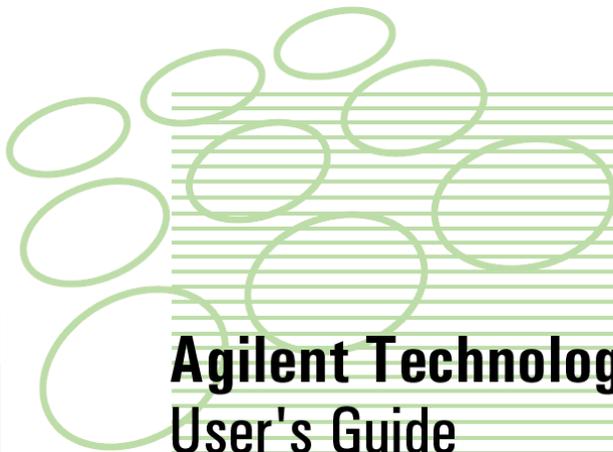




Agilent Technologies

Innovating the HP Way



Agilent Technologies 2100 Bioanalyzer User's Guide



Welcome

**Troubleshooting
and Diagnostics**

Maintenance



WARNING

For details of safety, see the *Site Preparation and Safety Manual* for the Agilent Technologies 2100 Bioanalyzer.

The Agilent Technologies 2100 Bioanalyzer is marked with this symbol when the user should refer to the *Site Preparation and Safety Manual* in order to protect the Agilent Technologies 2100 Bioanalyzer against damage.

Welcome

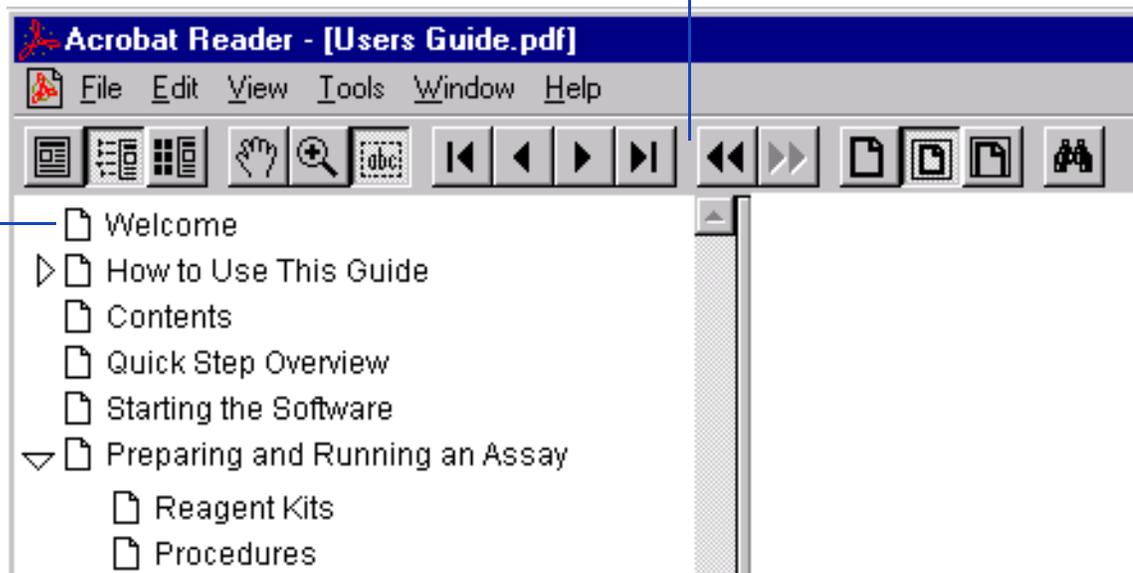
Welcome to the User's Guide for the Agilent Technologies 2100 Bioanalyzer. This online manual provides novice and advanced users with information needed to successfully run assays with the Agilent Technologies 2100 Bioanalyzer.

A quick look at **How to Use This Guide—4** explains how easy it is to use this online manual and helps you to get started.

How to Use This Guide

Use the interactive bookmarks in this frame to choose your desired topic.

Use Acrobat Reader's navigation bar to move around within a topic, see **Navigating within Acrobat Reader**



Click here to go to the table of contents

Click here to go to the index

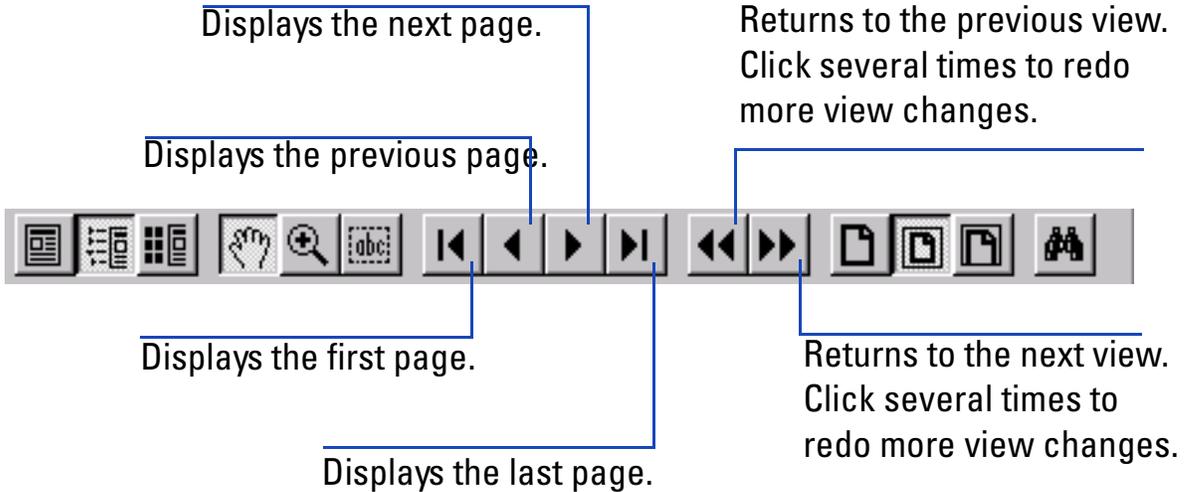
Here's the current page number

▲ displays previous page

▼ displays next page

Navigating within Acrobat Reader

When you've chosen a topic with the bookmarks, use the buttons in Acrobat Reader's tool bar to move around within a topic.



For more information, see the Reader Online Guide in the Help menu.

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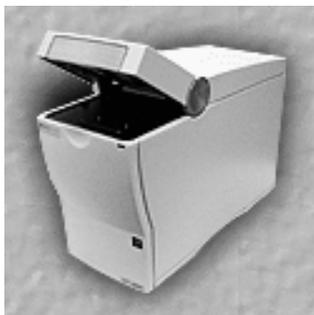
Quick Step Overview

- 1 Make sure the Agilent 2100 Bioanalyzer is connected to line power and connected to the computer.

NOTE

If the Bioanalyzer is not connected to the computer, refer to the printed instructions accompanying the instrument regarding how to set it up.

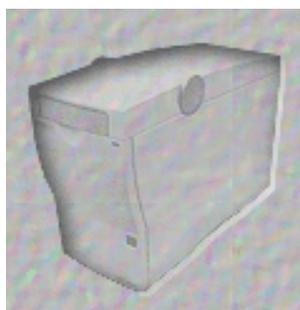
- 2 Turn on the line switch at the rear panel. The status indicator at the front of the Agilent 2100 Bioanalyzer comes on and shows green.
- 3 Start the Agilent 2100 Bioanalyzer software. After startup, the Agilent 2100 Bioanalyzer icon on the screen shows the status:



Lid open

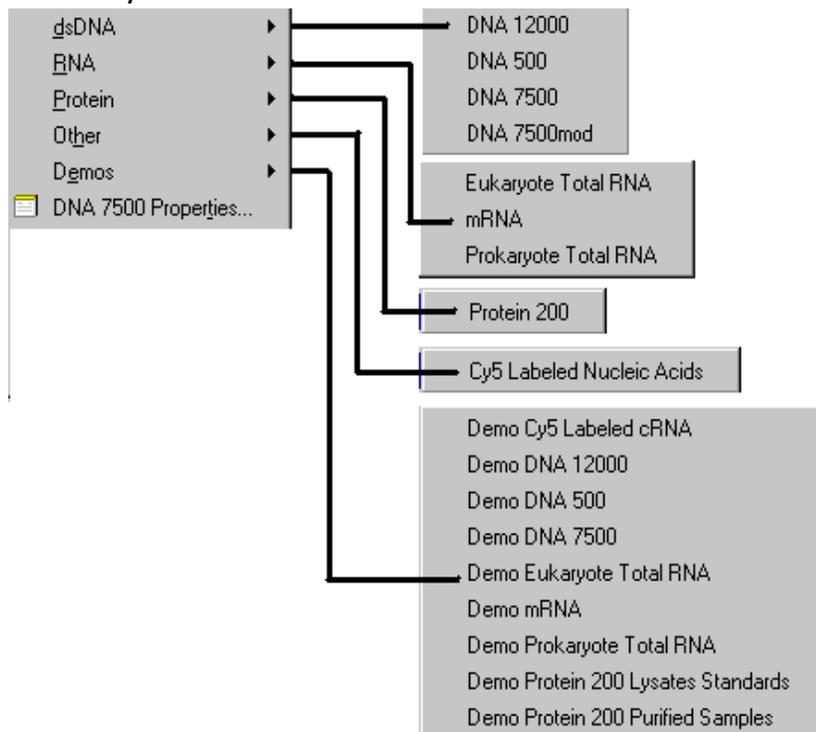


Lid closed but no chip inserted



Dimmed icon: instrument switched off or not communicating properly, see **Troubleshooting and Diagnostics—109**

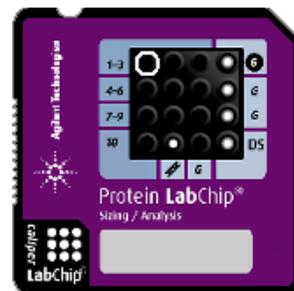
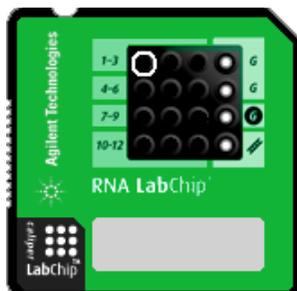
4 Use the assay run previously (the default assay showing) or choose a new one from the Assay menu.



5 Prepare the buffers, samples and chip.

For more information, see your appropriate Reagent Kit Guide.

- 6 Place the chip in the Agilent 2100 Bioanalyzer and close the lid. The Agilent 2100 Bioanalyzer icon changes to show a chip that looks like this:

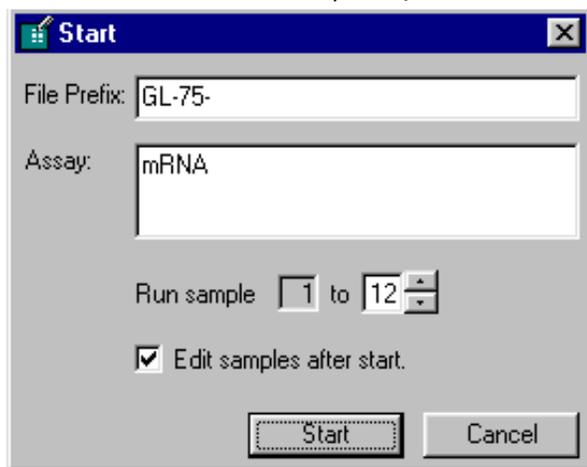


NOTE If the icon did not change to show a chip, then the chip is not detected.

- 7 Click the Start button located above the chip icon.



- 8 The Start dialog box will open. You can change the File Prefix (used as the beginning of the saved filename) and/or enter notes about the run.



- 9 Click the [Start] button on the Start dialog box. The assay begins (it will take approximately five minutes before data appears on the screen). The status indicator on the front of the Agilent 2100 Bioanalyzer flashes during the assay and the chip icon and other displays on the screen are updated to show which well is being read.

- 10 Leaving the checkbox next to "Edit samples after start" enabled on the Start dialog box causes the Edit Samples dialog box to appear. You can enter information in this dialog box while the chip is primed.

Sample Info: Expected Frag./Digest

#	Name	Comment	BP
1	Sample 1		
2	Sample 2		
3	Sample 3		
4	Sample 4		
5	Sample 5		
6	Sample 6		
7	Sample 7		
8	Sample 8		
9	Sample 9		
10	Sample 10		
11	Sample 11		
12	Sample 12		

Chip Notes:

Reset Apply to all

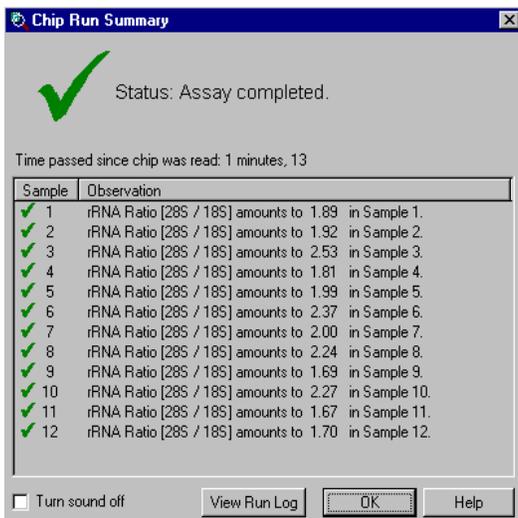
Import.. Export.. OK Cancel

You can change the names of the samples, add comments, expected basepair values, and/or notes about the chip or run, if desired.

