the installation will automatically start. If not, you can start the installation by opening **My Computer**, navigating to your CD-ROM drive, selecting the ***Windows*** directory and running ***Setup.exe***.

As you move through the installation, be sure to read each step. If you have questions or problems with the license agreement, please contact INCOGEN and we will help you in any way possible. Once you have installed Magelan, you can review the license agreement by navigating to the directory where you installed the software and opening the file ***license.txt***.

Once you have accepted the license agreement, you may select the directory in which to install Magelan (Figure 2.1.1). The default destination directory is:

C:\Program Files\INCOGEN\MAGELAN.

If you would like to change the destination directory, press **Browse** and select a new location.

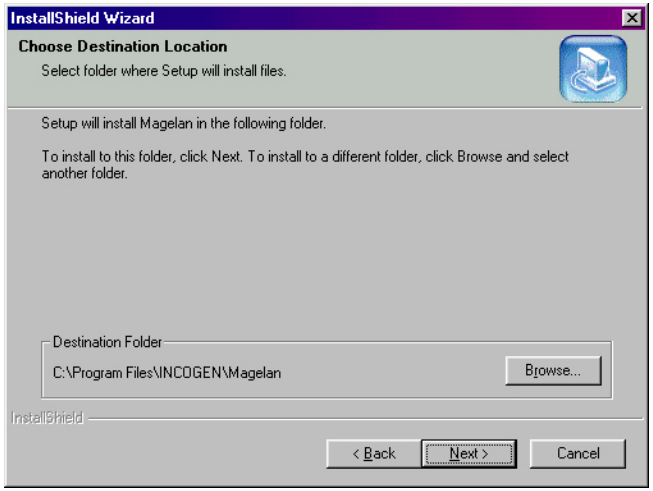

Figure 2.1.1: Choosing a destination directory.

After you have selected a location for the software, you will be asked to specify what type of installation you would like to perform: ***Typical***, ***Compact*** or ***Custom***. In the typical installation, the files necessary for running Magelan will be installed, as well as a set of sample files and an electronic copy of this tutorial. In the compact installation, only the files necessary for running the software will be installed. In the custom installation, you can choose which items you would like to install through a dialog like the one shown in Figure 2.1.2 below.

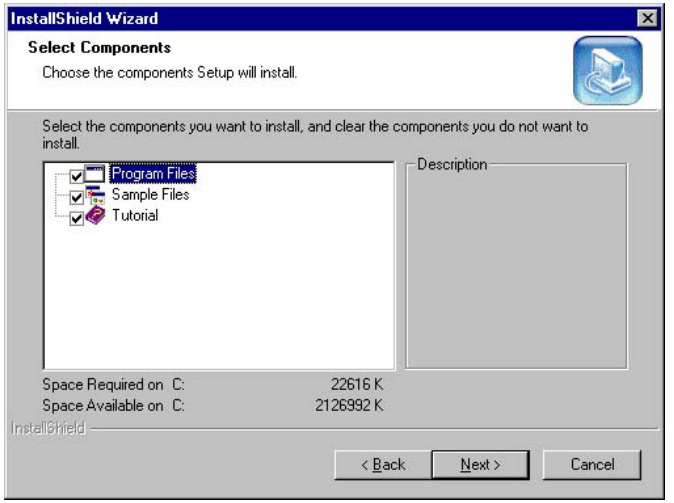

Figure 2.1.2: Selecting the components to install.

Finally, the installation will prompt you for a location to place a shortcut. The default location is on the **Start** menu under **Programs** in a group called **INCOGEN**. You can change this location if you desire. Press **Next** to begin copying the files from the CD to your hard drive. You will see a progress meter as files are copied and your system is configured. When this completes, press **Finish**. The software is now ready to use.

3 STARTING MAGELAN

3.1 Microsoft Windows

If, during the installation, you chose the default location for placing the shortcut, you can start Magelan by pressing **Start->Programs->INCOGEN-> Magelan** as shown in Figure 3.1.1. If you chose a different location for the shortcut, navigate to the appropriate folder to start Magelan.

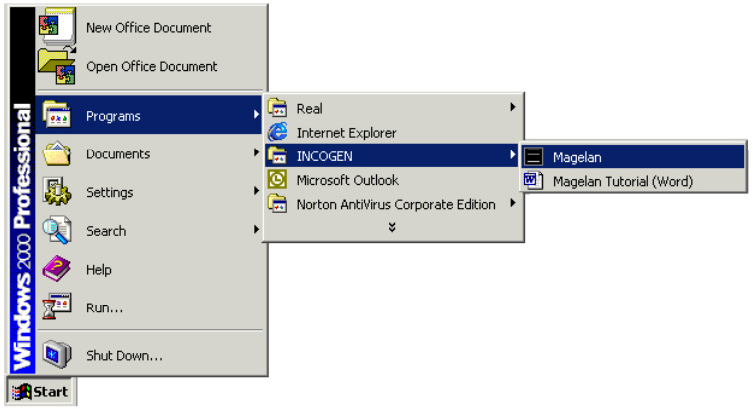

Figure 3.1.1: Starting Magelan under Microsoft Windows.

4 LOADING AN IMAGE

Magelan recognizes images of gels in the following formats: TIFF (TIF), GIF, JPEG (JPG). To open a new image, you can select **File->Import Image** from the Main Menu (Figure 4.1a), or click on the *FastFlow* **Image** button (Figure 4.1b).

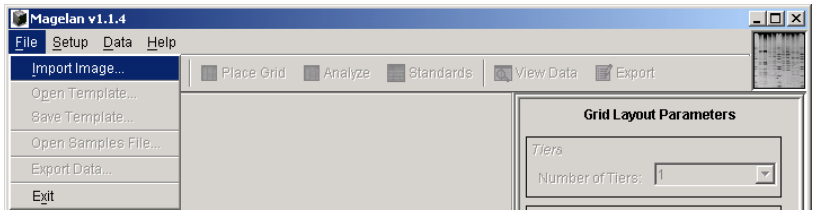

Figure 4.1a: Opening an image by using the **File** menu.

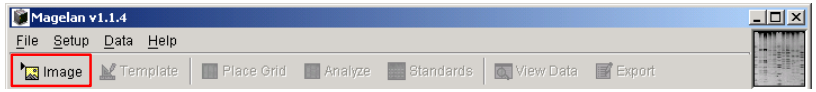

Figure 4.1b: Opening an image by using the *FastFlow* button.

Select the directory in which you installed Magelan. (For example, the default install directory for the Windows operating system is:
C:\PROGRAM FILES\INCOGEN\MAGELAN.)
That directory contains a sub-directory named ***samples*** which contains three sample image files:

1. ***SampleAllele.tif***
2. ***SampleFingerp.jpg***
3. ***SampleFragment.tif***

Double-click on the file called ***SampleFingerp.jpg*** to select it for analysis (Figure 4.2). It is possible that you may not see the image type extensions (.jpg, .tif) because you enabled the "Hide file extensions" feature in Windows.

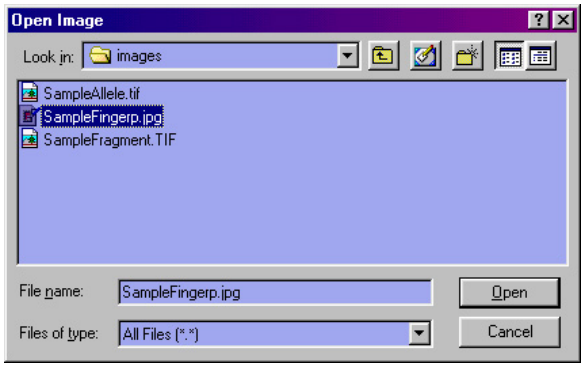

Figure 4.2: The file chooser window with the image file ***SampleFingerp.jpg*** selected for analysis.

After the image file has been selected and loaded into Magelan, it will appear inside of the image panel (Figure 4.3).

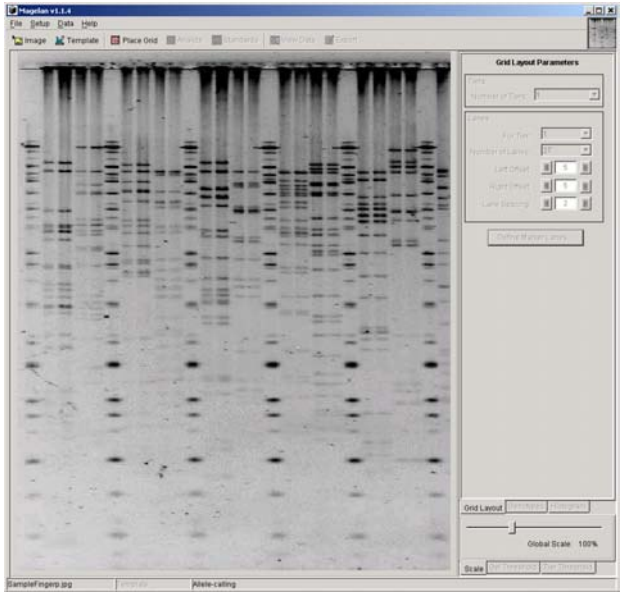

Figure 4.3: The sample image ***SampleFingerp.jpg*** opened inside of Magelan.

At this time, you have successfully loaded a gel image into Magelan. The image is displayed at its original size. You can scale the image (zoom in or out) by adjusting the "**Global Scale**" slider at the bottom right of the main window.

