Chapter 1 Getting Started

Chapter Overview

Installing NCSA GelReader

Preparing Your Image Data

The Tutorial

Loading the Image

Manipulating the Image

- Changing the Palette
- Finding Lanes and Bands
- Changing the Color of Lanes and Bands
- Adding and Deleting Lanes and Bands

Displaying the Lane Profiles

Using the Notebook

- Entering Standards Using the Command Line
- Using Macros

Generating a Report

Exiting NCSA GelReader

Chapter Overview

This chapter provides a brief tutorial to get you started using NCSA GelReader. The tutorial describes the basic steps involved in using NCSA GelReader:

- installing and invoking the program

- loading gel images from files
- changing palettes
- manipulating the placement of lanes and bands in a gel image
- designating standard lanes, and the molecular weights of the sites in those lanes
- displaying histograms of the lane profiles
- generating a report
- exiting the program

This chapter assumes that you are familiar with the Macintosh user interface. Specifically, to use NCSA GelReader successfully, you should know how to use the mouse, choose commands from menus, and make entries in dialog boxes. If you need further information regarding these subjects, refer to your Macintosh user's guide.

Installing NCSA GelReader

NCSA GelReader does not require any unusual installation procedures. As you would for any new Macintosh software, create a new folder and copy the contents of the NCSA GelReader disk to your hard disk. The application may also be run from a floppy disk; however, image data may exceed the capacity of floppy disks.

Preparing Your Image Data

NCSA GelReader generates images from datasets stored in raw binary or PICT files. Before using the program you need to obtain a digitized image of your stained gel or autoradiogram.

For the purposes of this tutorial, several sample image datasets of digitized gels have been provided on the NCSA GelReader disk. For instructions on creating your own image files, refer to the section, "Formatting You Image Files," in Chapter 3, "Windows and Technical Details"

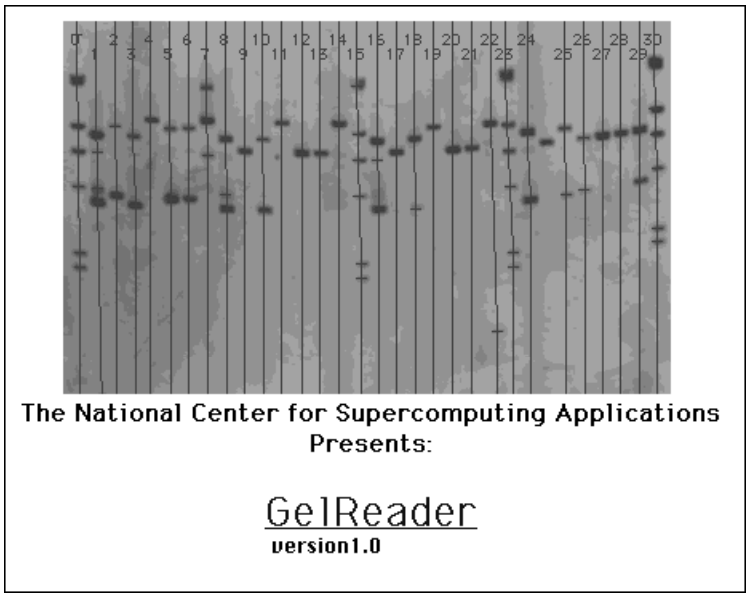
The Tutorial

Invoke NCSA GelReader by double-clicking on the NCSA GelReader program or file icon. The NCSA GelReader program icon is shown in Figure 1.1.

Figure 1.1 NCSA GelReader Program Icon

After you invoke the program, a startup dialog box appears (see Figure 1.2). This dialog box disappears whenever you begin your work.