11 Data is saved to a file with the name that is shown above the data display:

Data File: BioSizing_00000_2000-11-23_18-00-38
Read: 11/23/00,6:00:38 PM

12 When the assay is finished, the Chip Run Summary dialog box will appear and a sound will alert you (you can stop the sound by clicking the box at the bottom left of the dialog box next to "Turn sound off"). Remove the chip from the Bioanalyzer and dispose of it according to the guidelines established by your laboratory safety officer.



The dialog box shows the number of peaks (DNA and Protein), fragment ratios (Total RNA), or percent of rRNA contamination (mRNA) found in each sample and any assay-specific messages, such as results from a PCR fragment check. Any errors associated with the run will also be shown. You can view the Run Log by clicking the button at the bottom of the dialog box

NOTE

You can view the Chip Run Summary dialog box later by choosing Chip Run Summary from the View menu.

- 13 Follow the cleaning protocol for the particular assay you were running as described in the appropriate Reagent Kit Guide.
- 14 To view results for individual wells as data is acquired or after the run is finished, click a well in the chip icon, a single well on the large multiwell display, or a lane in the gel image. When you view a single well display, specific data for that well appears in a Results Table at the bottom of the window.
- 15 The Bio Sizing program can be set to print customized results automatically at the end of the run (see **Printing a Report** for more information). You can also choose to print a report manually which can contain different information (settings for the automatic and manual print functions are maintained separately).
- 16 The Bio Sizing program can be set to export data automatically at the end of the run. Settings for the automatic export function are customizable. You can also choose to export different information (settings for the automatic and manual export functions are maintained separately and both are remembered across invocations of the application).

For more information, see:

- **Data Analysis—DNA—27**
- **Data Analysis—RNA and Cy5-Labeled Nucleic Acids—54**
- **Data Analysis—Protein—61**

Further tasks include:

- **Changing Your Data Analysis—DNA—18**
- **Changing Your Data Analysis—RNA—57**
- **Changing Your Data Analysis—Protein—65**

You can print a report:

- **Printing an Assay Summary Report—114**
- **Printing a Gel Report—115**
- **Printing a Selected Graph Report—116**
- **Printing an All Graphs Report—117**

Or, you can export data:

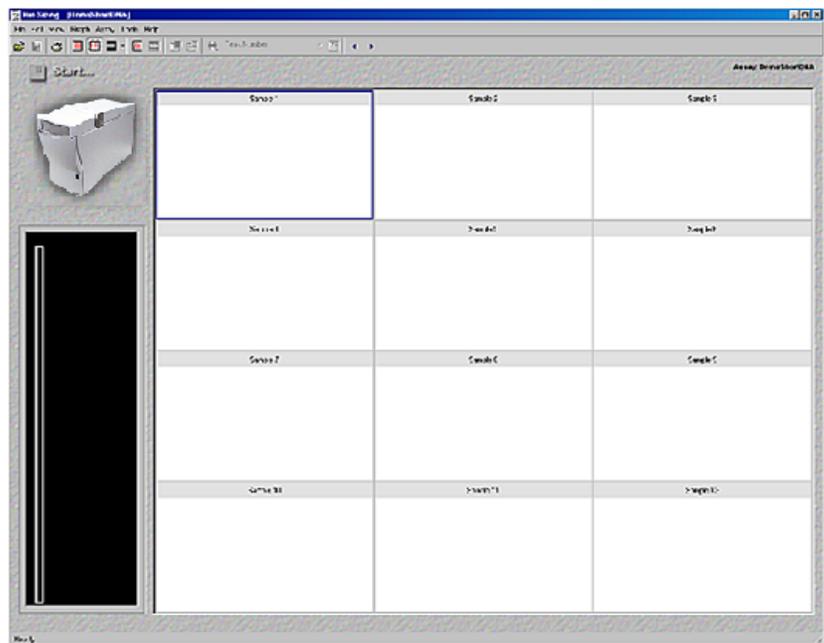
- **Exporting Data—120**

Starting the Agilent 2100 Bioanalyzer Software - Single instrument System

To start the software, go to your desktop and double-click the icon.



The main screen of the program appears. The Agilent 2100 Bioanalyzer or chip should be represented at the left side of the screen—what is shown depends on the status.



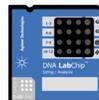
Lid closed, no chip
or chip empty



Lid open



Dimmed icon: no
communication



Lid closed, chip
inserted, DNA or
demo selected



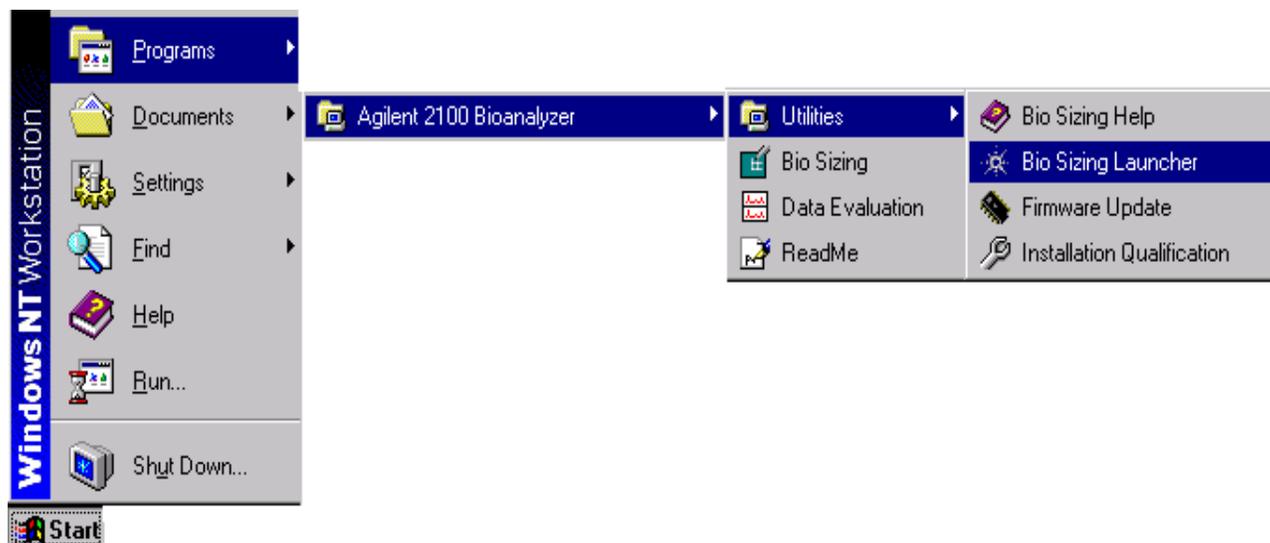
Lid closed, chip
inserted, RNA or
demo selected



Lid closed, chip
inserted, Protein or
demo selected

Starting the Agilent 2100 Bioanalyzer Software Multi-instrument System

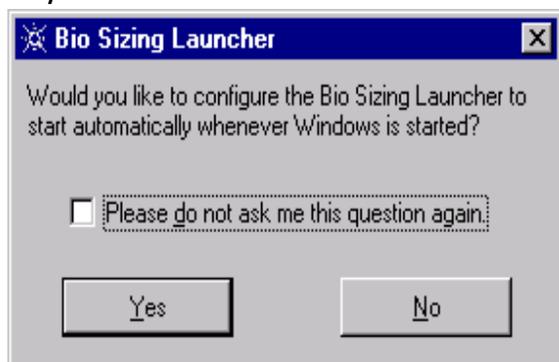
- 1 To start the multi-instrument software, you must start the Agilent 2100 Bio Sizing Launcher. You can invoke the Launcher by choosing Start > Agilent 2100 Bioanalyzer > Utilities > Bio Sizing Launcher.



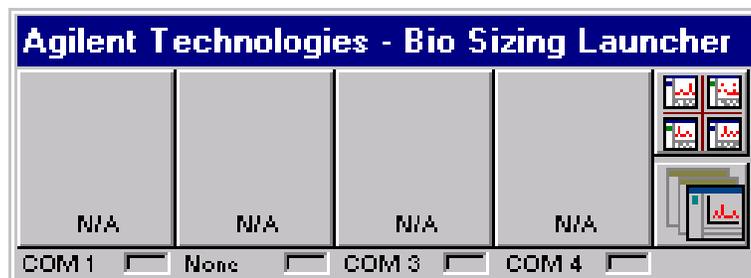
NOTE

You cannot start the Launcher when the Agilent 2100 Bio Sizing program is already open.

- 2 Upon starting the Launcher this way for the first time, a message will appear asking if you would like the Launcher to start automatically each time Windows is started:



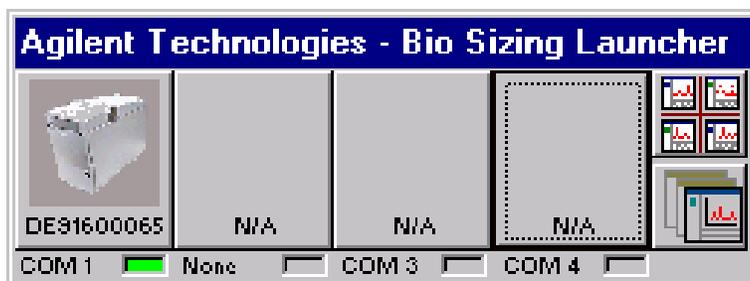
- 3 When the Launcher is started, it appears as shown below.



- 4 Click on the first instrument button to start an instance of the software. It will establish communication to the instrument connected to COM port 1. By clicking on the second, third, and/or fourth instrument buttons, you may start new instances of the software, establishing a connection to the instruments attached to those ports.

NOTE You may communicate with a maximum of four instruments using the Launcher.

- 5 After having established communication to the first instrument, the Launcher will look as shown below.



The Launcher can be described best by dividing it into the following sections:

- **Instrument Buttons:** Four boxes that represent the instances of the Bio Sizing program. Beneath each button is the status of the connection (or Demo Mode).
- **COM Ports:** Four COM port designations followed by LED representations showing the port to which the instrument associated with that instance of the Bio Sizing program is connected and that instrument's status.
- **Arrange Buttons:** On the right, two buttons allow you to display the instances of the program as either tiled or cascaded on the computer monitor.

Closing the Launcher

To close the Launcher, first you must close all open instances of the Agilent 2100 Bio Sizing software. Then position the mouse cursor over the Launcher icon and click the right mouse button. The following menu will appear:



Select Close to terminate the Launcher.

NOTE

You can change the port settings of the different Agilent 2100 Bio Sizing instances by choosing Change Serial Port Setting. Refer to the on-line help for more details.

Preparing and Running an Assay

DNA, RNA, and Protein Assays

- ① Check that you have everything listed in the appropriate **Reagent Kit Guide**
- ② Make sure you are familiar with the **Good Measurement Practices—22**
- ③ Prepare the reagents, load the chip and run the assay as described in the appropriate **Reagent Kit Guide**

Good Measurement Practices

This section lists all relevant hints regarding the handling of tools, chips, reagents, and the Agilent 2100 Bioanalyzer. For the latest information on assay-related hints, go to the Lab-on-a-Chip web site at:

<http://www.agilent.com/chem/labonachip>

Tools and Handling

- Always wear gloves when handling chips to prevent them from becoming contaminated.
- When pipetting sample, use pipette tips that are small enough. Pipette tips that are too large will lead to poor quantitation accuracy.
- Change pipette tips between two pipetting steps to avoid cross-contamination.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well can lead to bubbles and poor results.

Reagents and Reagent Mixes—General

- Keep all reagents and reagent mixes (for example, the gel-dye mixture) refrigerated at 4°C when not in use for more than 1 hour. Reagents might decompose, leading to poor measurement results.
- Allow all reagents and samples to equilibrate to room temperature before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Gel and Gel-Dye

- Use gel-dye mixture within three weeks of preparation. The gel-dye mixture might decompose and lead to poor measurement results.

Samples

- For optimal results, samples should be of pH 6 to 9 and should not have an ionic content greater than twice that of a PCR buffer.

Chips

- Prepared chips should be used within 5 minutes. Reagents might evaporate, leading to poor results.
- Vortex chips for the appropriate time of 1 minute. Improper vortexing can lead to poor results.
- Do not use force to press the chip in the receptacle of the Agilent 2100 Bioanalyzer. Proper placement of the chip should not require force. Improper placement of the chip could damage the electrode assembly when you close the lid.
- Do not touch wells of the chip. The chip could become contaminated, leading to poor measurement results.
- Do not leave any wells of the chip empty or the assay will not run properly. Add 1 μL of sample buffer to each unused sample well.
- Do not touch the underside of the chip.

Agilent 2100 Bioanalyzer

- Don't touch the Agilent 2100 Bioanalyzer during a run and never place it on a vibrating surface.
- Clean electrodes daily using the cleaning chip. For more details, see **Electrode Cartridge Maintenance—273**.
- Clean electrodes on a quarterly basis using a toothbrush and distilled water. For more details, see **Electrode Cartridge Maintenance—273**.
- Clean the focusing lens once a month (or after any liquid spill) using isopropanol. For more details, see **Lens Maintenance—275**.

Decontamination Procedure for RNA Assays

- Perform the following decontamination/cleaning procedure on a daily basis before running any RNA assays. See **Electrode Cartridge Maintenance—273** for more information regarding the use of the electrode cleaner and/or the procedures for cleaning and/or decontamination.

Decontamination:

- 1 Slowly fill an electrode cleaner with 350 μ L RNaseZAP. (Label this electrode cleaner "for RNase ZAP.")
- 2 Open the lid, place the electrode cleaner in the instrument, and close the lid for approximately 1 minute.

3 Open the lid, remove the RNase ZAP electrode cleaner, and store it for future use. You can reuse this electrode cleaner for all the chips in the kit. Empty the electrode cleaner for overnight storage.