Note: Magelan also contains a few simple image manipulation features. The **Setup->Image** menu will present options for rotating the image and adjusting the brightness and contrast of the image. The software expects the wells for the gel to be at the top of the image, as shown in Figure 4.3. Faint bands can be made more obvious by adjusting the contrast; more obvious bands are more likely to be found by Magelan. A little experimentation with this feature will help you to fine-tune your results.

5 ALIGNING THE GRID

The next step in the analysis process is the alignment of the grid to mark off tier and lane boundaries. To begin this step, press the **Place Grid** *FastFlow* button, shown in Figure 5.1 below.

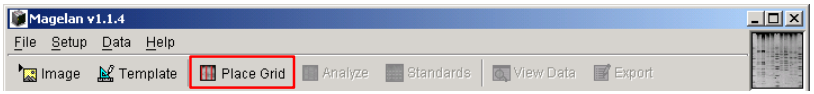

Figure 5.1: Drawing the grid using the *FastFlow* button.

A default grid will be drawn on the image, and the **Grid Layout Parameters** will become active on the right side of the main window (Figure 5.2). For this example, the gel has one tier and 27 lanes; however, we will mark 26 lanes since the 27th is partially cut off at the right of the image.

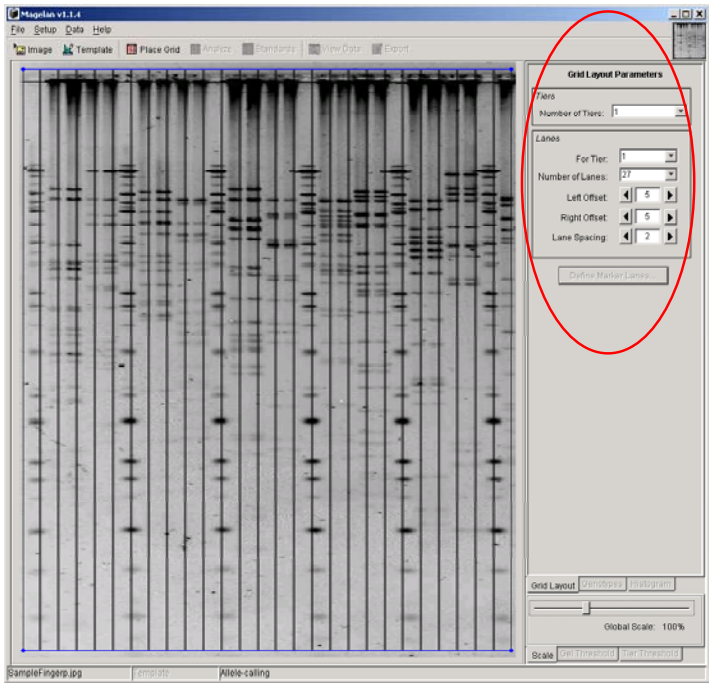

Figure 5.2: Setting the **Grid Layout Parameters**.

To reduce the amount of error in your results, you should isolate the useful part of the image as closely as possible by moving the tier lines. Anything outside the bounds of a tier will be ignored. To reduce the range of analysis for this image, position the mouse over the top blue tier line. The cursor will change to a vertical double-headed arrow. Drag this line downward until it is just above the first dark band. Next drag the bottom line upward until it is just below the last very dark band.

Next, position the lane lines such that they correspond to the lanes on the gel. Use the left and right arrows to change the values for the left and right offsets and lane spacing. Notice how the grid changes on the image. Set the left offset to 12, the right offset to 26, and the lane spacing to 3.

Note: You can also adjust the left and right offsets directly on the image. To adjust the left offset, place the mouse over the leftmost lane boundary line. The pointer will change to a horizontal double-headed arrow. Drag the line horizontally to the desired location, then release the mouse button. The same process can be used to move the right offset.

Notice also that the lanes shift slightly to the right toward the bottom of the image. To compensate for this, the grid can be skewed also. To skew it, position the mouse over the bottom-right blue control point (circled in Figure 5.3); the pointer will change to a horizontal double-headed arrow.

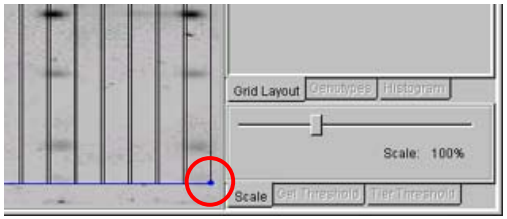

Figure 5.3: Grid-alignment control point.

Press the mouse button when you are over the control point and drag the point slightly to the right. The grid will realign. When you have aligned the rightmost lane line with the edge of the rightmost lane, slightly adjust the leftmost line to bring the rest of the lanes into alignment.

For illustration purposes, hold the CTRL key and click midway along the rightmost lane line. This will add another control point on both the rightmost and leftmost lines. You can now use these points to create a grid of a different shape. Drag the middle control point on the left line toward the right side of the image, and do the opposite for the right line. By adding control points, you can approximate curves and skews of any shape.

For this fingerprinting image, you only need the four original control points at the four corners. To remove the control points you added, hold the CTRL key and click on the points you wish to remove.

Note: Using the *CTRL* key with a mouse click adds or removes a *control* point.

Your image should look like the one shown below in Figure 5.4.

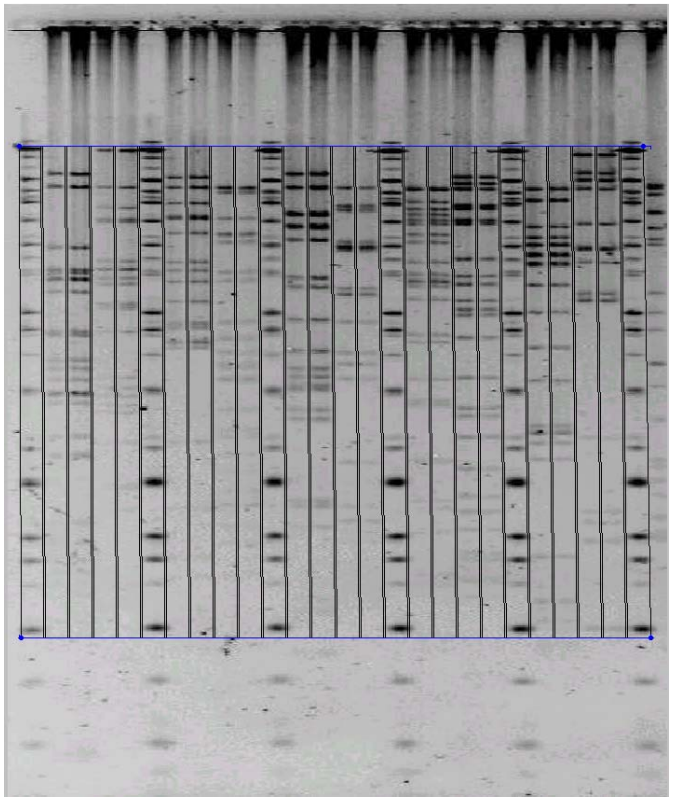

Figure 5.4: The skewed grid alignment.

Note: Lanes are numbered from left to right starting with lane 1. When a gel has multiple tiers, the tiers are numbered from top to bottom starting with tier 1.

6 ASSOCIATING WELLS OR SAMPLE NAMES

Note: This step is optional. It will not affect the analysis of the gel.

6.1 Associating Wells

If you prefer to refer to the lanes in your gel by the wells on a plate from which the samples came, you will want to define a plate-to-gel scheme. Before defining a scheme, take careful note of the layout of your gel. The gel in image ***SampleFingerp.jpg*** has a standards lane, four sample lanes, a standards lane, four sample lanes, etc., and ends with a standards lane.

To select or define a scheme, select **Setup->Plate-to-Gel Scheme** from the main menu (Figure 6.1.1).

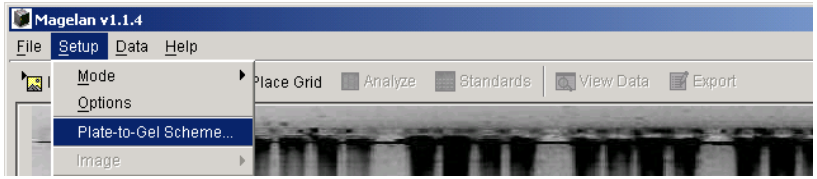

Figure 6.1.1: Menu for opening the **Plate-to-Gel Scheme** definition mechanism.

The **Plate-to-Gel Scheme** dialog will appear. There are three main areas of the dialog in which you will work. The master controls area (Figure 6.1.2) contains the controls for selecting an existing scheme, and loading and saving schemes.

Figure 6.1.2: The master controls of the **Plate-to-Gel Scheme** dialog.

The plate area (Figure 6.1.3) contains the controls through which you can define the layout of your plate and the process of picking samples from the plate with a pipette.

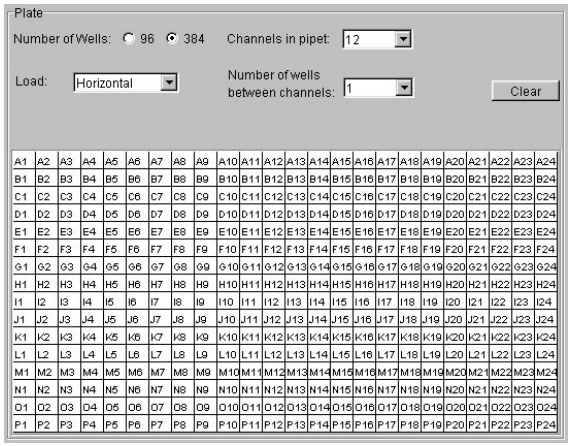

Figure 6.1.3: The plate area of the **Plate-to-Gel Scheme** dialog.