Figure 1.2 Startup Dialog Box



Loading the Image

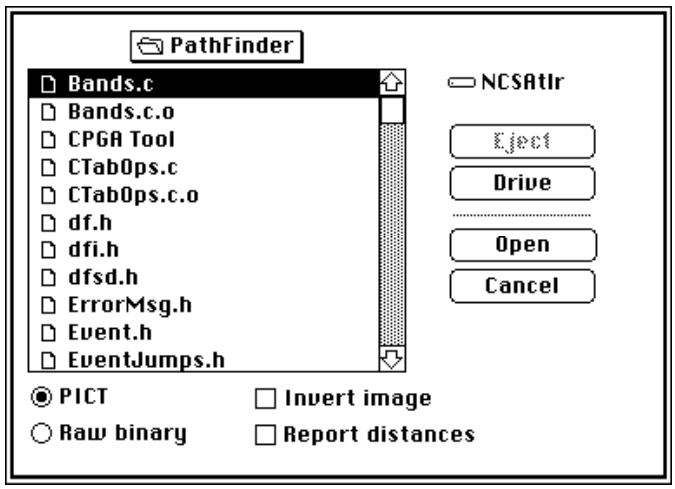
Now lets load a digitized gel image by following the steps below:

1. Choose Open from the File menu, as shown in Figure 1.3, or press -O.

Figure 1.3 File Menu

A directory dialog box appears, displaying the contents of your disk (the dialog box is shown in Figure 1.4).

Figure 1.4 Directory Dialog Box



In this dialog box you may select a file from which to load the data. Notice the radio buttons and check boxes in the dialog box. The two radio buttons allow you to select the type of file from which the gel image is loaded. *Raw binary* format and *PICT* format are supported. The check boxes allow you to specify characteristics of the gel.

- *Invert image* should be checked if bands are represented by lighter rather than darker shades.

- *Report distances* indicates the report form to be generated. If this item is checked, only the percentage of the distance down the length of the lane for each band is reported. Otherwise, the molecular weights are reported.

For the purposes of this tutorial, several datasets have been saved in a folder labeled Images on the NCSA GelReader disk. In this example, you will work with the file named image1(401*260) which is a raw binary image of an autoradiogram.

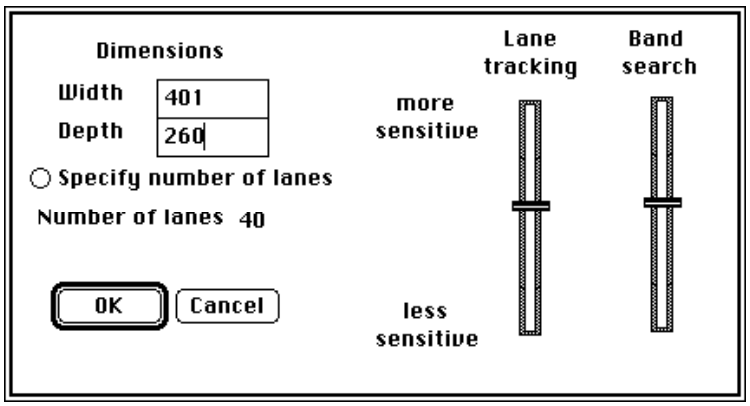
2. Select the Raw binary button for the file type.

Notice that the dimensions of the gel image are included in the filename, (401*260). This inclusion is a convenient mechanism by which you can avoid entering the dimensions of the image. If the dimensions are included in the filename as they were here, those dimensions are later used as the defaults.

3. Select and open the dataset named image1(401*260).

The parameters dialog box appears (Figure 1.5). The dimensions provided are correct (they were provided in the filename), so you need not change them. The two sliders on the right-hand side of the box allow you to change the sensitivity of the searching functions. The left slider changes the resolution of the search for lanes while the right slider controls the band searches.