4 Then follow the instructions below for cleaning the electrodes.

Cleaning:

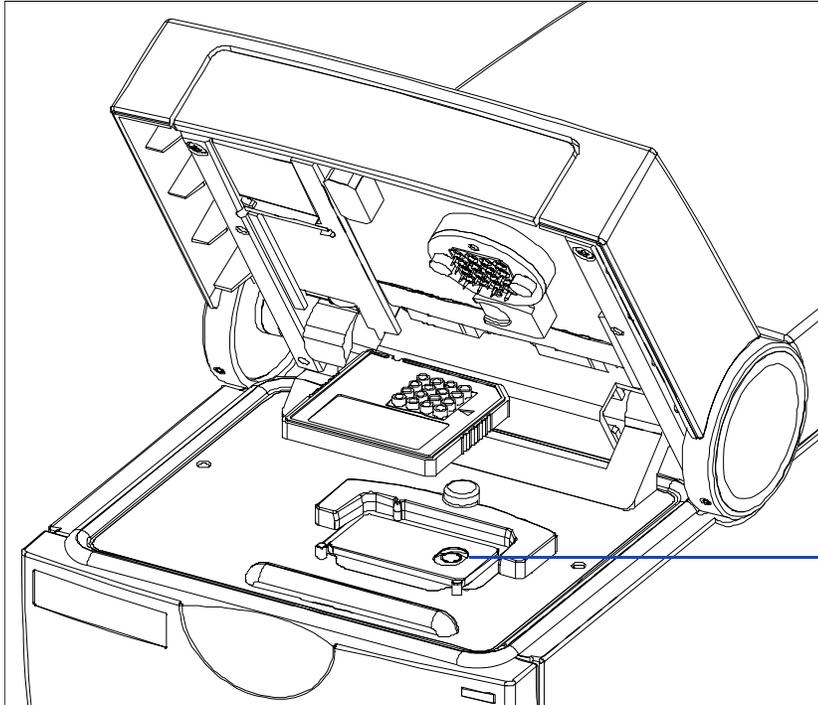
1 Slowly fill another electrode cleaner with 350 μ L RNase-free water. (Label this electrode cleaner "RNase-free water.")

2 Open the lid, load this electrode cleaner into the instrument, and close the lid, immersing the electrodes in the water.

3 After approximately 10 seconds, remove the electrode cleaner. Put this electrode cleaner aside for future use as well.

4 Wait another 10 seconds for the water on the electrodes to evaporate.

- Clean the focusing lens on a quarterly basis (or after liquid spill) using a lens tissue and isopropanol.



Focusing Lens

Data Analysis—DNA

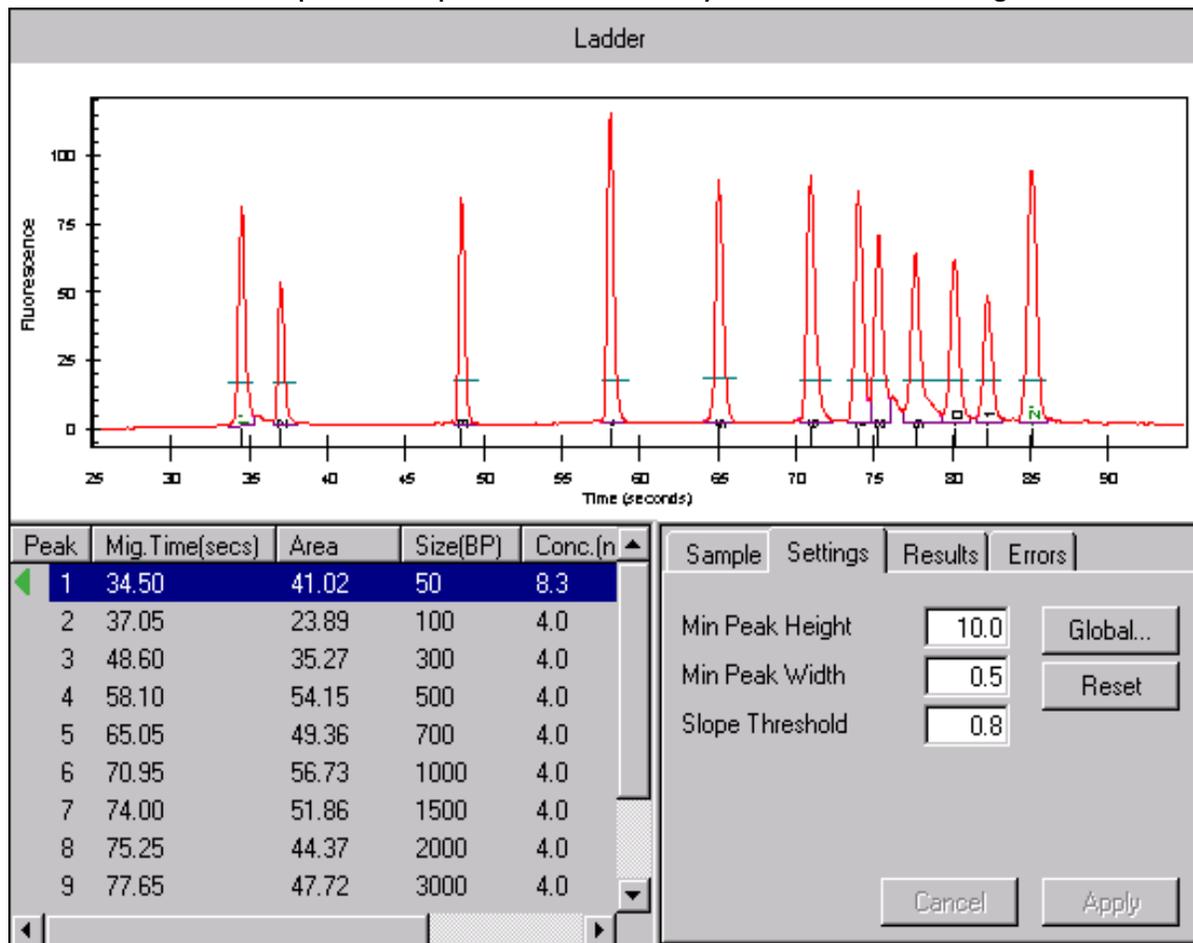
How the Agilent 2100 Bioanalyzer Software Analyzes Data

The purpose of Bio Sizing assays is to calculate the size and/or concentration of nucleic acid fragments. Results are calculated after all data for an individual well has been read.

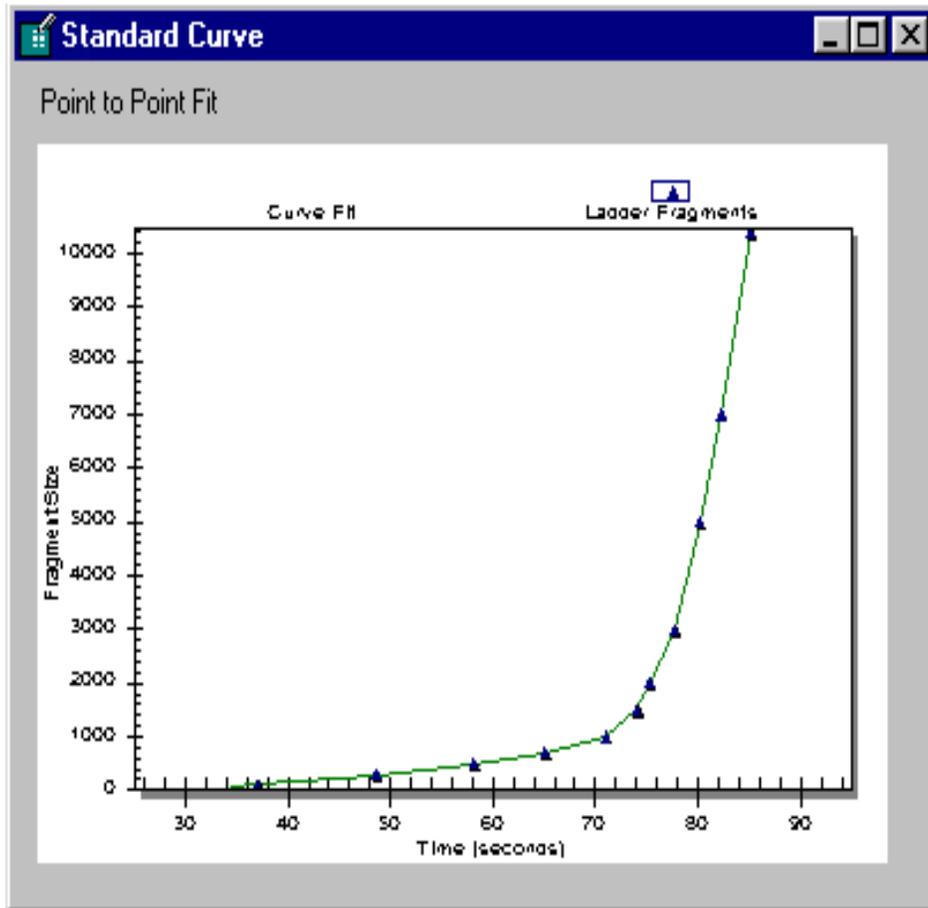
The data analysis procedure consists of the following steps:

- 1** Raw data is read and stored by the system for all the individual wells.
- 2** A software algorithm filters the data and plots the resulting electropherograms of all wells. You can change the settings of the filtering algorithm after the run and reanalyze your data.
- 3** Peaks are identified for all wells and tabulated by migration time. You can change the settings of the peak find algorithm after the run has finished and reanalyze your data.

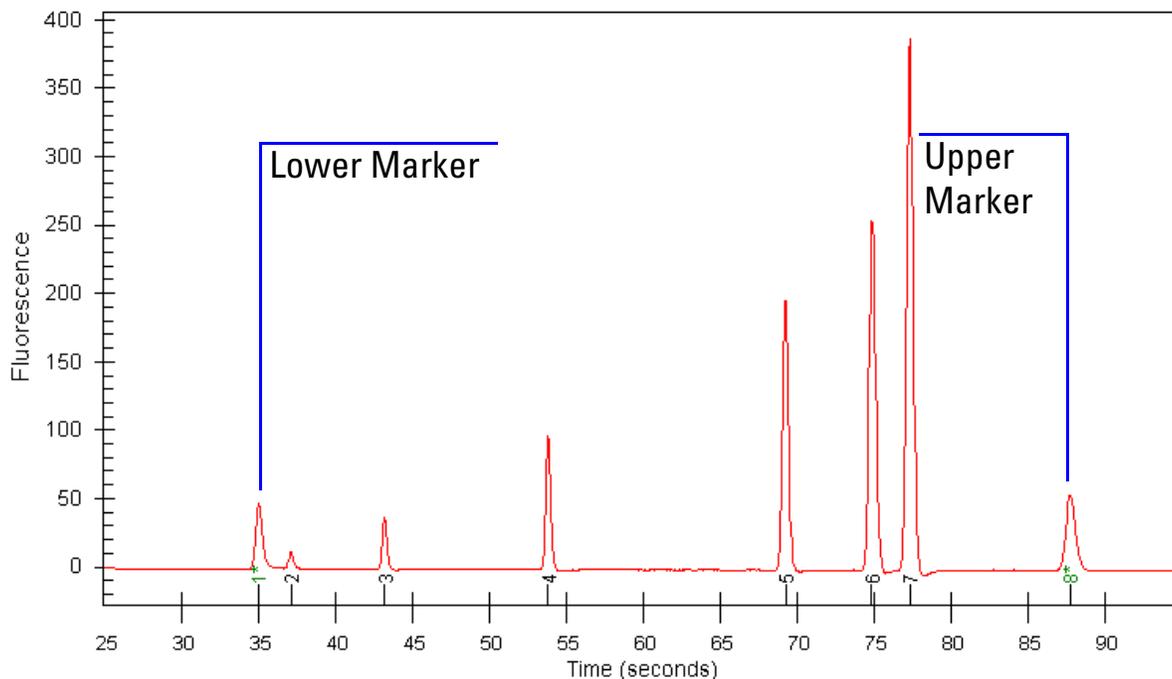
- 4 A Bio Sizing ladder—a mixture of DNA fragments of different sizes—is run first from the ladder well (see the electropherogram below). The concentrations and sizes of the individual base pairs are preset in the assay and can't be changed.



- 5 A standard curve of migration time against DNA size is plotted from the DNA sizing ladder by linear interpolation. The standard curve derived from the data of the ladder well should resemble the one shown below.



- 6** Two DNA fragments are run with each of the samples, bracketing the DNA sizing range. Called lower and upper markers, these are internal standards and are used to align the ladder well with the individual sample wells. The figure below shows an example of assigned marker peaks in a sample well.



NOTE

The software performs alignment automatically. You can turn off the alignment at the end of a run; however, no automatic data evaluation will occur until the alignment is turned on again.

- 7 The calibration curve (plotting migration time against DNA size), in conjunction with the markers, is then used to calculate DNA fragment sizes for each well from the migration times measured.
- 8 To calculate the concentration of the individual DNA fragments of all sample wells, the upper marker, in conjunction with an assay-specific concentration against base pair size calibration curve, is applied to the individual sample peaks of all sample wells.

NOTE

The software allows you to define upper and lower markers yourself. However, a change in the selection of the markers will lead to quantitative changes of the calibration procedure and will, therefore, alter the entire data evaluation.