The gel area (Figure 6.1.4) contains the controls through which you can define the layout of your gel and the process of loading the samples into the proper lanes.

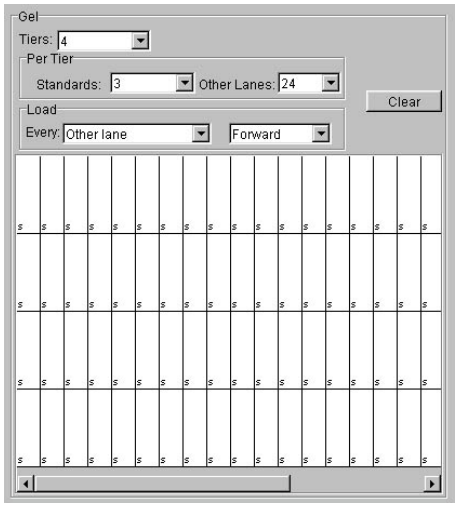

Figure 6.1.4: The gel area of the **Plate-to-Gel Scheme** dialog.

In general, if you want to use a predefined scheme, simply select it from the list. The details of the scheme will be shown for you to review. If it is acceptable for your gel, press **OK**. If you need to make changes, press the **Edit** button at the top right. When you have completed your changes, you can press **Save Changes** to commit the changes to this scheme, or type in a name and press **Save** to save it as a new scheme.

You'll need to define a new scheme for the current gel image. To define a new scheme, select "**Define Scheme**." The plate area and gel area will be activated. First fill in the gel area information as follows:

Standards	6
Other lanes	20
Load	Every Lane
Load	Forward
Number of Tiers	1

Initially on the gel, each lane has a small "**s**" at the bottom. This signifies a standards lane. When you load a sample into a lane, the lane then represents that sample, not standards.

Fill in the plate area information as follows:

Number of wells	96
Load	Horizontal
Channels in pipette	4
Wells between channels	0

The plate information is arbitrary for this gel. This step will only have meaning when you make an association for a real plate to a real gel. However, these steps are universal and should provide you with a feel for the workings.

To define how the wells on the plate correspond to the lanes on the gel, click on cell A1 on the plate to "pick up the samples." Four wells will change color, indicating that they have been loaded into the "virtual pipette." To place them on the gel, click the *second* lane in the gel area. The lanes will become the same color as the corresponding wells and the well names will appear at the bottoms of the lanes. We skipped the first lane because it is a standards lane on our gel.

Go back to the plate area and load four more samples into the virtual pipette by clicking on B1. Place these samples on the gel, starting with the seventh lane. Fill the remaining sets of four lanes through this same process. Pick your samples from C1, D1 and E1.

Your gel should look like the one shown in Figure 6.1.5 below.

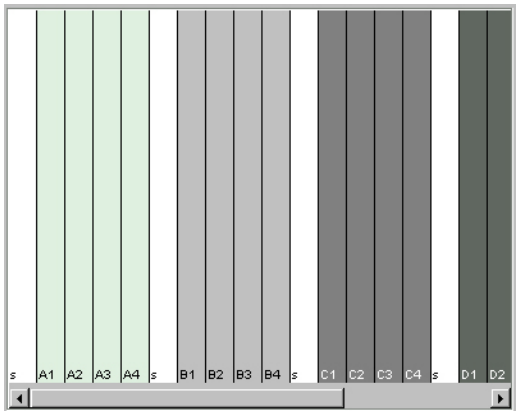

Figure 6.1.5: "Gel" with samples loaded.

Save this scheme as "Scheme 1." Press **OK**.

Note: Other functions are available to help you define your gel layout. By right-clicking on any lane, you will find a menu of other possibilities, such as inserting and removing standards if you misplace a sample. Also note that **Clear** buttons are available if you need to start over.

6.2 Loading Sample Names

If you prefer to refer to a lane on the gel by the name of the sample it contains, you can load a file that specifies the samples. A samples file for the fingerprinting image you have been working with would have 26 entries and would adhere to the following format:

<tier_number><tab><lane_number><tab><sample_name>

A samples file might start like this:

```
1    1    SampleName1
1    2    ThisIsSample2
1    3    NameForSample3
…
```

To load a samples file, select **File->Open Samples File…** as shown in Figure 6.2.1 below.

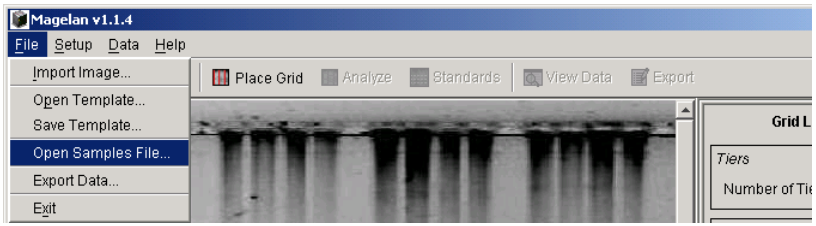

Figure 6.2.1: Loading a samples file from the menu.

7 FINGERPRINTING ANALYSIS

7.1 Setting the Mode

To put the software in fingerprinting mode, select **Setup->Mode-> Fingerprinting** from the main menu (Figure 7.1.1). Once selected, the status bar at the bottom of the main window will show that you are in fingerprinting mode. The dot on the menu also shows the current mode.

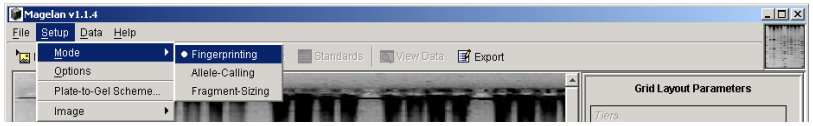

Figure 7.1.1: Entering fingerprinting mode.

7.2 Defining Standards

Next you will specify which lanes hold the standards. Click on lanes 1, 6, 11, 16, 21 and 26. The lanes will be highlighted in red, and the **Define Marker Lanes** button under the **Grid Layout Parameters** will be enabled.

Note: Later in this section, as well as sections 8.3 and 9.3, you will see that it is very important to have an equal number of bands in your standards lanes on each tier. To check this, find the top-most and bottom-most standard in one of the red lanes. Are those two bands in each and every other standards lane on the tier? If not, adjust the blue tier lines so that they are included; it is fine to include *more* above and below those bands, but you must included *at least* those bands.

Note: Your standards must completely cover your samples, too. To determine whether this condition has been met, find the sample (non-standards) lane in the tier with the highest band (closest to the well). Compare that band to the top bands in your standards lanes. If the standards bands are closer to the well than the band in the sample lane, you have done well. If not, you will need to adjust your tier lines to include more standards until this condition is met. Also check the bottom standards bands against the sample band that is farthest from the well. Figure 7.2.1a below shows a tier where these conditions have been met. In Figure 7.2.1b, the top condition has not been met; a sample band is higher than any standards band. In Figure 7.2.1c, the bottom condition has not been met; a sample band is lower than any standards band.

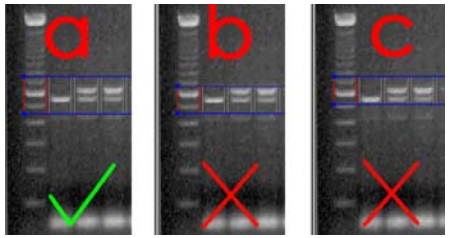

Figure 7.2.1a: Tier alignment that satisfies the standards-coverage requirement.
Figure 7.2.1b: Top standards-coverage requirement not met.
Figure 7.2.1a: Bottom standards-coverage requirement not met.

After selecting the lanes that hold the standards, press the **Define Marker Lanes** button to access the **Marker Information** dialog (Figure 7.2.2).

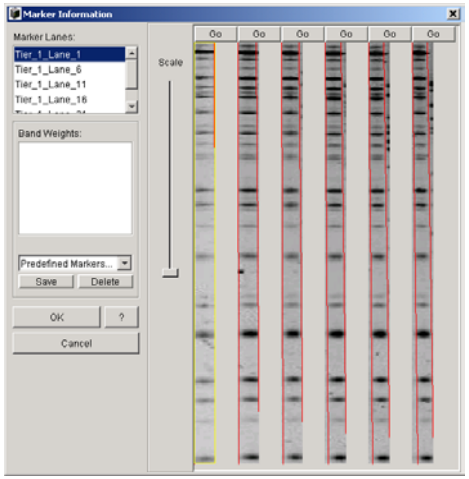

Figure 7.2.2: The Marker Information dialog.

The top left scrollbox lists the lanes you selected for standards lanes in order from left to right as they appear on the gel. Six images are displayed, one for each lane, in order from left to right. Lane 1 from tier 1 is highlighted in yellow and is also selected in the list.

If the lanes are long, you may not be able to see the bottoms of them. To see the bottoms, you can use to scrollbar at the far right of the dialog box. Also, you can grab the bottom edge of the dialog box and drag downwards until the lanes are fully shown.