Figure 1.5 Parameters Dialog Box



4. Move the slider on the right to about 2/3rds of the way up.

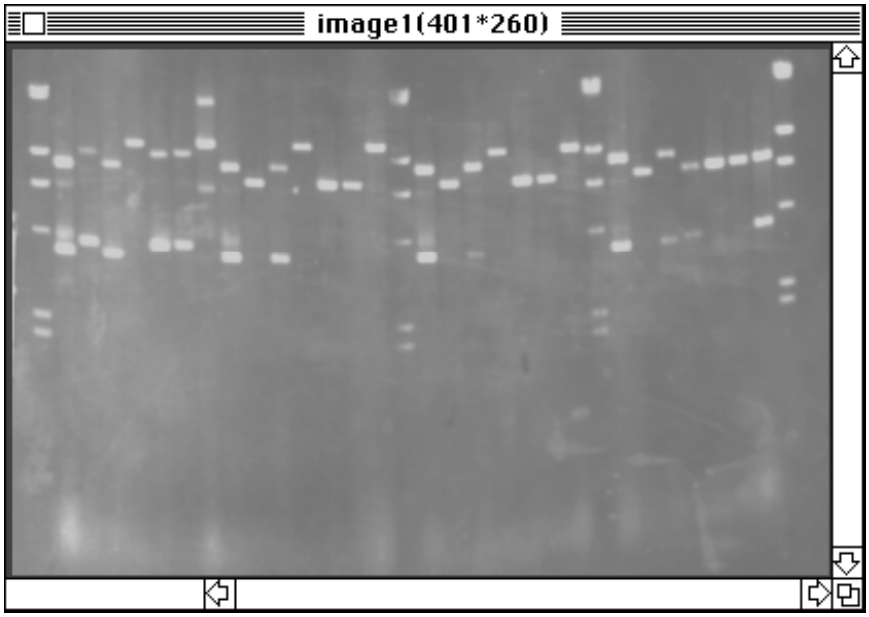
GelReader automatically determines the likely number of lanes in the image. Alternatively, you may specify the number of lanes expected. Do so now by following the steps below.

5. Click the Specify number of lanes button, and the Number of lanes field becomes modifiable.

6. Enter the value 40 in this field.

7. Click OK or press RETURN to view the image. The image appears in an image window (see Figure 1.6).

Figure 1.6 Image Window



Manipulating the Image

NCSA GelReader allows you to perform several operations on your image to facilitate analysis of your data. Specifically, you can do the following: (1) alter the gray scale or color representation of your image, (2) trim away meaningless parts of the image,
(3) magnify the entire image, (4) invoke searching algorithms to find lanes and bands, (5) add and delete lanes and bands from the display, and (6) apply image processing techniques to produce greater contrast in the image. You can also display a lane profile and the notebook window.

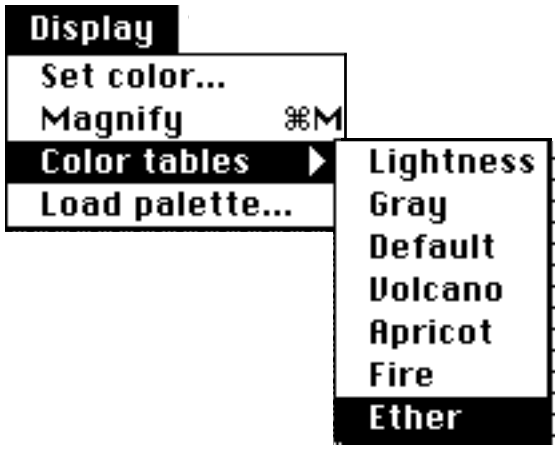
In this tutorial, you will allow the program to find the lanes and bands in the sample image and practice deleting lanes and bands. For information regarding the other imaging and analysis features of NCSA GelReader, refer to Chapter 2, “The Menus.”

Changing the Palette

NCSA GelReader allows you to initialize the *color table* according to any of a number of built-in or user-defined palettes. Palettes help you use color to yield the best perspective of your image. Because the use of the word “color” here includes grays, you may use gray scale color tables if you prefer.

Load a sample built-in palette. Choose Ether from the submenu contained under the Display menu (see Figure 1.7).

Figure 1.7 Display Menu



The color table is initialized and the individual values of your dataset are *color-mapped*, or displayed, according to the new palette.