Changing Your Data Analysis—DNA

Changing the Settings of the Data Evaluation Algorithm

Different sets of parameters can be changed in the software in order to modify the data evaluation for sample analysis:

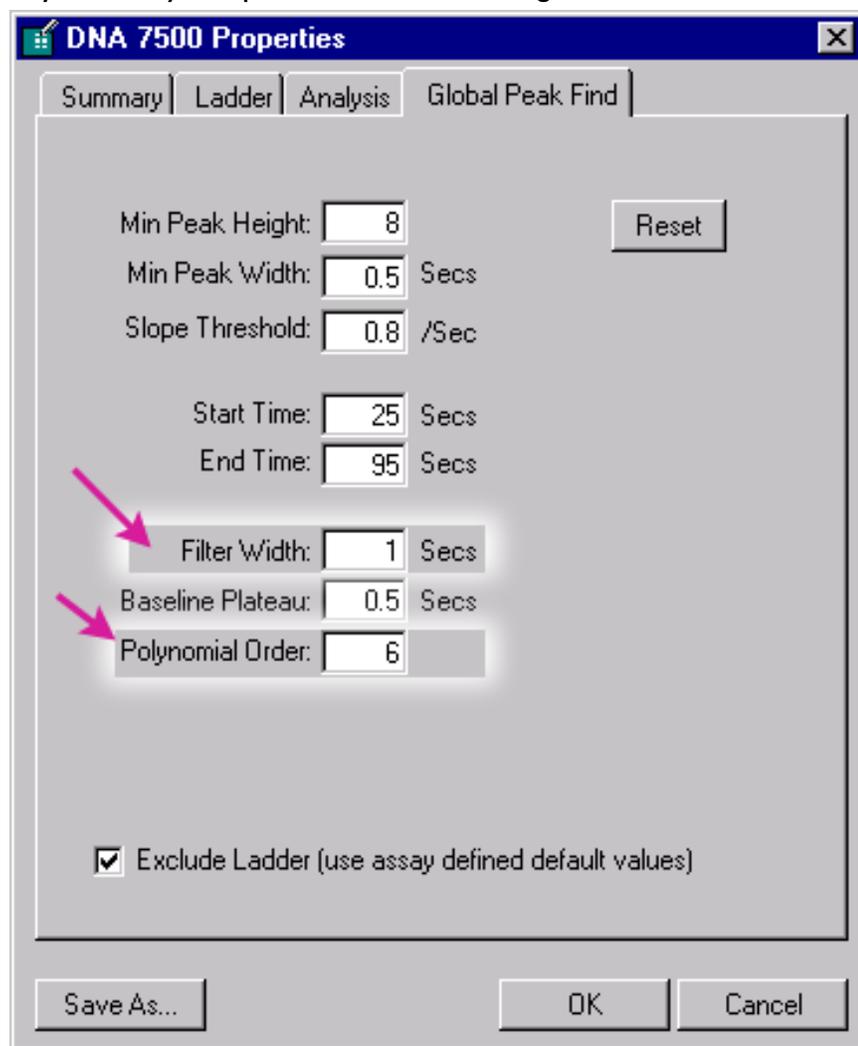
- filtering parameters
- peak find parameters for all wells; peak height for individual wells
- time window for analysis
- assigning upper and lower marker peaks
- aligning or unaligning marker peaks

Changes can be made before a new run is started or to reanalyze the data from a previous run.

Filtering Parameters

The first step the software takes in analyzing the data is to apply data filtering. Highlighted in the figure below are the two filtering parameters that can be changed: Polynomial Order and Filter Width.

You can access the Global Peak Find settings in the software by going to: Assay > Assay Properties and choosing the Global Peak Find tab.



The screenshot shows the 'DNA 7500 Properties' dialog box with the 'Global Peak Find' tab selected. The dialog has four tabs: 'Summary', 'Ladder', 'Analysis', and 'Global Peak Find'. The 'Global Peak Find' tab contains the following settings:

- Min Peak Height: 8
- Min Peak Width: 0.5 Secs
- Slope Threshold: 0.8 /Sec
- Start Time: 25 Secs
- End Time: 95 Secs
- Filter Width: 1 Secs
- Baseline Plateau: 0.5 Secs
- Polynomial Order: 6

A 'Reset' button is located to the right of the 'Min Peak Height' field. At the bottom of the dialog, there is a checked checkbox labeled 'Exclude Ladder (use assay defined default values)'. The dialog also features 'Save As...', 'OK', and 'Cancel' buttons at the bottom.

The Polynomial Order setting is used to define the power series applied to fit the raw data. The higher the number you set, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.

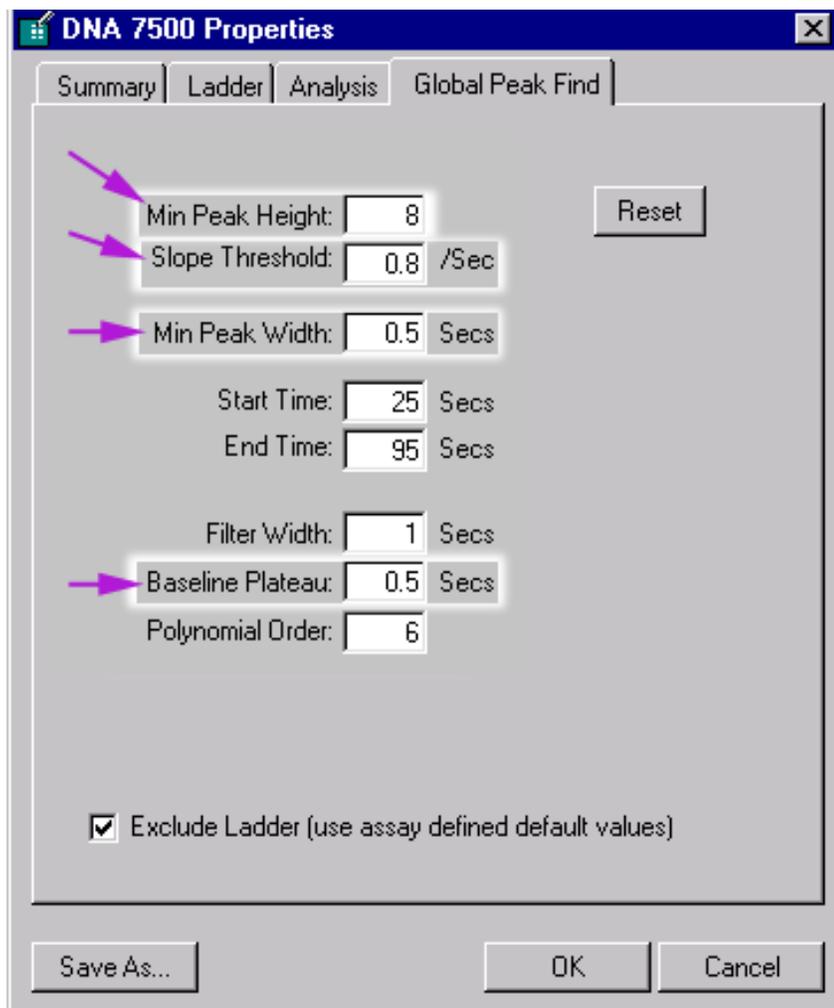
Filter Width defines the data window, given in seconds, used for averaging. The broader the filter width, the more raw data points are used for averaging. As a result, the noise level will decrease but peaks will become lower and broader.

Overall, changing the Filter Width has more effect on the result of the filtering procedure that is applied than does changing the Polynomial Order.

Peak Find Parameters

After data filtering, the Peak Find algorithm locates the peaks and calculates the local peak baselines. The algorithm begins by finding all the peaks above the noise threshold in order to determine the baseline, after which any peaks below the noise threshold are rejected. A local baseline is calculated for each peak to allow for baseline drift.

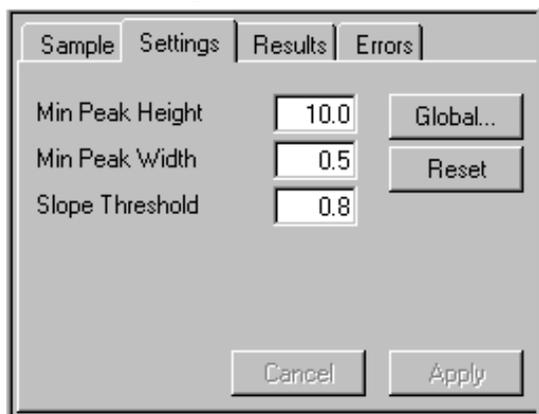
The four peak find parameters that can be changed, Min Peak Height, Slope Threshold, Min Peak Width and Baseline Plateau, are shown below. Choosing Apply sets the parameters for all the wells.



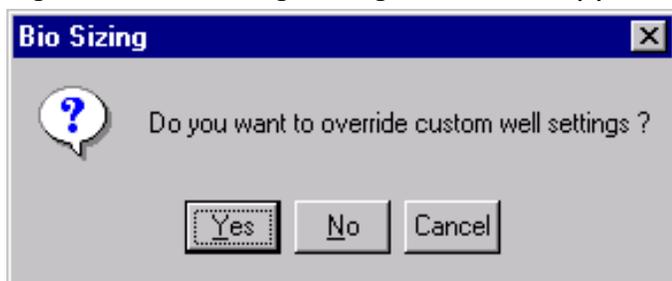
The Reset button sets the Global Peak Find values back to the factory settings.

Min Peak Height	Determines the threshold for the peak find algorithm. For each peak, the difference between the start point value and the center point value (local baseline) must be greater than the Minimum Peak Height value.
Slope Threshold	Determines the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.
Min Peak Width	Determines the minimum amount of time that must have elapsed after threshold was exceeded.
Baseline Plateau	A parameter that assists in finding peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the Slope Threshold setting (either positive or negative) for longer than the time set for the Baseline Plateau. This setting rejects brief, low slope areas such as those found between non-baseline-resolved peaks.
Exclude Ladder	(Default: enabled) This setting causes the Bio Sizing program to use the values defined by the assay for ladder data instead of data obtained from the ladder run with the assay.

You can change all peak find settings except the Baseline Plateau for individual wells. In the lower right pane of the single-well display (to the right of the Results Table) are four tabs. The Settings tab shows the peak find settings that are currently in effect for that well. Changing the settings shown on this tab will affect this well only (to change the settings that affect all wells, click the [Global...] button to open the Assay Properties dialog box and then click the Global Peak Find tab).



If you change the Global peak find settings after making individual well setting changes, the following dialogue box will appear:

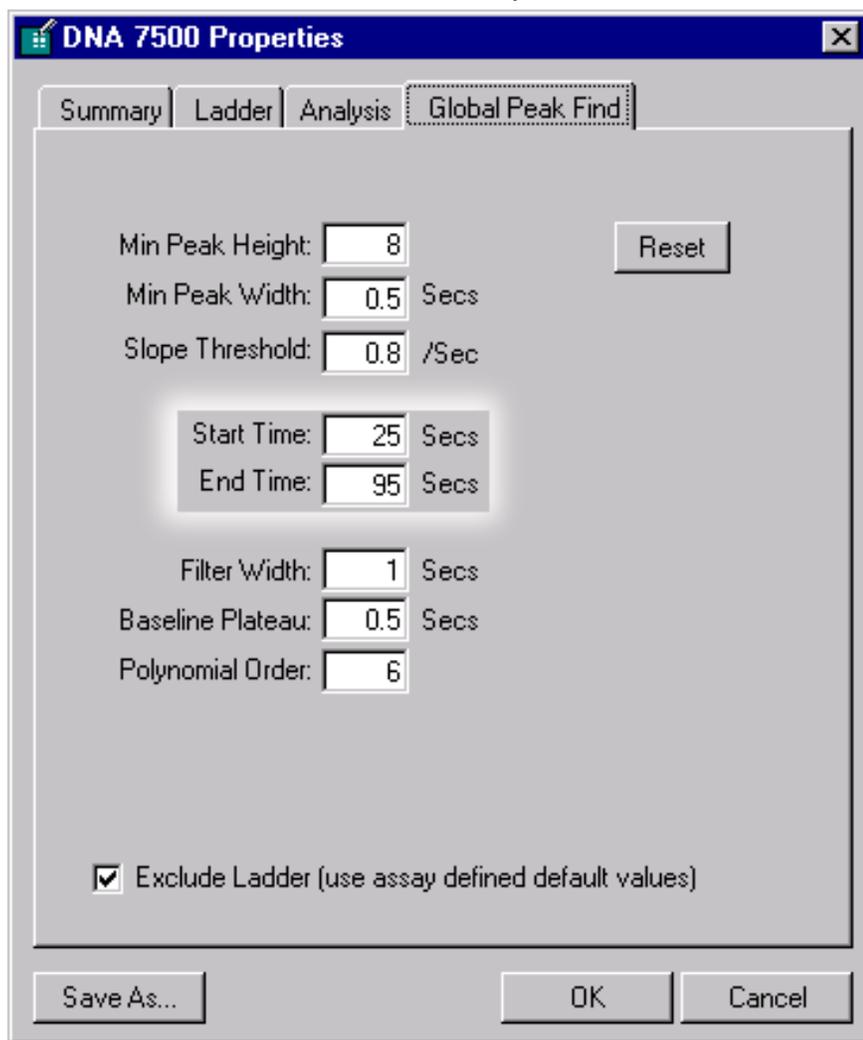


Choosing [Yes] causes any changes made to the peak find settings for individual wells to be discarded and applies the global peak find settings to all wells. Choosing [No] allows individual wells to retain changed peak find settings.

NOTE This dialogue appears whenever at least one of the samples has different local settings.

Time Window for Analysis

The Start Time and EndTime parameters on the Global Peak Find tab (see figure below) define the time window within which peaks will be found.