Enter the following molecular weights into the **Band Weights** text field: 40000, 36500, 22300, 19000, 12500, 9600, 8400, 7000, 6600, 6000, 5200, 5000, 4400, 4000, 3600, 3000, 2500, 2000, 1550, 1400, 1000

Press the **Save** button. This allows you to save this set of molecular weights for future use. In the dialog that appears (Figure 7.2.3), type "Standards Set 1" and press **OK**.

Figure 7.2.3: Saving a set of molecular weights.

Next press the **Go** button above Tier_1_Lane_1 (leftmost). Pink lines will appear on the image, showing where Magelan found peaks in this lane. You entered 21 molecular weights, and Magelan selected the 21 strongest peaks to label as bands. Figure 7.2.4a (right) shows the lane with the bands marked in pink. Figure 7.2.4b shows a simplified version of the fingerprint. Figure 7.2.4c shows the correct calls for this lane. Two bands have been misplaced (marked with red arrows). To correct this, click on the bands that were misplaced to toggle them off, then click in the areas where the bands should be placed (marked with blue arrows) to toggle them on.

Note: You can also use the keyboard to manipulate the placement of bands. When the lane is selected (outlined in yellow), press the up and down arrows to jump between bands. A green crosshair will show your location. Press the spacebar to toggle a band on or off. If a band has been placed just slightly above or below its rightful place, hold CTRL and press the up or down arrow to nudge it slightly. To move to an area where there is no band, hold SHIFT and press the up or down arrow to move the crosshair pixel by pixel.

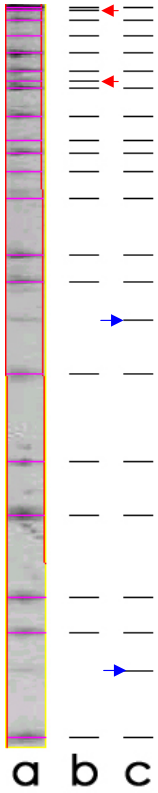

Figure 7.2.4a: Digital fingerprint generated by Magelan.
Figure 7.2.4b: Simplified digital fingerprint before manipulation.
Figure 7.2.4c: Simplified digital fingerprint after manipulation.

Important note: All molecular weights for the other lanes in the gel will be based on the molecular weights and relative positions you define through this interface. The accuracy of the results can only be as good as the precision and care you use in this crucial step.

If any other bands have been misplaced, use the mouse and/or keyboard techniques to replace them so that the fingerprint looks like the one shown above in Figure 7.2.4c. The software will try to place the bands in subsequent lanes that use the same marker according to the positions you choose in the first lane. When you are satisfied with the first lane, press the **Go** button for the next lane and make any necessary adjustments. Repeat this process for each marker lane.

Note: The bands in neighboring lanes will be "connected" in much the same manner as you may have seen in the Image package. Use these connections to help you find bands that might need to be adjusted.

When all of the lanes are finished (Figure 7.2.5), press **OK**. If the **OK** button is disabled, you have not marked 21 bands in one or more of the lanes. Press the **?** button to discover which lane has a problem, and fix that problem. Repeat this process as necessary. When you are finished, your dialog should resemble Figure 7.2.5.

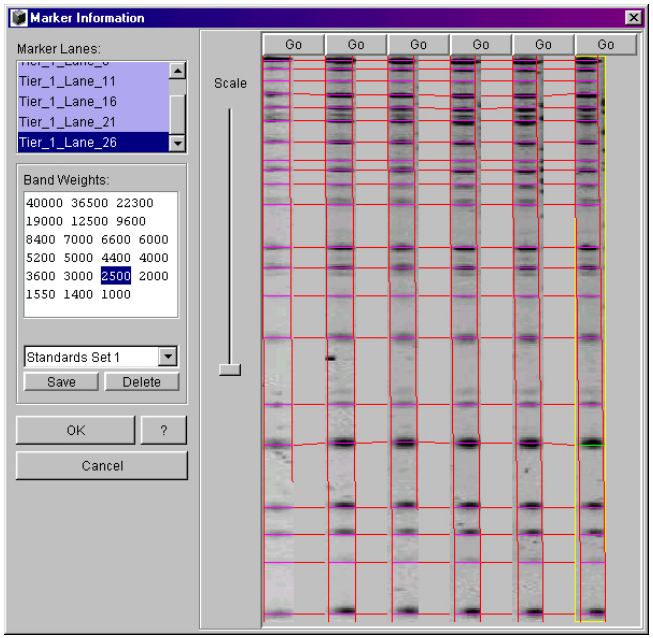

Figure 7.2.5: Marker Information dialog with all standards lanes marked.

Note: When you highlight a particular molecular weight, it will be drawn in green on the active lane. In Figure 7.2.5, the weight of 2500 was selected, and is shown in green (fifth band from the bottom in rightmost lane).

After you press **OK**, the standards lanes will be marked on the full image.

7.3 Saving and Loading a Template

Magelan provides an easy way for the same gel layout and marker selection to be applied to multiple images through **templates**. A template can record the locations of the tiers and lanes, the location of standards lanes and the weights of bands in the standards lanes. A template can easily be opened and used for other images that fit the saved format.

You can save a template at any point. To do so, select **File->Save Template** from the main menu (Figure 7.3.1). Save the template as ***fingerprint.mtf***. The **.mtf** extension means **Magelan Template File**.

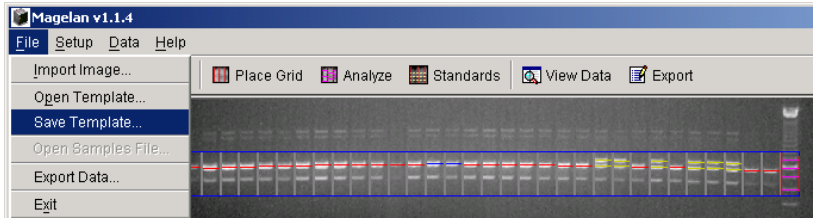

Figure 7.3.1: Saving a template from the File menu.

Now, to see that it has worked, reopen the image ***SampleFingerp.jpg*** as discussed in Chapter 4. All work up to this point will be cleared and the fresh image will be loaded. To replace that work, you can open the template you just created. To open a template, select **File->Open Template** or press the **Open Template** *FastFlow* button (Figure 7.3.2). Find ***fingerprint.mtf*** that you saved previously and select it.

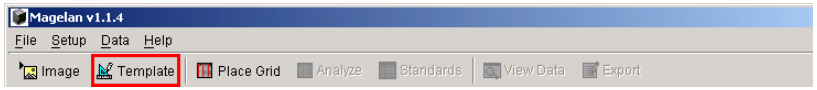

Figure 7.3.2: Opening a template using the **Open Template** *FastFlow* button.

After the template has been loaded, you will see the grid aligned as before with the markers selected and filled with bands. For verification, you can go back into the Marker Information dialog via the **Define Marker Lanes…** button and check that the weights are correct and that the bands are properly placed.

Note: A single template may be applicable to many gels, but it is nearly impossible to have many gels run identically. Minor adjustments can be made to the position of the grid after loading a template to accommodate these variations, either through the Grid Layout Parameters or by using the mouse to drag lane and tier lines to their desired positions. Changes can also be made to the marker lanes via the Marker Information dialog.

7.4 Calling Bands

The next step is to perform the analysis on the remaining lanes. Press the **Analyze** *FastFlow* button (Figure 7.4.1).

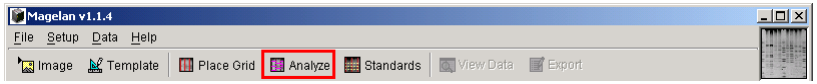

Figure 7.4.1: *FastFlow* **Analyze** button.

Magelan will spend a few seconds processing the image and determining where peaks are located in each lane. When it has finished, all bands that were found and that have an intensity value greater than the default intensity threshold are marked. A histogram is shown for the active lane at the top right of the window, as well as the image of the lane.

The histogram, drawn in blue, shows the intensity of each row of the image. The current histogram is of Tier_1_Lane_1, your first standards lane. Short yellow lines are drawn on the histogram to denote that a band has been called at that point.

Click once on Tier_1_Lane_2, just to the right of the leftmost standards lane, to make it the active lane. It will be highlighted in green, and the histogram view will be updated to reflect the information associated with it. Now you will see a red line on the histogram as well. This line represents the intensity threshold. Any peaks that extend beyond that threshold are called as bands.

7.5 Thresholding

You can choose to use a "pseudo-exponential" function or a linear function for the threshold by selecting the "**exp**" (exponential) or "**linear**" radio button. You can raise and lower the threshold to exclude or include more peaks by adjusting the threshold slider. You can make these choices for the entire gel using the **Gel Threshold** controls, or for only the active lane by using the **Local Threshold** controls (Figure 7.5.1 at the right).

Note: In general, you should adjust the gel controls first and get the most accurate results you can this way. If you have multiple tiers, it may be useful to adjust the threshold on a tier-by-tier basis (tab next to Gel Threshold), particularly if the contrast of the image changes from top to bottom. Then, fine tune individual lanes using the local controls. Remember, global adjustments apply to every lane on every tier; tier adjustments apply to every lane in a specific tier; local adjustments apply only to the active lane. Tier-specific and lane-specific changes will be lost if you make changes for the whole gel; any local adjustments will be lost after a tier change. Also, you can mix and match threshold settings. For instance, your results overall may be better using a linear threshold, yet for a particular lane the result may be better using the exponential threshold.

Take a few minutes to adjust the threshold controls -- first global, then local -- to include as many bands as possible while eliminating as many smudges, run-overs and other blemishes as possible. With practice, you'll become adept at this process and minimize the time you spend on manual addition and removal of bands.