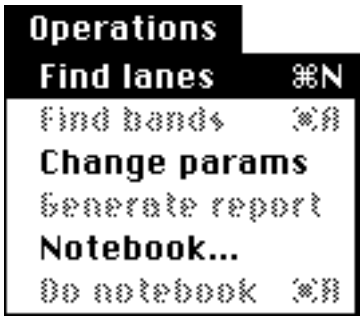
NOTE: NCSA GelReader never alters your actual datasets, only the visual representations of them. That your image data is *mapped according to* a new palette means only that the data is represented with colors from the new color palette. For example, because the 36th entry of the palette may be blue for one palette and green for another, the data value

assigned to this entry is represented by blue when the former palette is used and by green when the latter is used.

Finding Lanes and Bands

NCSA GelReader requires that lanes in an image have been located before bands can be placed. Choose Find Lanes from the Operations menu or press -N to initiate an automated lane search (Figure 1.8).

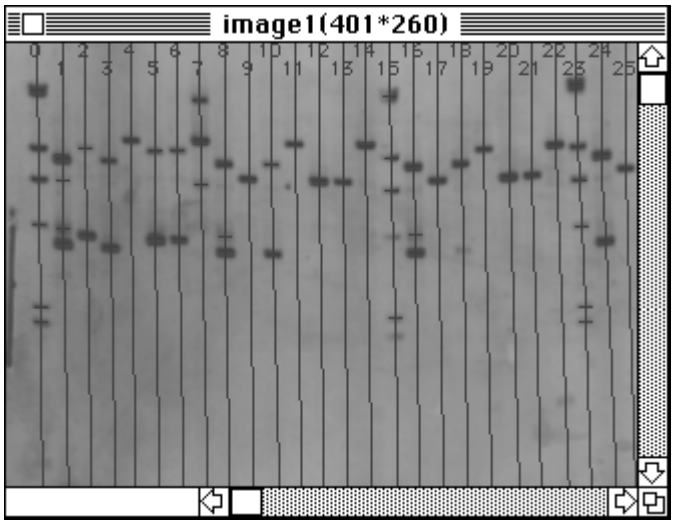
Figure 1.8 Operations Menu



Numbered vertical lines appear in the image, highlighting the lanes that NCSA GelReader detected (see Figure 1.9).

Find bands in the image by selecting Find Bands from the Operations menu or by pressing -A. Horizontal segments appear in the image highlighting the bands that NCSA GelReader detected.

Figure 1.9 Lanes in an Image



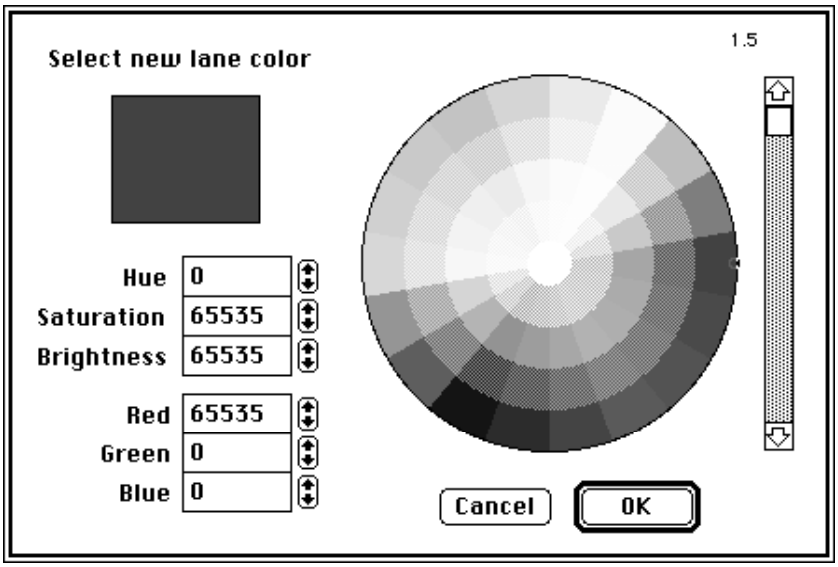
Changing the Color of Lanes and Bands

Sometimes, as with this sample palette, the default band and lane color are difficult to see. Change the color of your lanes and bands by executing

the steps below:

1. Choose Set Color from the Display menu. A color wheel dialog box, shown in Figure 1.10, appears on the screen.

Figure 1.10 Color Wheel Dialog Box



2. Scroll up or down, respectively, to increase or decrease the brightness of the colors in the wheel. Here, for example, you would scroll down to make the colors darker.