The image shows a screenshot of the "DNA 7500 Properties" dialog box, specifically the "Global Peak Find" tab. The dialog box has a title bar with a close button (X) and a menu icon. Below the title bar are four tabs: "Summary", "Ladder", "Analysis", and "Global Peak Find". The "Global Peak Find" tab is selected and highlighted. The main area of the dialog contains several input fields and a "Reset" button. The input fields are: "Min Peak Height" (8), "Min Peak Width" (0.5 Secs), "Slope Threshold" (0.8 /Sec), "Start Time" (25 Secs), "End Time" (95 Secs), "Filter Width" (1 Secs), "Baseline Plateau" (0.5 Secs), and "Polynomial Order" (6). A "Reset" button is located to the right of the "Min Peak Height" field. At the bottom of the dialog, there is a checkbox labeled "Exclude Ladder (use assay defined default values)" which is checked. Below the checkbox are three buttons: "Save As...", "OK", and "Cancel".

DNA 7500 Properties

Summary | Ladder | Analysis | **Global Peak Find**

Min Peak Height:

Min Peak Width: Secs

Slope Threshold: /Sec

Start Time: Secs

End Time: Secs

Filter Width: Secs

Baseline Plateau: Secs

Polynomial Order:

Exclude Ladder (use assay defined default values)

Assigning Upper and Lower Marker Peaks

For each sample, the upper and lower marker peaks are assigned first and then the data is aligned so that the well markers match the ladder markers in time. This allows the size and concentration of the sample peaks to be determined.

The first peak is assigned to be the lower marker and is then offset to match the lower marker in the ladder. The upper marker is then assigned either to the last peak in the sample well or to the peak nearest to the ladder's upper marker. See **Aligning or Unaligning the Marker Peaks—42** for an example of assigned marker peaks.

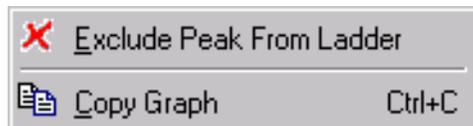
If you see unexpected peaks in the ladder analysis or the markers are set incorrectly, you can exclude peaks manually from the ladder or choose a peak to be used as a marker.

NOTE

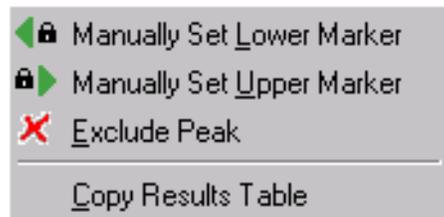
Excluding a peak or manually setting a peak to be an upper or lower marker may cause errors in analysis.

You can move the boundary between the Results Table and the well graph up or down to increase or reduce the amount of space allotted to the Results Table, making it possible to see all of the results at once.

Right-clicking in the result table of a *ladder* well causes this pop-up menu to appear:

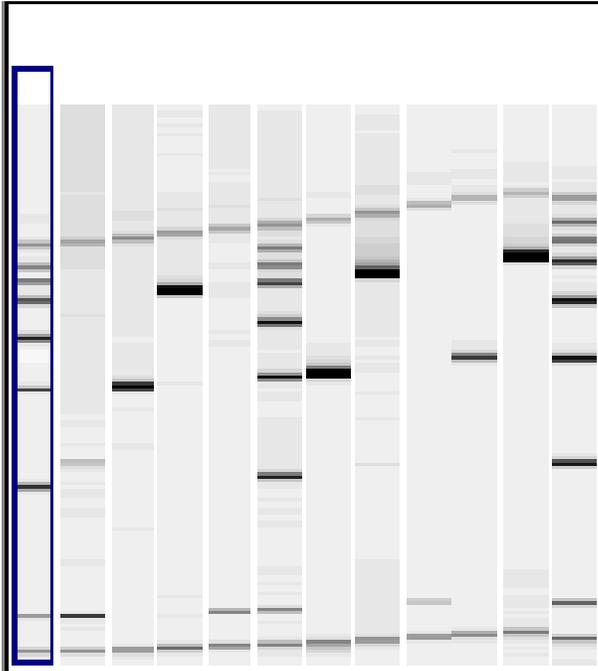


Right-clicking in the result table of a *sample* well causes this pop-up menu to appear:

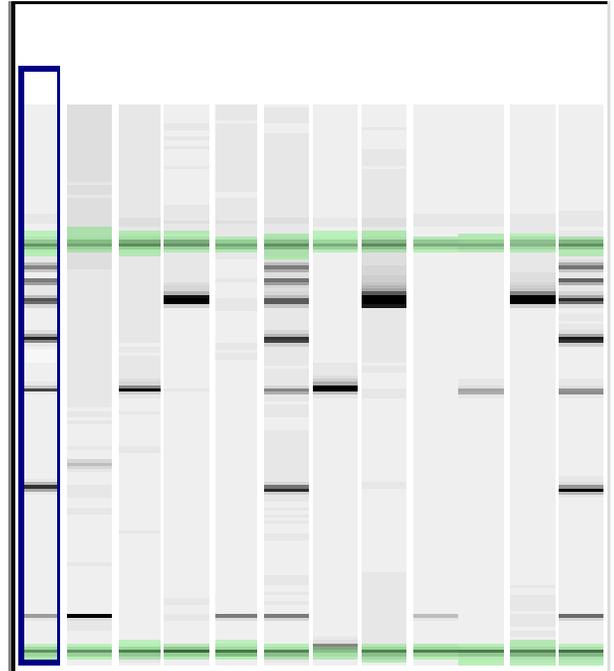


Aligning or Unaligning the Marker Peaks

The upper and lower markers are then aligned to the ladder markers by resampling the well data in a linear stretch or compression using a point-to-point fit.



Data before alignment



Markers aligned to ladder

If the sample marker peaks are either more than twice as far apart or less than half as far apart as the ladder markers, they are assumed to be the wrong peaks and analysis of the well stops, producing the error “Marker peaks not detected.”

NOTE

With DNA assays, the height of marker peaks is assay dependent. Ladder peaks are analyzed to calculate a marker peak threshold which is used to locate the marker peaks in the sample wells. If the marker peaks found using this calculated method fail to align with those of a sample, the Bio Sizing software will use the minimum peak height threshold setting instead (if this value is lower than the value for the marker peak).

For example, the calculated threshold might be too high to find the sample's markers if they happen to be very small. Either no markers will be found or the wrong peaks will be assumed to be markers and these may not align with the ladder markers.

Consequently, the software attempts to use the minimum peak height threshold which, if it is set low enough, will locate the real markers, allowing the sample to align.

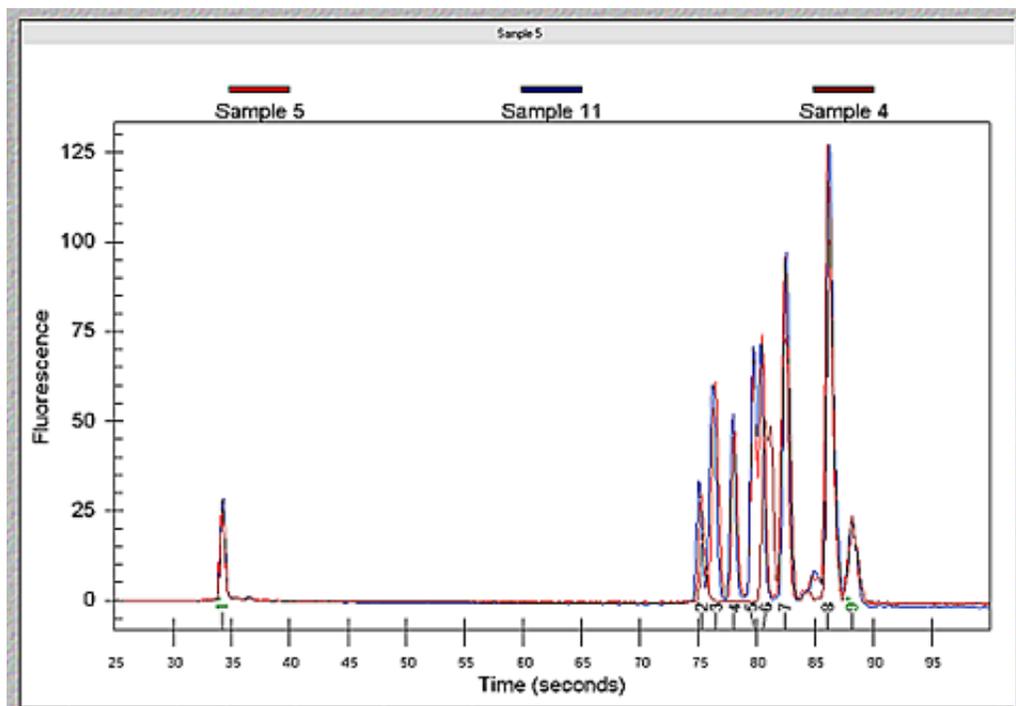
While the actual peak times are those shown in the unaligned data, the Bio Sizing program cannot perform analysis without alignment so "relative migration times" are used (aligning the markers to the ladder peaks causes a shift in the rest of the peak times).

Changing the View of the Results

A number of different options are available for viewing the data after it has been acquired by the Agilent 2100 Bioanalyzer. None of these options change the raw data but rather provide different means of viewing the results.

Overlaying Well Graphs

Data from multiple wells can be overlaid within the single-well large display view. Hold down the CTRL key and then click the left mouse button on other lanes in the gel image in the small display.



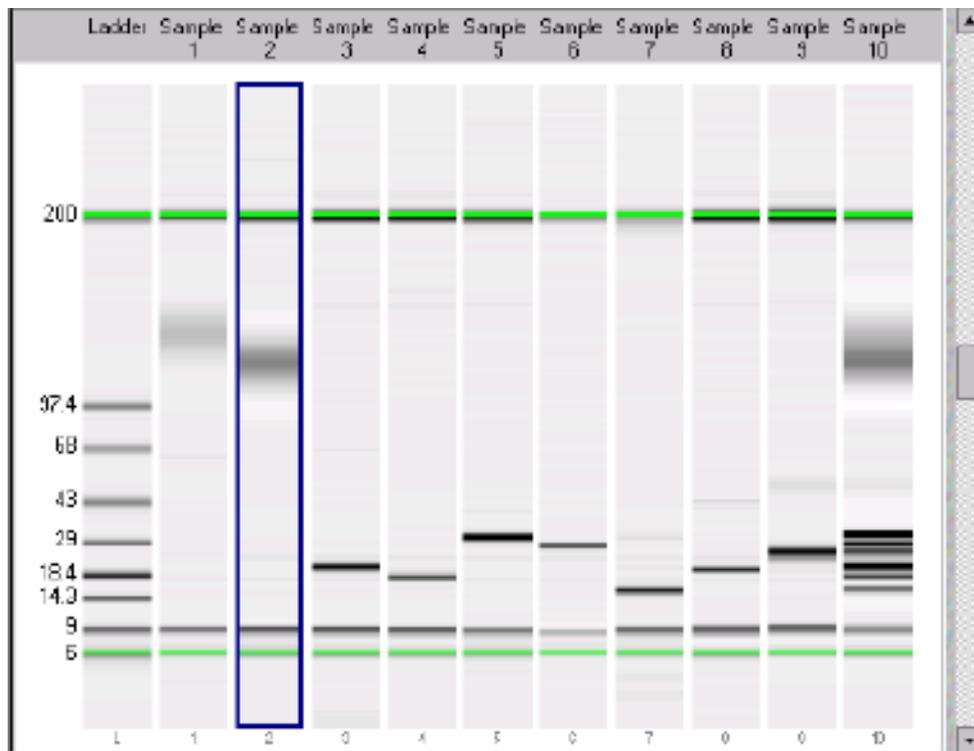
Bounding boxes will appear around the gel lanes signifying which wells are shown overlaid. Each peak graph will be shown in a different color and line style with a legend at the top of the window. You can remove wells from the overlay by CTRL+clicking the corresponding lane in the small gel display (the bounding box will disappear).

A stand-alone program called " Data Evaluation" is also included with the Bio Sizing software and can be accessed by choosing Tools > Compare Results....

This program allows you to compare the results from the same or different runs and even different assays within a single window and provides tools that allow you to manipulate the comparison of the data in different ways. Documentation and help for the Data Evaluation program are available within that program.

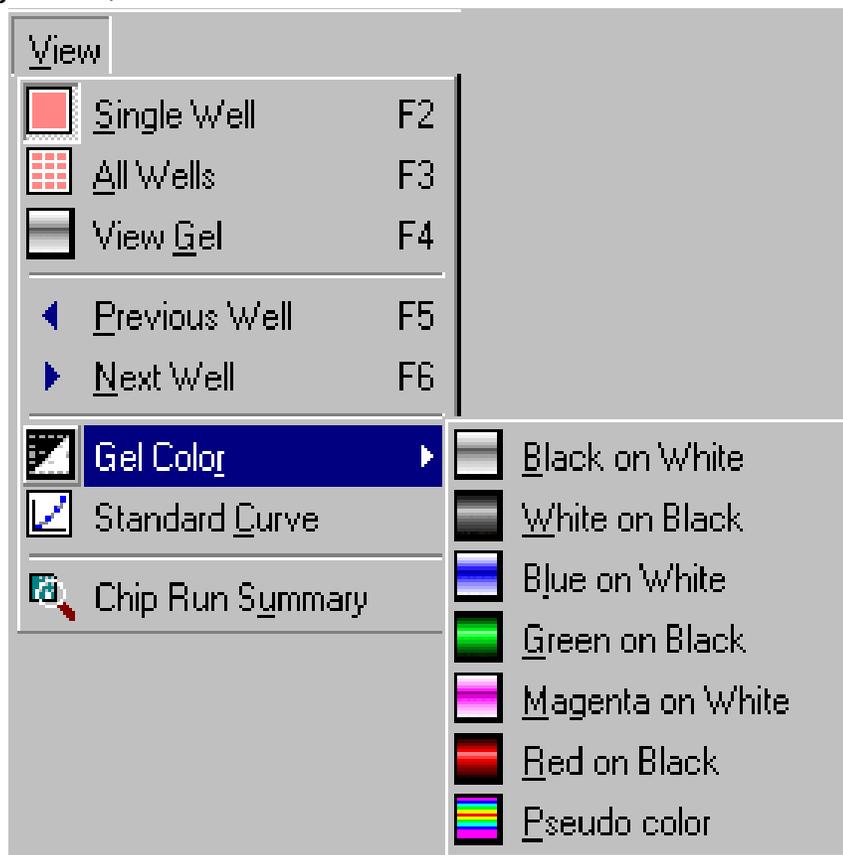
Changing to Gel View

To see an overview of you data in a gel-like image, switch to the Gel view. In the menu bar, click on View > View Gel. The main window will change and display the results in a format as would be generated by a slab gel device.