Note: The peaks on the histogram will serve as a guide.

For the lane shown in the image to the right, poor contrast is responsible for so few peaks.

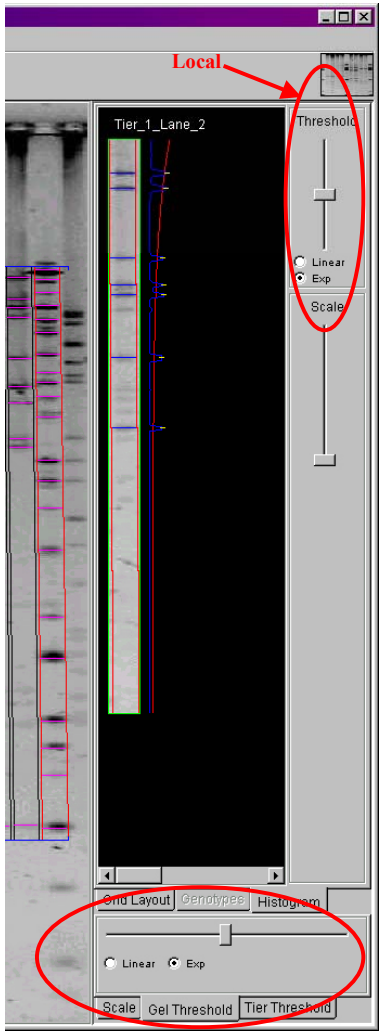

Figure 7.5.1: Gel and local thresholds.

Note: When a threshold slider is selected, you can use the arrow keys to make very fine changes to the threshold.

7.6 Adding, Removing and Adjusting Bands

It may be necessary for you to manually add or remove bands. The threshold controls should get you close to a well-called gel, but it can't account for very faint bands or blemishes on the gel.

The techniques for altering called bands here are the same as on the Marker Information dialog. To remove a band that was miscalled, first click on the lane once to activate it. Then click on the band to toggle it off. You can click in an empty area to toggle a band on.

7.7 Data Visualization

When you have called the bands throughout the image, you can view the calculated molecular weights for each band. Press the **View Data** *FastFlow* button (Figure 7.7.1) to see a simple spreadsheet of data (Figure 7.7.2).

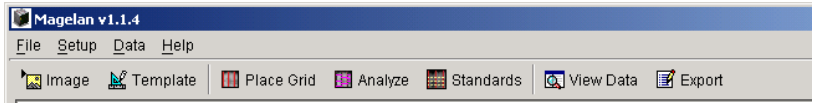

Figure 7.7.1: **View Data** *FastFlow* button.

The left-hand column gives the distance from the top of the tier to the band. The right-hand column gives the corresponding molecular weight. Click on a row in the table. The corresponding band will be drawn in green in the active lane. When you make another lane active, the table will be updated with that lane's information.

Note: While viewing data, you can still add and remove bands on the gel. The table will automatically update to reflect your changes.

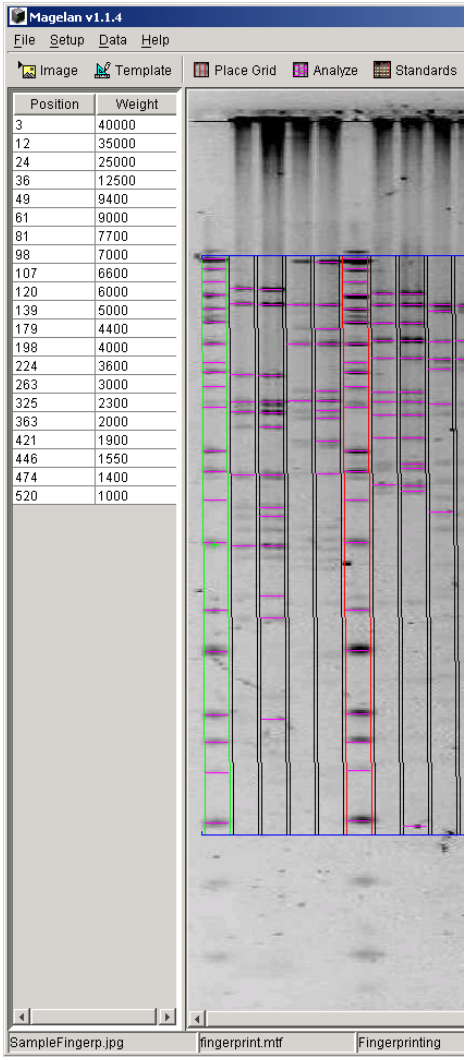

Figure 7.7.2: Viewing data for a standards lane.

7.8 Exporting Data

NOTE: THIS FEATURE IS ONLY AVAILABLE IN THE FULL RELEASE.

You can export your results to a human-readable file. The default extension for a Magelan Data file is ***.mdf***. To export the data, select **File->Export Data** from the main menu (Figure 7.8.1) or use the **Export** *FastFlow* button (Figure 7.8.2).

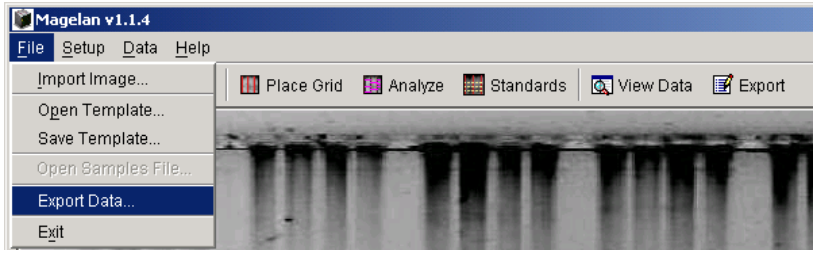

Figure 7.8.1: Menu selection to export data.

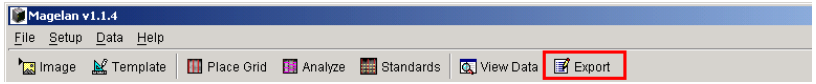

Figure 7.8.2: **Export** *FastFlow* button.

You will be prompted to choose a location and file name for your data file. Once that has been selected, you will have the opportunity to enter extra information concerning the gel and project through the **Gel Information** dialog (Figure 7.8.3). This information is optional. After pressing **OK** on this dialog, your data will be saved.

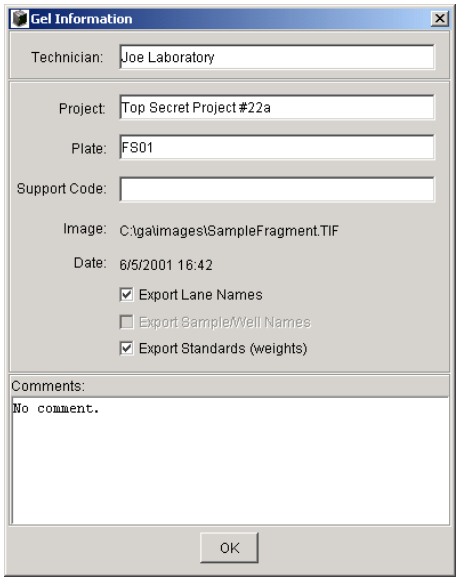

Figure 7.8.3: The Gel Information dialog.

8 FRAGMENT-SIZING ANALYSIS

8.1 Preparing for the Example

Open the image ***SampleFragment.TIF***. This is a black image with white bands. Press the **Place Grid** *FastFlow* button. The grid was drawn with the parameters used in the fingerprinting example, and the lane lines are difficult to see. At the top right of the main window is a small icon that displays black bands on a white background (Figure 8.1.1). Click on that icon. The icon image inverts, and now the grid lines are easier to see.

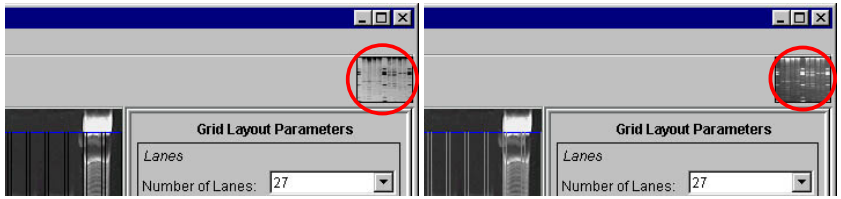

Figure 8.1.1: The clickable image format icon, before (left) and after (right).

Note: The internal processing of the image depends on the image format. Be sure to set the image format by clicking the icon before performing any analysis. If your results are ever drastically illogical, an incorrect image format is likely the culprit.

Enter the following values for the Grid Layout Parameters:

Number of Lanes	14
Left Offset	27
Right Offset	15
Lane Spacing	5
Number of Tiers	1

Adjust the tier (blue horizontal) lines to isolate the meaningful fragments as shown in Figure 8.1.2 below.

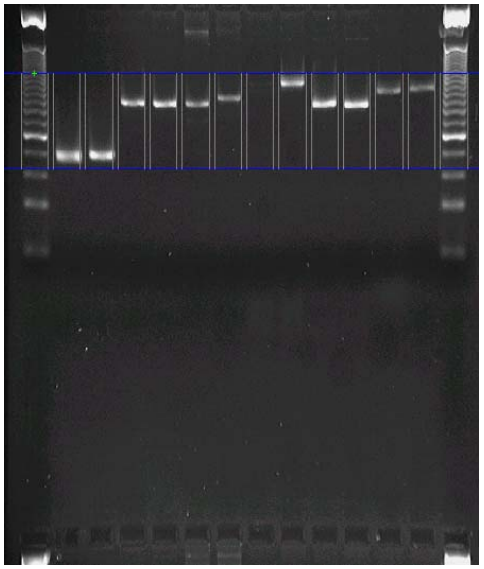

Figure 8.1.2: Fragment-sizing gel with one tier enveloping the meaningful fragments.