3. Select the desired color by clicking on it in the color wheel. For example, click on a dark color like black or navy blue. The color you select appears in the top rectangle located under the heading Select new lane color.

4. Click OK or press RETURN. GelReader applies the color you choose to the bands and lanes in the image.

For additional information on using the color wheel dialog box, refer to your *Macintosh System Software User's Guide.*

Adding and Deleting Lanes and Bands

NCSA GelReader may not always locate the appropriate number of lanes or bands, because of noise in the image, extraneous data, poor contrast in the image, etc. For this reason, you are allowed to delete and add lanes and bands manually.

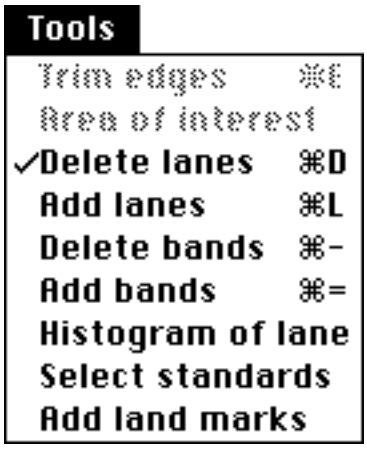
Deleting a Lane or Band

Delete a lane or band by following the steps below:

1. Choose Delete lanes (-D) or Delete bands (—) from the Tools menu (see

Figure 1.11). The cursor becomes a delete lanes or bands tool, respectively.

Figure 1.11 Tools Menu



2. Center the tool over the lane or band you wish to delete and click the mouse button. The lane or band is removed from the display.

Adding a Lane

Add a lane by completing the steps below:

1. Choose Add lanes from the Tools menu or press -L. The cursor becomes an add lanes tool.

2. Position the tool where you wish the new lane to begin, and click the mouse button.

After initially clicking the mouse, notice that as you move the mouse around on the image, the cursor motion is tracked by a shaded line. Clicking the mouse again adds the point at which you clicked to the lane's path. Notice that a new vector emanates from the initial point to the new end point. This vector reflects the path the lane will follow.

3. Click the center of the lane about halfway down the gel image.

4. Move the mouse to where you wish the new lane to end and double-click the mouse-button. A lane is drawn between the points at which you clicked, and the shaded tracking line disappears.

NOTE: You may specify as many points in the path of the lane as you like, creating a very complex lane curvature. The last point in the path is the point where you double-clicked the mouse button. Single click on non-terminal points in the lane's path.

Adding a Band

Add a band by doing the following:

1. Choose Add bands from the Tools menu, or press -=. The cursor becomes an Add bands tool.

2. Position the tool on the lane where you wish to insert the band, and click the mouse button. A band is drawn at the location at which you clicked.