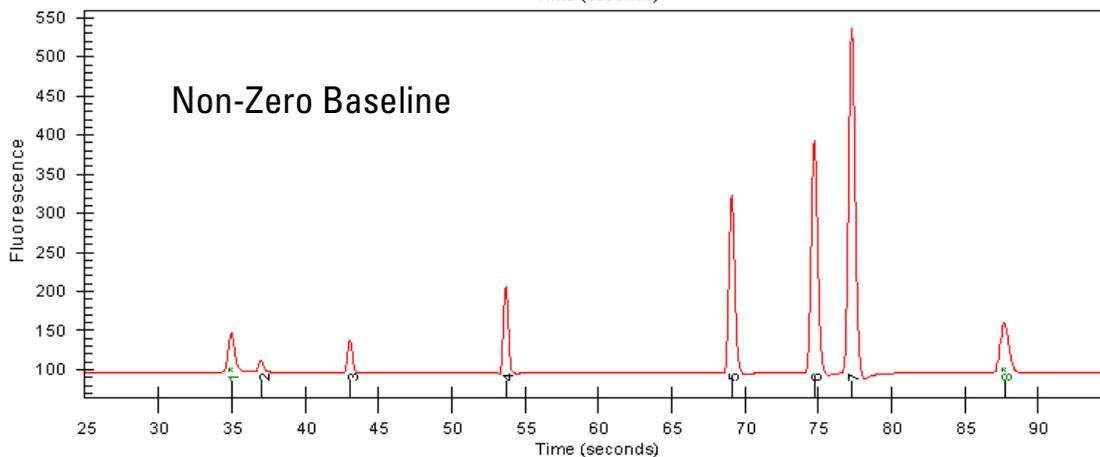
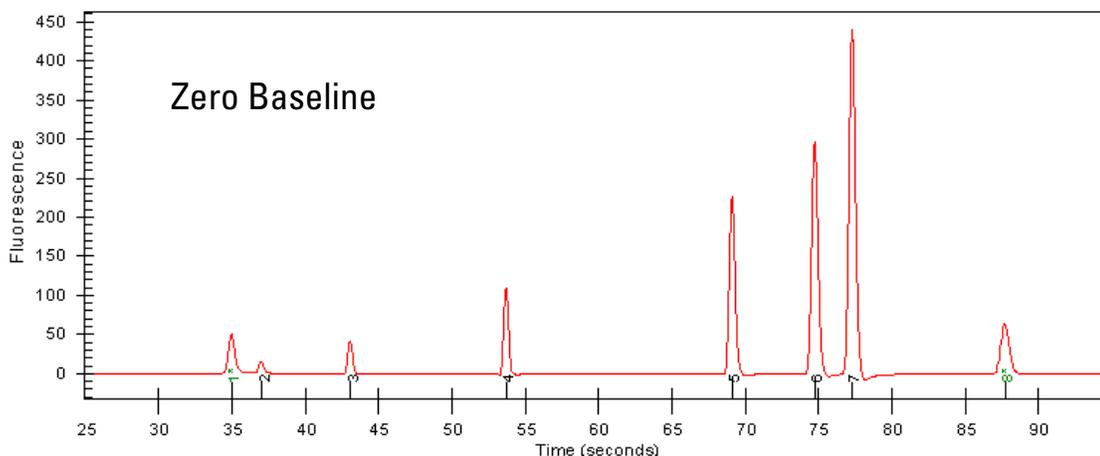
Different gel display colors are available by choosing View > Gel Color and then choosing one of the color schemes from the drop-down menu:

The colors are designed to approximate actual gel staining and imaging techniques. Blue on White, for example, simulates a Coomassie gel often used with proteins. The Pseudo color choice provides more detail (1,280 colors) since it maps the signal into a larger color space than is available with the other monochrome options (256 levels of brightness).



Force Baseline to Zero

Since all electropherograms show some amount of background fluorescence, the Bio Sizing software automatically sets the baseline to zero fluorescence units. To remove the zeroing, select Tools > Options > Advanced and uncheck the Zero Baseline box. .

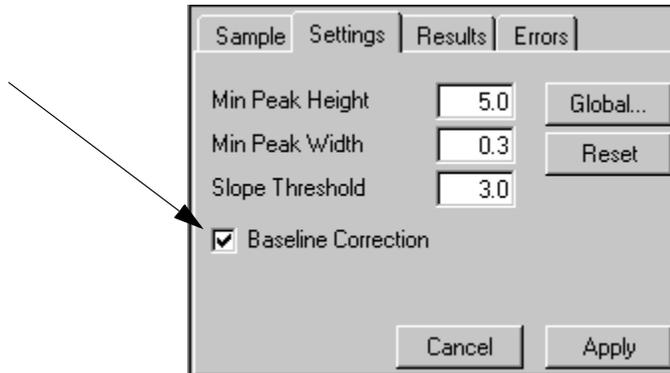


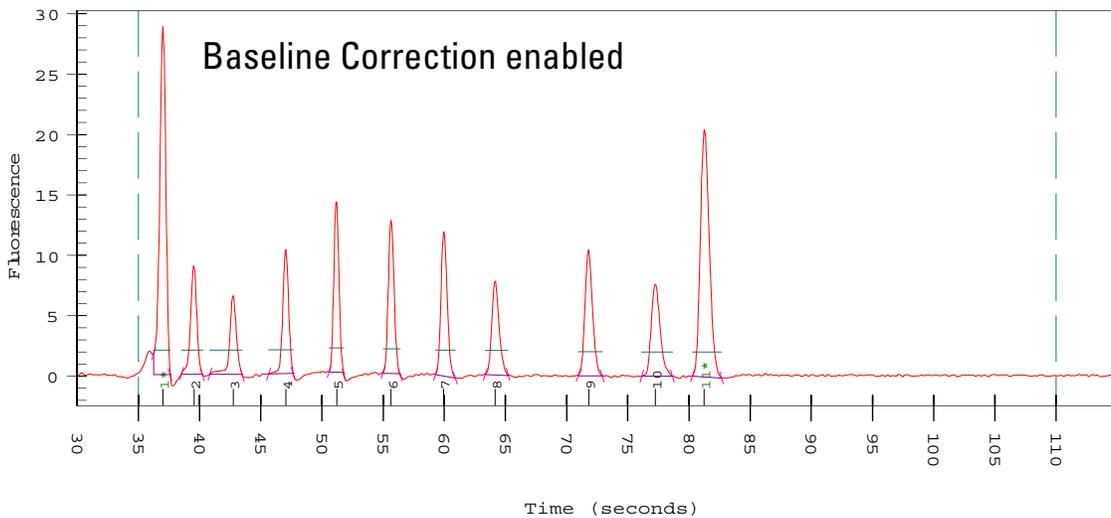
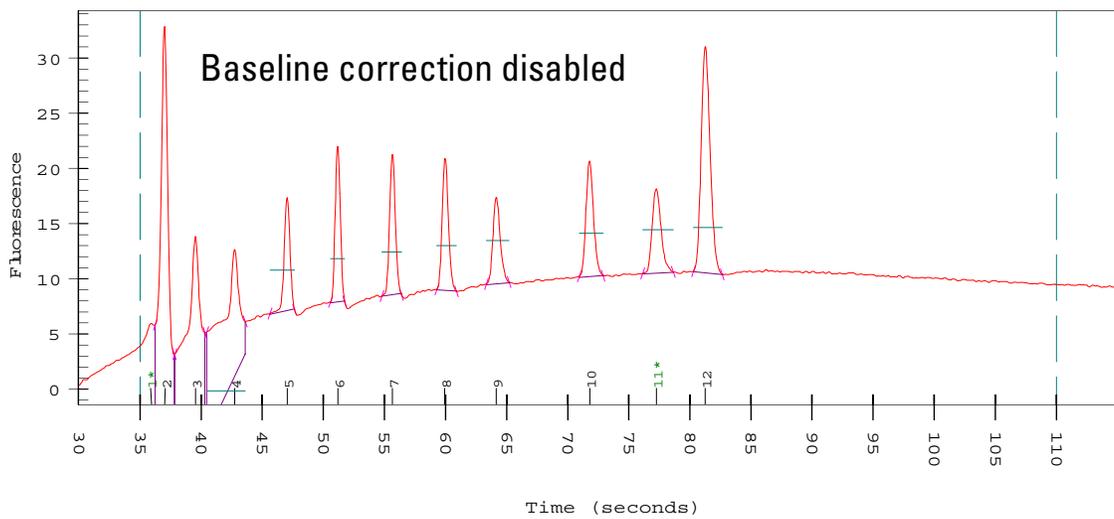
Baseline Correction (Ladder)

The individual sample settings tab for the ladder well in a DNA assay shows a checkbox for Baseline Correction (enabled by default).

In case of bend ladder baselines the baseline correction algorithm sets the baseline to zero fluorescence units.

To apply the baseline correction, label the check box and press the Apply button.





The Results Table

The Results Table appears below the single well view in the large display area. This table provides the following information:

Peak Number	The order in which the peaks were detected.
Mig. Time (seconds)	The migration time is the amount of time from sample injection to the detection of a particular nucleic acid fragment.
Area	The area under the peak. If the sample has been aligned, the area of the aligned peaks is reported.
Size (bp)	The size is the number of calculated DNA base pairs.
Conc (ng/μL)	The concentration in nanograms per microliter for each fragment (derived from the area/conc. relationship with the upper marker, the same for all ladder peaks).
Molarity (nM)	$\text{Molarity} = \frac{\text{Concentration} \times 10^6}{660 \times \text{Size}}$ <p>where: molarity is measured in nanomoles per liter (nmol/L) concentration is measured in nanograms per microliter (ng/μL) size is measured in base pairs (bp).</p>
Observations	Additional information about the peak such as possible comigration or expected fragment indication.

Reanalyzing a Data File

Occasionally you may wish to open and view or reanalyze a data file that was run and saved previously. The raw data values are saved in the data file, along with the analysis settings that were chosen for the run, so that the data can be reanalyzed with different settings.

To do this:

- 1 Click File > Open.
- 2 Choose the filename from the list of data files.
- 3 Click OK. If you have no unsaved data currently open, the chosen file will open, allowing you to view/edit the results. If you have unsaved data open, a dialog box will ask if you want to save the current data first.

The items that can be changed for reanalysis are:

- Global Peak Find settings
- Individual sample peak find settings (chosen in the sample information pane to the right of the Results Table in the single well view window) (see Settings Tab)
- Expected base pair size for certain assays

- Gel color
- Sample names and comments
- Exclude peaks from analysis
- Reassign upper/lower markers
- Alignment or no alignment with ladder peaks
- Use of ladder run with samples or use of internal assay ladder
- Assay (you can save the changed settings under a new assay name, if desired)

NOTE

If you save the data file after making changes, it will keep a record of the assay (if a new assay name has been saved, it will use the settings from this assay the next time the file is opened), gel color, well names, and peak find settings that were in effect at the time the file is resaved. If you don't want to change the original file, choose Save As... and give the file a new name or save it to a different location.

Data Analysis—RNA and Cy5-Labeled Nucleic Acids

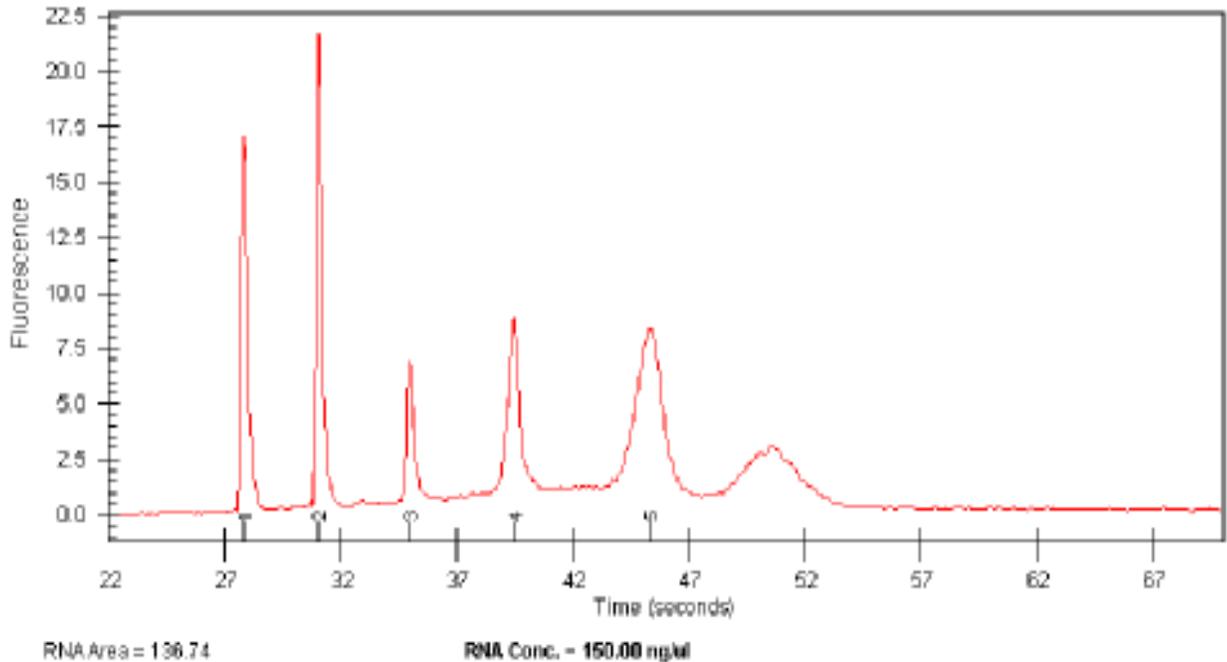
How the Agilent 2100 Bioanalyzer Software Analyzes Data

The purpose of Bio Sizing assays is to calculate the size and/or concentration of nucleic acid fragments. Results are calculated after all data for an individual well has been read.