8.2 Setting the Mode

To put the software in fragment-sizing mode, select **Setup->Mode->Fragment Sizing** from the main menu (Figure 8.2.1). Once selected, the title bar of the main window will show that you are in fragment-sizing mode.

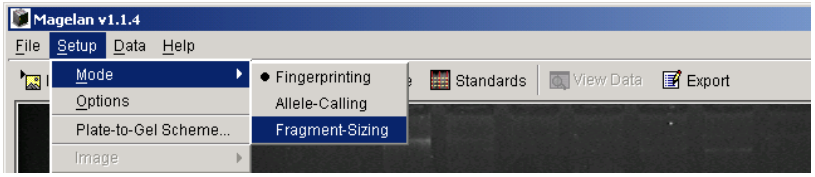

Figure 8.2.1: Entering fragment-sizing mode.

8.3 Defining Standards

Next you will specify which lanes hold the standards. Click on the leftmost and rightmost lanes. The lanes will be highlighted in red, and the **Define Marker Lanes** button under the **Grid Layout Parameters** will be enabled.

After selecting the lanes that hold the standards, press the **Define Marker Lanes** button to access the **Marker Information** dialog. Adjust the scale so that you can distinctly see ten bands, counting from the bottom. At the top of each lane, you'll most likely see the bottom of the eleventh band. However, if you see more than just a sliver of that band, exit the dialog and move the top tier line down until you include at most ten complete bands, then reenter the dialog.

For a detailed description of the Marker Information dialog, see "Defining Standards" in Fingerprinting, section 7.2.

Enter the following molecular weights into the **Band Weights** text field:

75, 100, 125, 150, 175, 200, 225, 250, 275, 300

Save these weights for future use.

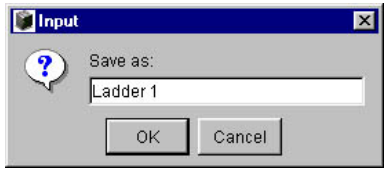

Figure 8.3.1: Saving a set of molecular weights.

Mark both lanes by pressing the **Go** button over each and adjusting as necessary.

Important note: All molecular weights for the other lanes in the gel will be based on the molecular weights and relative positions you define through this interface. The accuracy of the results can only be as good as the precision and care you use in this crucial step.

When both lanes are finished (Figure 8.3.2), press **OK**. If the **OK** button is disabled, you have not marked ten bands in one or more of the lanes. Press the **?** button to discover which lane has a problem, and fix that problem. Repeat this process as necessary. When you are finished, your dialog should resemble Figure 8.3.2.

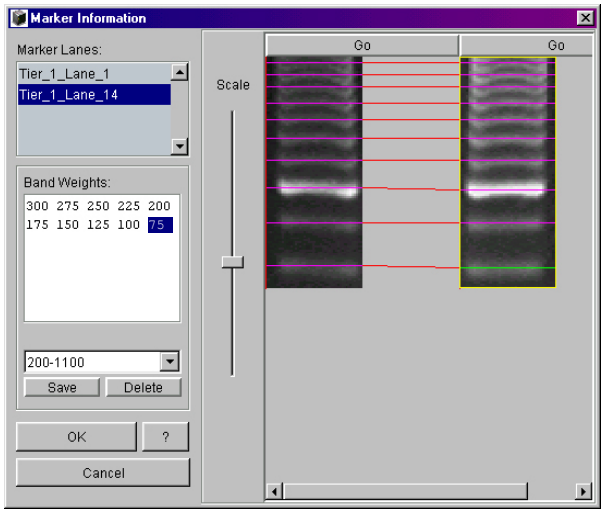

Figure 8.3.2: Marker Information dialog with both standards lanes marked.

After you press **OK**, the standards lanes will be marked on the full image.

8.4 Saving and Loading a Template

Magelan provides an easy way for the same gel layout and marker selection to be applied to multiple images through templates. A template can record the locations of the tiers and lanes, the location of standards lanes and the weights of bands in the standards lanes. This template can easily be opened and used for other images that fit the saved format.

For information on creating and using templates, see "Saving and Loading a Template" in Fingerprinting, section 7.3.

8.5 Calling Bands

The next step is to perform the analysis on the remaining lanes. Press the **Analyze** *FastFlow* button (Figure 8.5.1).

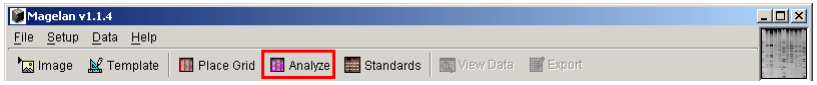

Figure 8.5.1: **Analyze** *FastFlow* button.

Magelan will spend a few seconds processing the image and determining where peaks are located in each lane. When it has finished, all bands that were found and that have an intensity greater than the default intensity threshold are marked. A histogram is shown for the active lane at the top right of the window, as well as the image of the lane.

Note: This analysis expects to find at most one peak in each non-standards lane.

The histogram, drawn in blue, shows the intensity of each row of the image. The current histogram is of Tier_1_Lane_1, your first standards lane. Short yellow lines are drawn on the histogram to denote that a band has been called at that point.

Click once on Tier_1_Lane_2, just to the right of the leftmost standards lane, to make it the active lane. It will be highlighted in green, and the histogram view will be updated to reflect the information associated with it. Now you will see a red line on the histogram as well. This line represents the intensity threshold. Any peaks that extend beyond that threshold are called as bands.

The remaining steps – Thresholding; Adding, Removing and Adjusting Bands; Data Visualization; and Exporting Data – are the same as the steps for fingerprinting, with the restriction that only one band can be present in each lane. See sections 7.4 through 7.8 for thorough descriptions of these steps.

9 ALLELE-CALLING ANALYSIS

9.1 Preparing for the Example

Open the image ***SampleAllele.tif***. This is a three-tiered black image with white bands. Press the **Place Grid** *FastFlow* button. The grid was drawn with the parameters used in the last example. If the lane lines are not gray, click the small icon that displays the image format at the top right of the main window, which displays the current image format (Figure 9.1.1). Click on that icon. The icon image inverts, and now the grid lines are easier to see.

Figure 9.1.1: The clickable image format icon, before (left) and after (right).

Note: The internal processing of the image depends on the image format. Be sure to set the image format by clicking the icon before performing any analysis. If your results are ever drastically illogical, an incorrect image format is likely the culprit.

Enter the following values for the **Grid Layout Parameters**:

Number of Lanes	34
Number of Tiers	3
Left Offset	12
Right Offset	14
Lane Spacing	2

Notice that these parameters are fairly accurate for Tier 1, but are not so well aligned on the right-hand side for Tiers 2 and 3. You should fix this individually for each tier by selecting it from the tier list (Figure 9.1.2) and adjusting the parameters as necessary. Tier 2 should have a right offset of 10, and Tier 3 should have a right offset of 9.

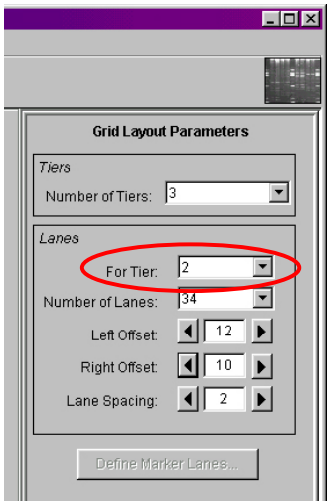

Figure 9.1.2: Grid Layout Parameters for Tier 2.

Adjust the tier (blue horizontal) lines to isolate the meaningful fragments as shown in Figure 9.1.3 below. We'll focus on the pair of alleles in the middle of each tier, although we'll include the single allele as well. The standards at either end of each tier are good landmarks to go by. For this example, we'll be including four bands in the standards lanes, the second of which is slightly more intense than the others.

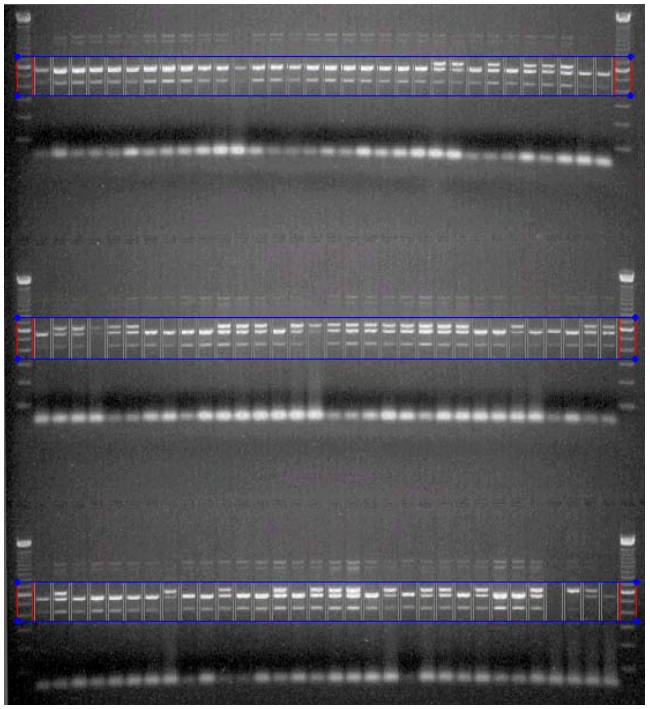

Figure 9.1.3: Allele-calling gel with tiers enveloping the meaningful fragments.