Displaying the Lane Profiles

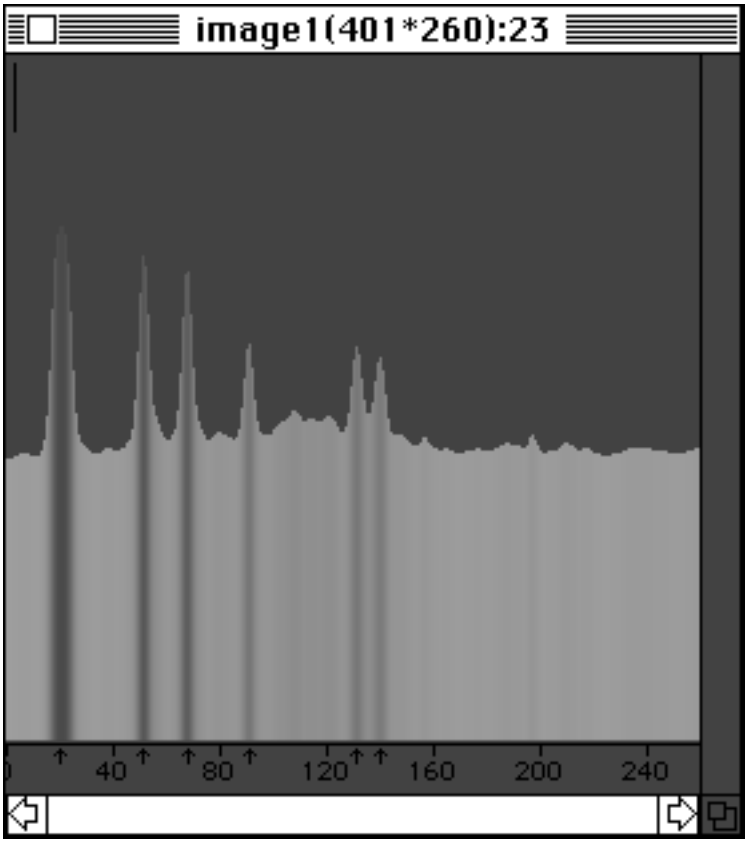
GelReader may also calculate and display a lane profile of the values down the lane. A *lane profile* is a histogram of the averaged value of each row in the lane.

1. Select the Lane profile tool from the Tools menu.

2. Move the cursor into the gel image window. Notice the cursor changes to the Histogram tool.

3. Place the cursor over the lane labeled 23 and click. A lane profile window appears, similar to the one displayed in Figure 1.12.

Figure 1.12 Lane Profile Window



Notice the scale and arrows at the bottom of the window. The arrows indicate the positions of identified bands. You may place new bands in the gel image using this window. Do so by following these steps:

1. Move the cursor into the histogram window. Press and hold down the mouse button. A vertical white line appears.

2. Move the cursor back and forth across the lane profile. The white line follows.

Notice that a bar also is displayed in the gel image window. This bar slides up and down the axis of the lane to indicate the position where the band would be placed if you were to release the mouse button.

3. Release the button around 200 in the scale. A new band is placed in the location of the white line in the lane.

As you can see, an arrow appears in the scale at the bottom of the window, indicating the placement of the new band which has also appeared in the gel image window.

Using the Notebook

GelReader contains a *notebook* that allows you to define macros and configure standard lanes. A *standard lane* is a lane that contains a set of DNA fragments for which molecular weights are known. In future releases, this notebook will be useful for storing information associated with the gel image.

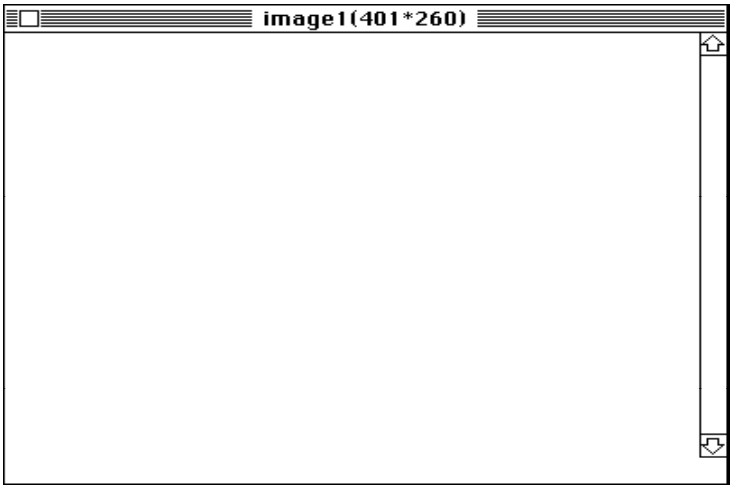
The notebook window appears as a typical text window as shown in Figure 1.13. Open the notebook by doing the following:

1. Select the gel image window.

2. Select Notebook from the Operations menu.

The blank notebook appears in front of all other windows.

Figure 1.13 The Notebook Window



NOTE: The notebook's contents are not saved from one session to the next. Once you quit a GelReader session, all lines within the window are lost; however, although no longer listed in the notebook, any macros that you created using that window are permanently saved with that session. For more information on creating macros, refer to Chapter 3, "Windows and Technical Details."