The data analysis process for RNA and the Cy5-labeled nucleic acids assays consists of the following steps:

- 1** Raw data is read and stored by the system for all the individual wells.
- 2** A software algorithm filters the data and plots the resulting electropherograms of all wells. You can change the settings of the filtering algorithm after the run and reanalyze your data.
- 3** Fragments are identified for all wells and tabulated by migration time. You can change the settings of the peak find algorithm after the run has finished and reanalyze your data.

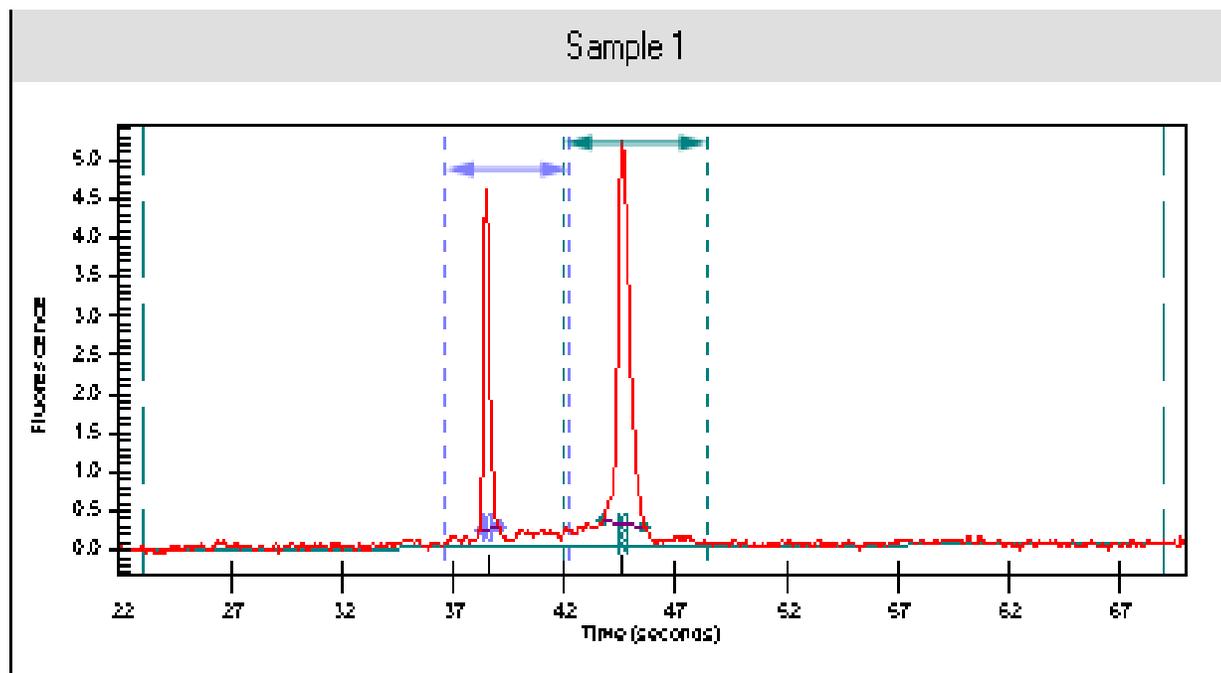
- 4 A Bio Sizing ladder—a mixture of RNA fragments of different size but having the same concentration—is run first from the ladder well (see the electropherograms below). The ladder information is preset in the assay and can't be changed.



Sample RNA ladder (Total RNA)

- 5 For the Eukaryote or Prokaryote Total RNA assay, two time windows (that are determined dynamically based on the ladder run and cannot be changed) are used to assist in detecting the RNA fragments (either 18S and 28S for eukaryotic RNA or 16S and 23S for prokaryotic RNA). These windows are delineated by short-dashed lines shown in the same color as the fragment designator; actual detection occurs

within the window area plus 15% on either side. After detection, the ratio of the fragment areas is calculated and displayed.



- 6 To calculate the concentration of the RNA, the area under the entire RNA electropherogram is determined. The ladder, which provides the concentration/area ratio, is applied to transform the area values into concentration values.

Changing Your Data Analysis—RNA

Changing the Settings of the Data Evaluation Algorithm

Different sets of parameters can be changed in the software, in order to modify the data evaluation for sample analysis:

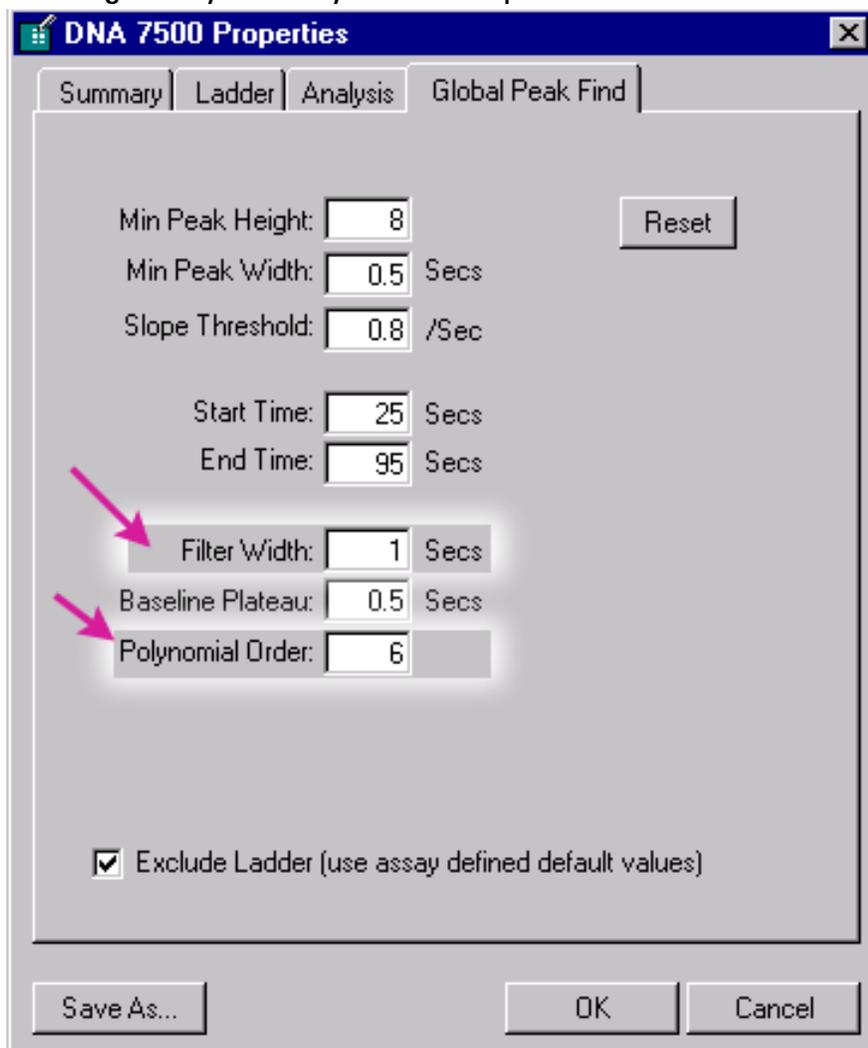
- filtering parameters
- peak find parameters for all wells; peak height for individual wells
- time window for analysis
- setting the baseline
- adding or deleting fragments

Changes can be made before a new run is started or to reanalyze the data from a previous run.

Filtering Parameters

The first step the software takes in analyzing the raw data is to apply data filtering. Highlighted in the figure below are the two filtering parameters that can be changed: Polynomial Order and Filter Width.

One way you can access the Global Peak Find tab of the Assay Properties dialog box is by choosing Assay > Assay Name Properties > Global Peak Find.



The Polynomial Order setting is used to define the power series applied to fit the raw data. The higher the number you set, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.

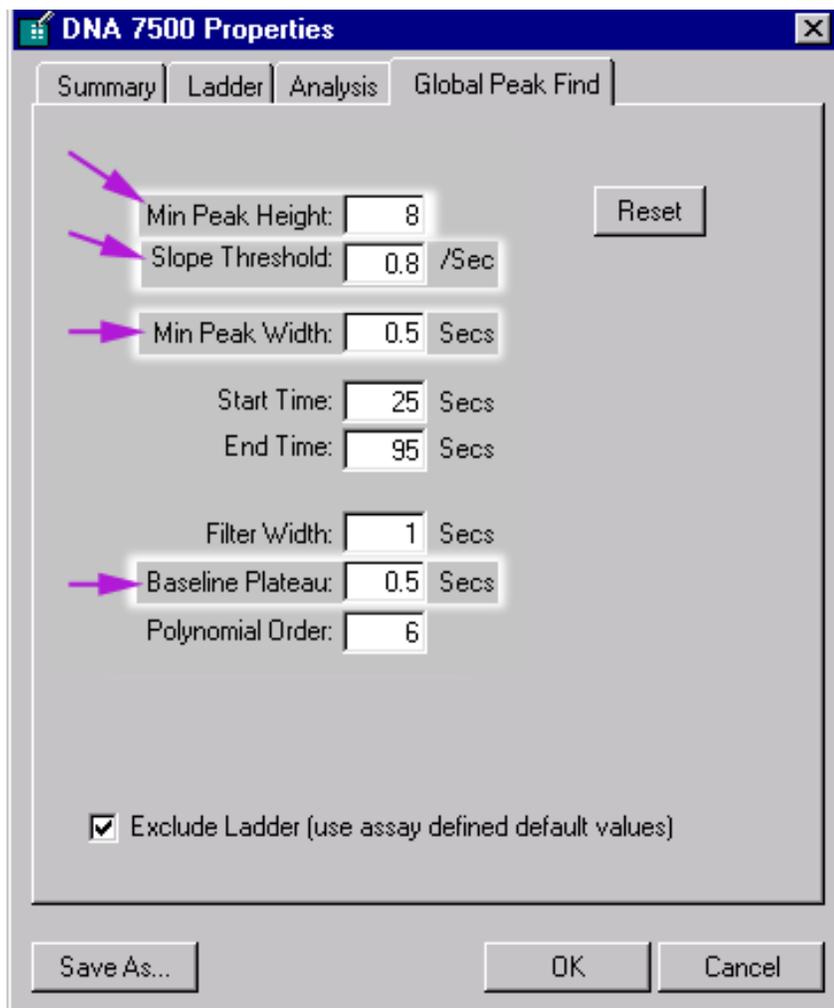
Filter Width defines the data window, given in seconds, used for averaging. The broader the filter width, the more raw data points are used for averaging. As a result, the noise level will decrease but peaks will become lower and broader.

Overall, changing the Filter Width has more effect on the result of the filtering procedure that is applied than does changing the Polynomial Order.

Peak Find Parameters

After data filtering, the Peak Find algorithm locates the peaks and calculates the local peak baselines. The algorithm begins by finding all the peaks above the noise threshold in order to determine the baseline, after which any peaks below the noise threshold are rejected. A local baseline is calculated for each peak to allow for baseline drift.

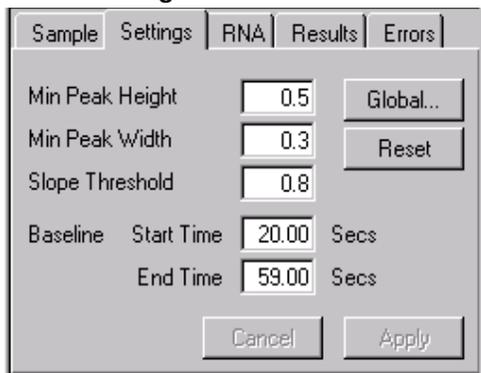
The four peak find parameters that can be changed, Min Peak Height, Slope Threshold, Min Peak Width and Baseline Plateau, are shown below. Choosing Apply set the parameters for all the wells.



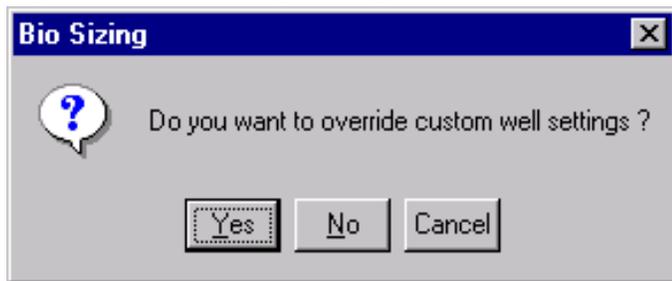
The Reset button sets the Global Peak Find values back to the factory settings.