9.2 Setting the Mode

To put the software in allele-calling mode, select **Setup->Mode->Allele Calling** from the main menu (Figure 9.2.1). Once selected, the title bar of the main window will show that you are in allele-calling mode.

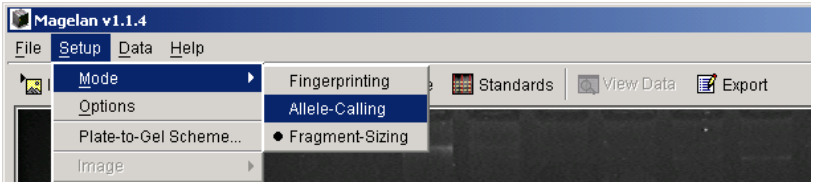

Figure 9.2.1: Entering allele-calling mode.

9.3 Defining Standards

Next you will specify which lanes hold the standards. Click on the leftmost and rightmost lanes of each tier, for a total of six. The lanes will be highlighted in red, and the **Define Marker Lanes** button under the **Grid Layout Parameters** will be enabled.

Press the **Define Marker Lanes** button to access the **Marker Information** dialog. Adjust the scale so that you can distinctly see four bands, counting from the bottom. At the top of some lanes, you'll most likely see the bottom of the fifth band. However, if you see more than just a sliver of that band, exit the dialog and move the top tier line down until you include at most four complete bands, then reenter the dialog.

For a detailed description of the Marker Information dialog, see “Defining Standards” in Fingerprinting, section 7.2.

Enter the following molecular weights into the **Band Weights** text field: 100, 125, 150, 175. Save this set of molecular weights for future use as described in “Defining Standards”, section 7.2.

Mark all six lanes by pressing the **Go** button over each and adjusting as necessary.

Important note: All molecular weights for the other lanes in the gel will be based on the molecular weights and relative positions you define through this interface. The accuracy of the results can only be as good as the precision and care you use in this crucial step. Any smiling or frowning of the image must be specified through this interface to Magelan; therefore, it is wise to place a standards lane in the very center of each tier of your physical gels.

When you are finished, the dialog should look like Figure 9.3.1 below. If not, make adjustments, keeping in mind that your precision here will impact the rest of the analysis.

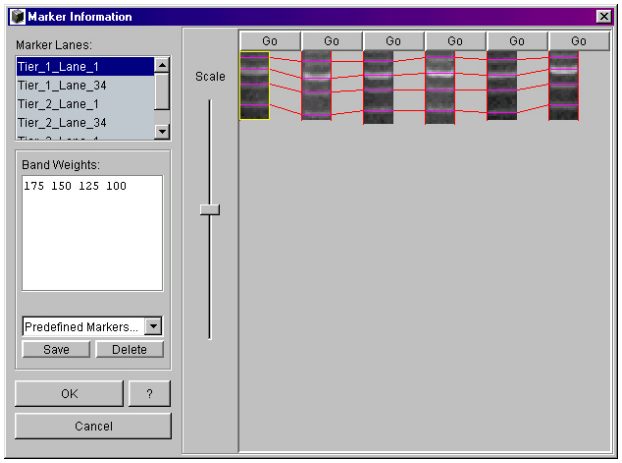

Figure 9.3.1: **Marker Information** dialog with all standards and control lanes marked.

If the **OK** button is disabled, you have not marked four bands in one or more of the lanes. Press the **?** button to discover which lane has a problem, and fix that problem. Repeat this process as necessary. When you are finished, your dialog should resemble Figure 9.3.1.

After you press **OK**, the standards will be marked on the full image.

9.4 Defining Genotypes

After pressing **OK** on the Marker Information dialog, the Genotypes tab will be active on the right side of the window (Figure 9.4.1).

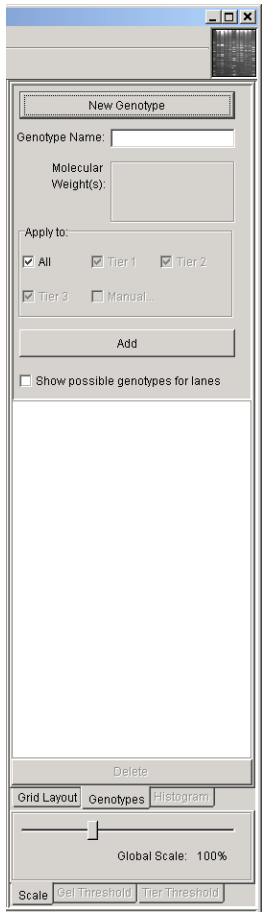

Figure 9.4.1: Genotype Definition controls.

For this example, you will define four genotypes for the four possibilities: Allele from parent A (top band – A), allele from parent B (bottom band – B), neither allele (-), or both alleles (heterozygote – H). This example will ignore the single band at the bottom of the tier.

To define a new genotype, press the **New Genotype** button. The controls beneath the button will become enabled. Type "A" (no quotation marks) in the space for the genotype name. Next, click on any lane that contains allele A (top band); for instance, the next to the last lane on Tier 2. That lane will become active. Click on allele A. It will be marked and its molecular weight (157) will be filled in on the genotype controls.

Note: If your molecular weight does not read 157, toggle the band off and click again slightly above or below your previous mark. If your standards are not exactly as shown in figure 9.3.1, you may not be able to achieve a band at 157. Get as close as possible.

Next, select the tiers on which to search for this genotype by checking all applicable checkboxes. "All" is appropriate for this gel, but take note that different genotypes can be defined for different tiers by way of these checkboxes.

Note: You can select portions of a tier by choosing "Manual" from the set of checkboxes; then click on any lanes that might have that genotype. The lanes will display a large yellow dot. You can select many adjacent rows at once by selecting the leftmost lane then while holding the SHIFT key selecting the rightmost lane. All lanes that are selected will have the yellow dot. You will also be shown any other genotypes that are candidates for the selected lanes.

To finish this genotype, press the **Add** button. An entry will be made in the formerly empty list that says "A" and the molecular weight.

Next, check the "Display Genotypes" checkbox. This will show you which lanes will be searched for the "A" genotype by drawing an "A" on the lane.

Note: When you highlight a genotype in the list, the bands that were selected when defining it will be marked in blue on the gel.

Repeat the steps above to define a genotype called "B" with molecular weight 141; "-" with no molecular weights (simply press **New Genotype**, type in "-", press **Add** and click **Yes**); and "H" with molecular weights 157 and 141.

Note: The molecular weights for your "H" genotype should match your A and B genotype molecular weights, even if they do not match this example.

Note: You can define multiple sets of weights to be the same genotype. For instance, consider the lanes shown in Figure 9.4.2 below. In this case, three bands are present in the heterozygote. Lane a shows all three bands with bright intensities. Lane b shows two bands clearly, but the top band is faint. The top band in Lane b may not be found by the software. However, since the presence of the two lower bands still qualifies it as the heterozygote, Lane b should be scored as the same as Lane a. To achieve this, you would define two H genotypes: one with all three bands, and one with the lower two. This provides a greater range of accuracy when used correctly, but can severely harm your results if used improperly.

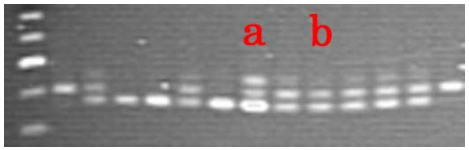

Figure 9.4.2: Three-banded heterozygotes with poor intensities.

9.5 Saving and Loading a Template

Refer to section 7.3 for information on creating and using templates. For allele-calling analyses, templates can be saved with only grid information or with grid information and genotype information, depending on whether the template is saved before or after the genotype definition step.

9.6 Calling Bands

The next step is to perform the analysis on the remaining lanes. Press the **Call Bands** *FastFlow* button.

Magelan will spend a few seconds processing the image and determining where peaks are located in each lane. When it has finished, all bands that were found and that have an intensity greater than the default intensity threshold *and* that fit a defined genotype are marked. A histogram is shown for the active lane at the top right of the window, as well as the image of the lane.

Note: Only bands that fit a defined genotype will be marked, regardless of intensity relative to the threshold.

Note: Only one genotype will be called in each lane.

The histogram, drawn in blue, shows the intensity of each row of the image. The current histogram is of Tier_1_Lane_1, your first standards lane. Short yellow lines are drawn on the histogram to denote that a band has been called at that point.

Click once on Tier_1_Lane_2, just to the right of the leftmost standards lane, to make it the active lane. It will be highlighted in green, and the histogram view will be updated to reflect the information associated with it. Now you will see a red line on the histogram as well. This line represents the intensity threshold. Any peaks that extend beyond that threshold are called as bands, if they fit a genotype.

9.7 Thresholding

Refer to section 7.5, "Thresholding," for a thorough explanation of this essential function.

9.8 Restricting Allele Candidate Range

Initially, the software will assign the genotype that fits the best for the lane, regardless of overall error. In general, every lane will have a genotype. This is the loosest analysis. Frequently, however, you will want to limit the overall error, or, more precisely, limit local errors by defining the valid range in which a band might be found.