There are several ways to use the notebook to define standards. This tutorial outlines two of them. For a more detailed discussion on the notebook, see Chapter 3, "Windows and Technical Details."

Entering Standards Using the Command Line

The syntax for entering the molecular weights for standards using a notebook command (Figure 1.13) is as follows:

std(*Lane#*)=*MolWt1,...,MolWtn*

where Lane# is replaced by the number of the lane that you wish to set up as a standard. MolWt1 to MolWtn are the molecular weights where n is the number of bands in the lane. The molecular weights are assigned to consecutive bands in the lane. The number of weights, n, should correspond to the number of bands in the lane.

NOTE: By entering just the std(Lane#) command, you can query the values associated with a standard lane.

Define a standard lane for the gel image you have displayed.

1. Enter the command:

   std(1)=23134,9415,6553,4361,2322,2027

2. Click and drag the mouse over the entire line to select.

3. Select the Do notebook item from the Operations menu, or press -R.

You have now set up a standard lane for this image. To find out the molecular weights you assigned to lane 1, do the following:

1. Enter the command:

```
std(1)
```

2. Use the mouse to select the line.

3. Select the Do notebook item from the Operations menu or press -R.

The notebook displays the following executable command:

```
std(1)=23134,9415,6553,4361,2322,2027
```

Using Macros

As an alternative to using the notebook command line for defining standard lanes, you can create macros to represent commonly used standards. You may also use the macro capabilities of NCSA GelReader to assign standards. Specifically, a *macro* is a symbolic name that can be used in place of a list of molecular weights. Once you have defined a macro, it is saved with the application so that you may use it whenever you are running that copy of NCSA GelReader. For more detailed information on macros, refer to Chapter 3, "Windows and Technical Details."

Creating Macros

This section gives procedures for defining several macros by using the notebook and the following syntax:

```
MacroName = MOLWT1+...MOLWTn
```

where MOLWTs are the molecular weights that you want to associate with the macro. This is an effective way of storing the standard so that you can recall it by a MacroName. *MacroName* is the symbolic name that represents the list of weights associated with the standard.

You will now create a few macros.

1. Enter the following line in the notebook:

```
1/HindIII=23134,9415,6553,4361,2322,2027
```

2. Select Do notebook from the Operations menu, or press -A.

3. Enter the following line in the notebook:

```
pBR233/Hinf1=1631,517,506,396,344,298,221,220,154
```

4. Select Do notebook from the Operations menu, or press -A.

5. Enter the following line in the notebook:

```
BRL123=1476,1353,1230,1107,984,861,738,615,492,369
```

6. Select Do notebook from the Operations menu, or press -A.

You have now defined macros by the names l/HindIII, pBR233/Hinfl, and BRL123.

Listing Macros

List all of the macros currently known by the system to confirm that the software has actually retained these standards and that no entry errors exist.

1. Enter the following line in the notebook:

```
Macros
```

2. Select the line.

3. Select Do notebook from the Operations menu.

The following standards are displayed in the notebook:

```
l/HindIII=23134,9415,6553,4361,2322,2027
pBR233/Hinfl=1631,517,506,396,344,298,221,220,154
BRL123=1476,1353,1230,1107,984,861,738,615,492,369
```

You now know that the macros are properly set up and that you can use them to define standard lanes.

Using Macros to Define Standards Interactively

Assign the standard l/HindIII to the remaining standard lanes by doing the following:

1. Select the gel image window.

2. Choose Select standards from the Tools menu.

A new menu entitled Standards appears. This menu contains a list of all of the macros that you have defined.

3. Select the l/HindIII item from the Standards menu. This item is the name of the macro standard that you will use for the rest of the standard lanes.

4. Click the mouse on lanes 6, 24, and 32. If the standard is assigned to the lane, then the character ‘S’ appears before the lane number.

Your standards are now all defined.