To specify this limitation, right-click on any non-standards lane. The Allele Range dialog will appear. On the right is the image of the lane. For the range restriction, the blue bracket on the right and the top slider on the left are applicable.

The blue bracket is centered around an allele. By adjusting the slider, the bracket can grow or shrink, representing a larger or smaller window in which a band can be considered that allele.

After adjusting the range to the desired value, press **Apply**. The image in the main window will be reprocessed. If you like the results, press **OK**. If not, you can adjust the slider

again and press **Apply**. If no adjustment improves the original analysis, check "Don't restrict" to remove the range restriction, press **Apply**, and then press **OK** to return to the main window.

9.9 Adding, Removing and Adjusting Bands

It may be necessary for you to manually add or remove bands. The threshold controls and range-restriction should get you close to a well-called gel, but they cannot account for very faint bands or blemishes on the gel.

The techniques for altering called bands here are the same as for fingerprinting, as detailed in section 7.6. Keep in mind that a band you mark manually will only be shown if it falls into a defined genotype.

9.10 Data Visualization

When you have called the bands throughout the image, you can view the genotype for each lane. Press the **View Data** *FastFlow* button to see a simple spreadsheet of data (Figure 9.10.1, right).

Information for all lanes will be shown at once. On the "**Raw**" data tab, the left-hand column tells gives the name of the lane. The middle column gives the name of the well or sample, if defined. The right-hand column lists the genotype that is expressed in the lane.

Note: While viewing data, you can still add and remove bands on the gel. The table will automatically update to reflect your changes. Standards and allele control lanes are designated by red text on a gray background.

Occasionally, the wrong genotype is chosen. For instance, the slight frowning on this gel contributes to the miscalling of several lanes on Tier 1. Because of the slope, some expressions of genotype B (molecular weight 141) are slightly higher on the image and therefore, from the computer's point of view based on the standards, have slightly larger molecular weights. The allele in Tier 1 Lane 9 most likely has a molecular weight of 150, which makes it a closer match to genotype A (157, error of 7) than to genotype B (141, error of 9).

Note: This would not have been an issue if there were a standards lane in the middle of the gel.

In this case, you will have to manually override the software's decision. To do so, click on the genotype in the table. A drop-down list will appear, from which you can select the appropriate genotype.

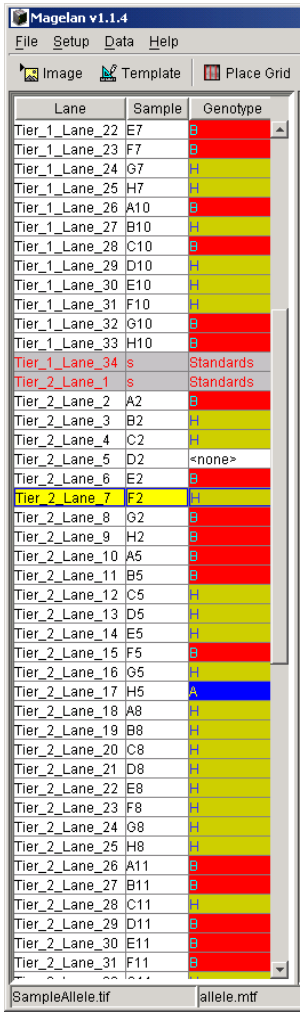

Figure 9.10.1 (right): Data Table with allele-calling results

9.11 Exporting Data

NOTE: THIS FEATURE IS ONLY AVAILABLE IN THE FULL RELEASE.

You can export your results to a human-readable file. The default extension for a Magelan Data file is ***.mdf***. To export the data, select **File->Export Data** from the main menu (Figure 9.11.1).

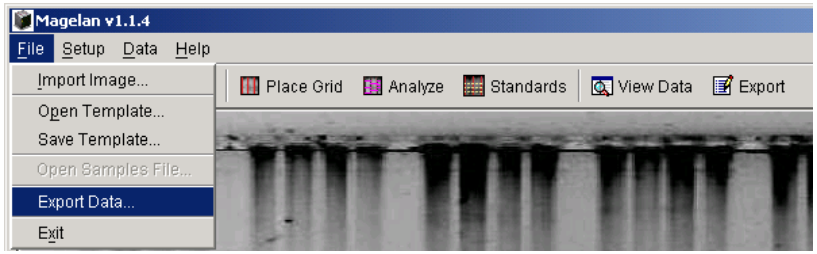

Figure 9.11.1: Menu selection to export data.

Figure 9.11.2: Options for the format of exported data.

There are several formats for exporting allele information (Figure 9.11.2, above). A "**Formatted**" export creates a tab-delimited file that gives the lane name (optional), the well or sample name ("?" if undefined) and the genotype expressed in that lane. Standards lanes have no entry for genotype. This file can easily be imported into Microsoft Excel or other spreadsheet software. An excerpt from the formatted export for this example is attached as Appendix A (a Plate-to-Gel scheme was defined).

A "**Plate**" export creates a file with a grid whose dimensions match the dimensions of the plate defined in the Plate-to-Gel Scheme; if no scheme is defined, this option is not available. Each position in the grid is filled in with the corresponding lane's genotype (Appendix B).

After selecting the format for your data, you will be prompted to choose a location and file name for your data file. Once that has been selected, you will have the opportunity to enter extra information concerning the gel and project through the **Gel Information** dialog. This information is optional. After pressing **OK** on this dialog, your data will be saved.

10 SUMMARY

By following the examples in this tutorial you should have obtained a working knowledge of all essential and some optional functions of Magelan. This information should allow you to use the program to analyze images of your own gels. If you have any additional questions regarding Magelan, or would like to comment on the program, please do not hesitate to contact INCOGEN by phone at 800-286-6599 or email at mailto:magelan@incogen.com. We encourage you to submit any bugs or requests for features at http://www.incogen.com/php/userLogin.php (username: magelan, password: new_issue). We hope you will enjoy using Magelan and will find it helpful in your research. We look forward to your feedback!

APPENDIX A

(formatted export)

```
Project:    Top Secret Project #9
Plate:      48F
Image:      C:\ga\images\SampleAllele.tif
Date:       6/4/2001 22:26
Technician: Joe Laboratory
Task:       Allele-Calling
Comments:   These are the comments for this experiment.

Lane    Well    Genotype
Tier_1_Lane_1   s
Tier_1_Lane_2   A1      B
Tier_1_Lane_3   B1      B
Tier_1_Lane_4   C1      B
Tier_1_Lane_5   D1      B
Tier_1_Lane_6   E1      B
Tier_1_Lane_7   F1      B
Tier_1_Lane_8   G1      B
Tier_1_Lane_9   H1      B
Tier_1_Lane_10  A4      B
Tier_1_Lane_11  B4      B
Tier_1_Lane_12  C4      B
Tier_1_Lane_13  D4      B
Tier_1_Lane_14  E4      B
Tier_1_Lane_15  F4      A
Tier_1_Lane_16  G4      A
Tier_1_Lane_17  H4      B
Tier_1_Lane_18  A7      B
Tier_1_Lane_19  B7      B
Tier_1_Lane_20  C7      B
Tier_1_Lane_21  D7      B
Tier_1_Lane_22  E7      B
Tier_1_Lane_23  F7      B
```


APPENDIX B

(Plate export)

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	B	B	B	B	H	H	B	B	H
B	B	H	H	B	B	B	B	H	H	H	B	B
C	B	H	B	B	H	H	B	H	B	B	H	B
D	B	<none>	B	B	H	B	B	H	H	H	B	H
E	B	B	B	B	H	B	B	H	B	H	B	<none>
F	B	H	B	A	B	H	B	H	H	H	B	B
G	B	B	B	A	H	B	H	H	H	B	H	<none>
H	B	B	H	B	A	H	H	H	B	B	B	<none>

APPENDIX C – FINGERPRINTING QUICKLIST

1. Load the image.
2. Load a template (optional; skip to 10).
3. Check image format.
4. Set mode to fingerprinting.
5. Align the grid.
6. Setup up Plate-to-Gel Scheme (optional; skip to 8).
7. Import sample names (optional).
8. Define standards.
9. Save template (optional).
10. Call bands.
11. Adjust threshold, if necessary.
12. Manually add, remove or adjust bands, if necessary.
13. View data (optional).
14. Export data (optional).

APPENDIX D – FRAGMENT-SIZING QUICKLIST

1. Load the image.
2. Load a template (optional; skip to 10).
3. Check image format.
4. Set mode to fragment-sizing.
5. Align the grid.
6. Setup up Plate-to-Gel Scheme (optional; skip to 8).
7. Import sample names (optional).
8. Define standards.
9. Save template.
10. Call bands.
11. Adjust threshold, if necessary.
12. Manually add, remove or adjust bands, if necessary.
13. View data (optional).
14. Export data (optional).

APPENDIX E – ALLELE-CALLING QUICKLIST

1. Load the image.
2. Load a template (optional; skip to 11).
3. Check image format.
4. Set mode to allele-calling.
5. Align the grid.
6. Setup up Plate-to-Gel Scheme (optional; skip to 8).
7. Import sample names (optional).
8. Define standards.
9. Define genotypes.
10. Save template.
11. Call bands.
12. Adjust threshold, if necessary.
13. Manually add, remove or adjust bands, if necessary.
14. View data (optional).
15. Manually select genotypes, if necessary.
16. Export data (optional).