Generating a Report

When you generate a report, the report may take one of two forms. It may consist of the molecular weights of all the bands in the lanes that are not

standards, or it may consist of the percentage of the distance a fragment has traveled down a lane. For the purpose of this example, you will generate a molecular weight report.

After you have entered the information required for the standard lanes, you may generate a report of the approximate molecular weights of the bands. To do this, the gel must contain several standard lanes.

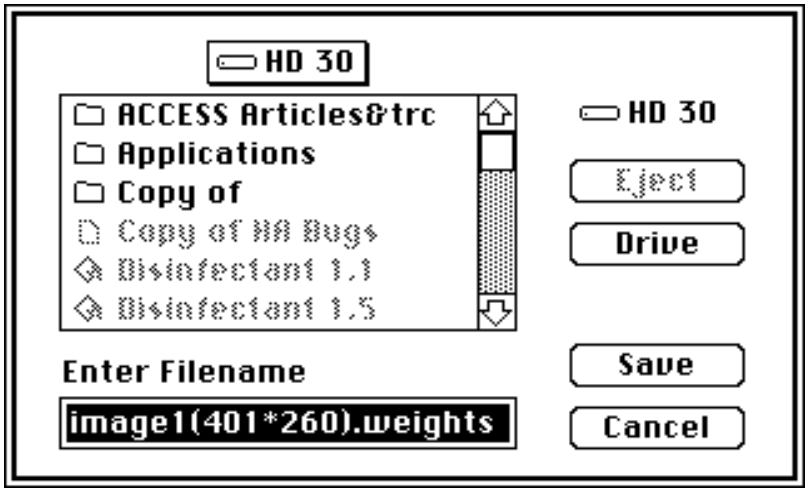
NCSA GelReader uses standard lanes to interpolate the molecular weights of the bands it finds in the lanes that appear between the standards in the image. NCSA GelReader knows both which lanes are standards and what the weights of the bands in these lanes are because you have already entered them as described in the previous section, “Using the Notebook.”

Generate a molecular weight report by following the steps below.

1) Choose Generate report from the Operations menu.

A dialog box appears as shown in Figure 1.14.

Figure 1.14 Output File Selection Dialog Window



2) Select the directory, and enter the filename of the file to contain the molecular weight report.

The last band in each standard lane should extend further into the gel than the bands whose weights are to be determined. In addition, the outermost lanes should be standards.

The *report* is a standard text file that can be edited with a word processor. Figure 1.15 depicts your generated report as it appears when you open it in an application that reads text files. The header of the molecular weight report contains the name of the original image file, the date, the time, and the number of lanes found in the image. The report that follows lists molecular weights by lane number and band number within the

lane.

For more information on generating reports, refer to the section, “Generating Reports” in Chapter 3.

Figure 1.15 Sample Molecular Weight Report

Report for HD40:Edit.Docs:GelReader:image1(401*260)
Date : 07/11/1989 04:30:04

32 Lanes found:

Data as follows:

Lane#	Band#	Molecular weight	Height
2	1	8942	38
2	2	4099	55
3	1	9455	30
3	2	4265	41
4	1	8825	47
4	2	4039	33
5	1	9884	59
6	1	9476	51
6	2	4261	45
7	1	9483	30
7	2	4243	46
8	1	20811	52
8	2	10105	49
8	3	6621	34
9	1	8755	66
9	2	4041	39
10	1	7271	66
11	1	8920	48
11	2	3991	63
12	1	9929	42
13	1	7291	58
14	1	7138	64
15	1	9858	64
17	1	9027	63
17	2	4113	55
18	1	7254	69

Figure 1.15 Sample Molecular Weight Report (Continued)

19	1	8894	64
19	2	4039	−1
20	1	9578	64
21	1	7261	59
22	1	7297	62
23	1	9456	66
25	1	8918	58
25	2	4035	51
26	1	7239	37
27	1	8578	32
27	2	3974	31
28	1	7341	44
29	1	7240	67
30	1	7202	73
31	1	7276	66
31	2	4141	70

Exiting NCSA GelReader

Exit the NCSA GelReader application by choosing Quit from the File menu or by pressing -Q.