Min Peak Height	Determines the threshold for the peak find algorithm. For each peak, the difference between the start point value and the center point value (local baseline) must be greater than the Minimum Peak Height value.
Slope Threshold	Determines the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.
Min Peak Width	Determines the minimum amount of time that must have elapsed after threshold was exceeded.
Baseline Plateau	A parameter that assists in finding peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the Slope Threshold setting (either positive or negative) for longer than the time set for the Baseline Plateau. This setting rejects brief, low slope areas such as those found between non-baseline-resolved peaks.
Exclude Ladder	(Default: enabled) This setting causes the Bio Sizing program to use the values defined by the assay for ladder data instead of data obtained from the ladder run with the assay.

You can change all peak find settings except the Baseline Plateau for individual wells. In the lower right pane of the single-well display (to the right of the Results Table) are four tabs. The Settings tab shows the peak find settings that are currently in effect for that well. Changing the settings shown on this tab will affect this well only (to change the settings that affect all wells, click the [Global...] button to open the Assay Properties dialog box and then click the Global Peak Find tab).



If you change the Global peak find settings after making individual well setting changes, the following dialogue box will appear:



Choosing [Yes] causes any changes made to the peak find settings for individual wells to be discarded and applies the global peak find settings to all wells. Choosing [No] allows individual wells to retain changed peak find settings.

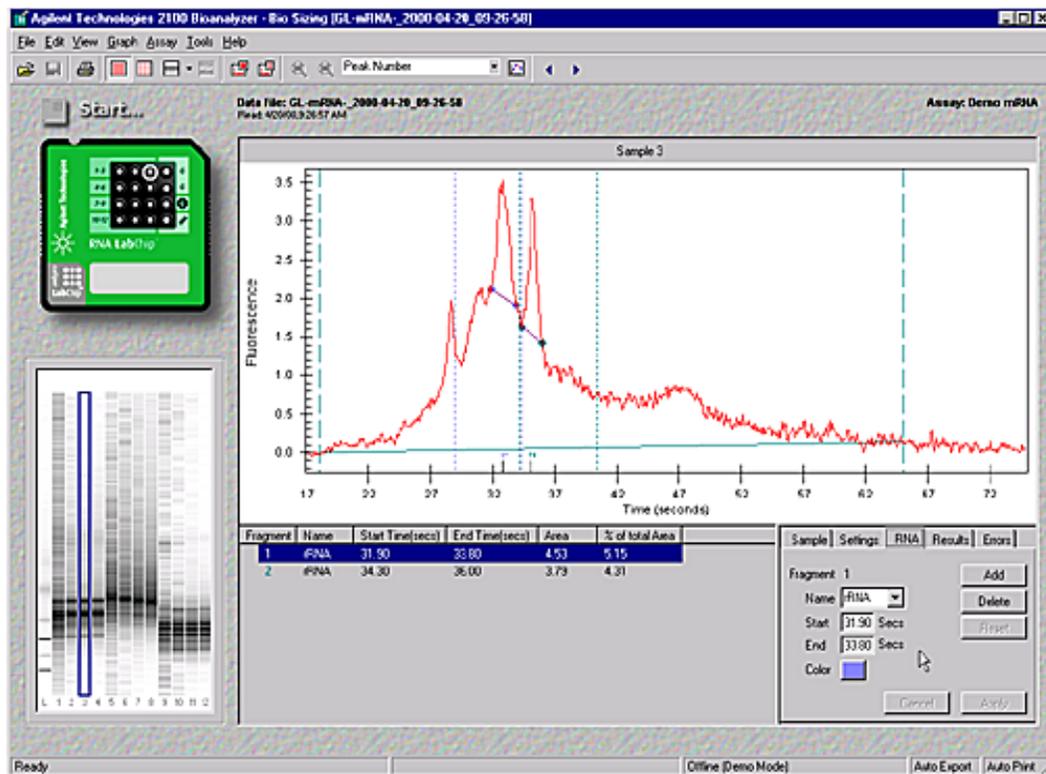
This dialogue appears whenever at least one of the samples has different local settings.

NOTE This dialogue appears whenever at least one of the samples has different local settings.

Manually Moving Peak Start and End Points

It is possible to manually alter the start and end points for individual peaks in an RNA or Cy5-labeled nucleic acids assay. Zooming in on the base of a particular fragment allows you to see the start and end points (color-coded to match the designator shown on the RNA tab of the sample information pane).

Positioning the cursor over one of these points changes the cursor to a pointing hand, allowing you to click and drag the point along the line of the fragment until it is positioned as desired.



Move any other start or end points as desired.

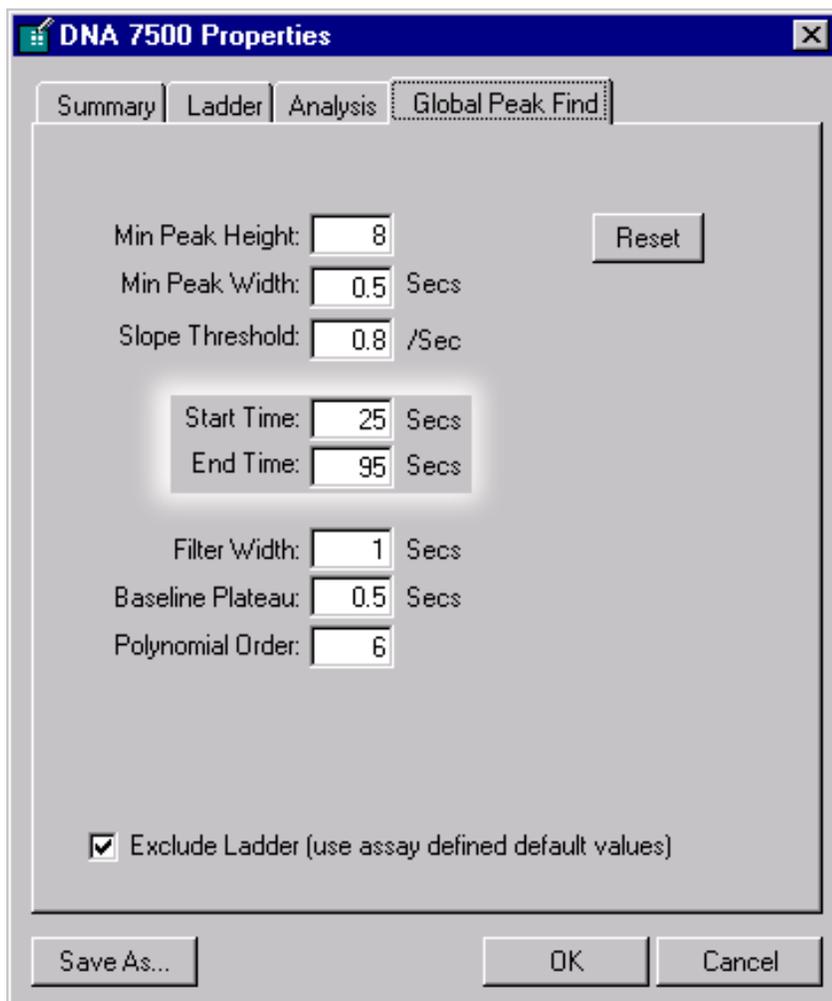
NOTE

Changing the start or end points of the fragment will change the calculated rRNA ratio.

Sample	Settings	RNA	Results	Errors
Corrected RNA Area		156.18		
RNA Concentration		187.81	ng/ul	
rRNA Ratio [28S / 18S]		1.92		

Time Window for Analysis

The Start Time and EndTime parameters on the Global Peak Find tab (see figure below) define the time window within which peaks will be found.



The image shows a software dialog box titled "DNA 7500 Properties" with a close button (X) in the top right corner. The dialog has four tabs: "Summary", "Ladder", "Analysis", and "Global Peak Find", with the "Global Peak Find" tab selected. The main area contains several input fields and a "Reset" button. The "Start Time" field is highlighted with a white glow. At the bottom, there is a checked checkbox labeled "Exclude Ladder (use assay defined default values)" and three buttons: "Save As...", "OK", and "Cancel".

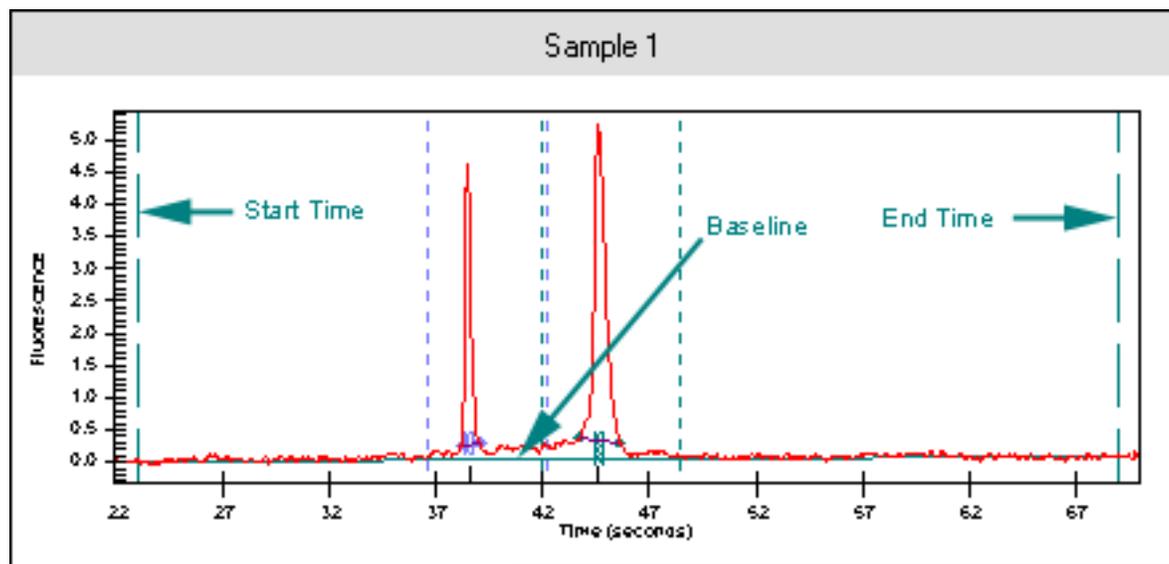
Parameter	Value	Unit
Min Peak Height	8	
Min Peak Width	0.5	Secs
Slope Threshold	0.8	/Sec
Start Time	25	Secs
End Time	95	Secs
Filter Width	1	Secs
Baseline Plateau	0.5	Secs
Polynomial Order	6	

Exclude Ladder (use assay defined default values)

Setting the Baseline for Calculation of RNA Concentration

At low signal-to-noise ratios, the baseline that defines the area used for calculating the concentration of RNA assays is highly dependent on the settings for the Start and End Time. You can adjust the Start and End Times (thereby adjusting the baseline) manually to ensure a good result even at very low signal-to-noise ratios.

Choose a single-well view. Two vertical long-dashed lines indicate the set points for the Start and End times (with the baseline drawn between them) are displayed in the window.



Move the cursor over the left long-dashed line (lower baseline set point) and drag the line to the desired position. Do the same with the right long-dashed line (upper baseline set point) until the baseline is flat.

NOTE

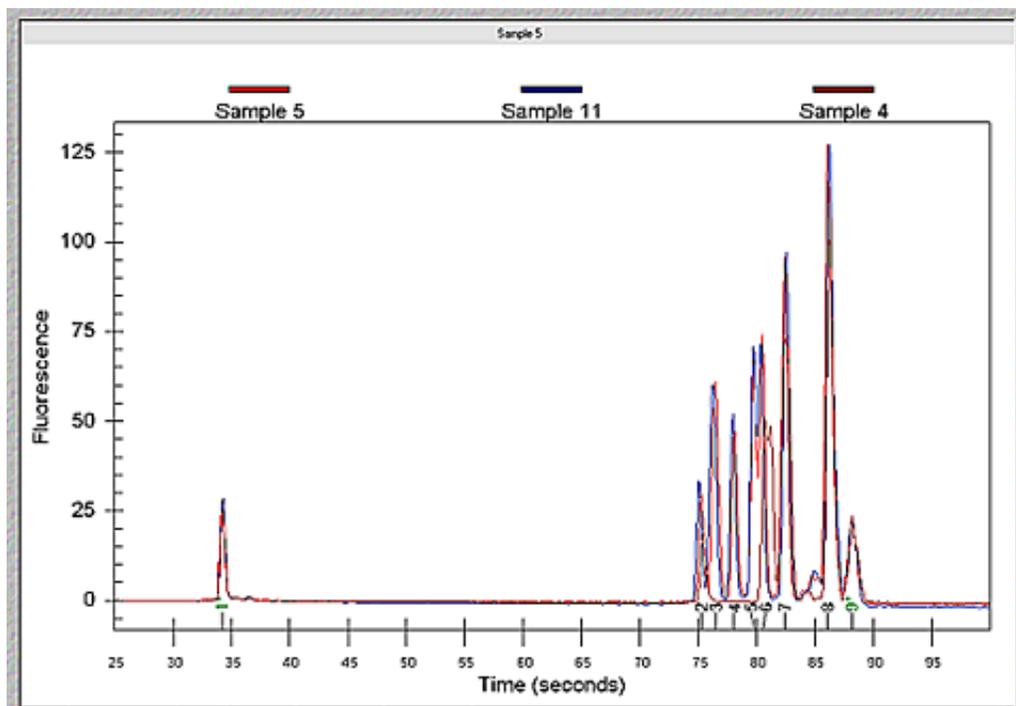
Changing the baseline set point will change the calculated RNA concentration.

Changing the View of the Results

A number of different options are available for viewing the data after it has been acquired by the Agilent 2100 Bioanalyzer. None of these options change the raw data but rather provide different means of viewing the results.

Overlaying Well Graphs

Data from multiple wells can be overlaid within the single-well large display view. Hold down the CTRL key and then click the left mouse button on other lanes in the gel image in the small display.



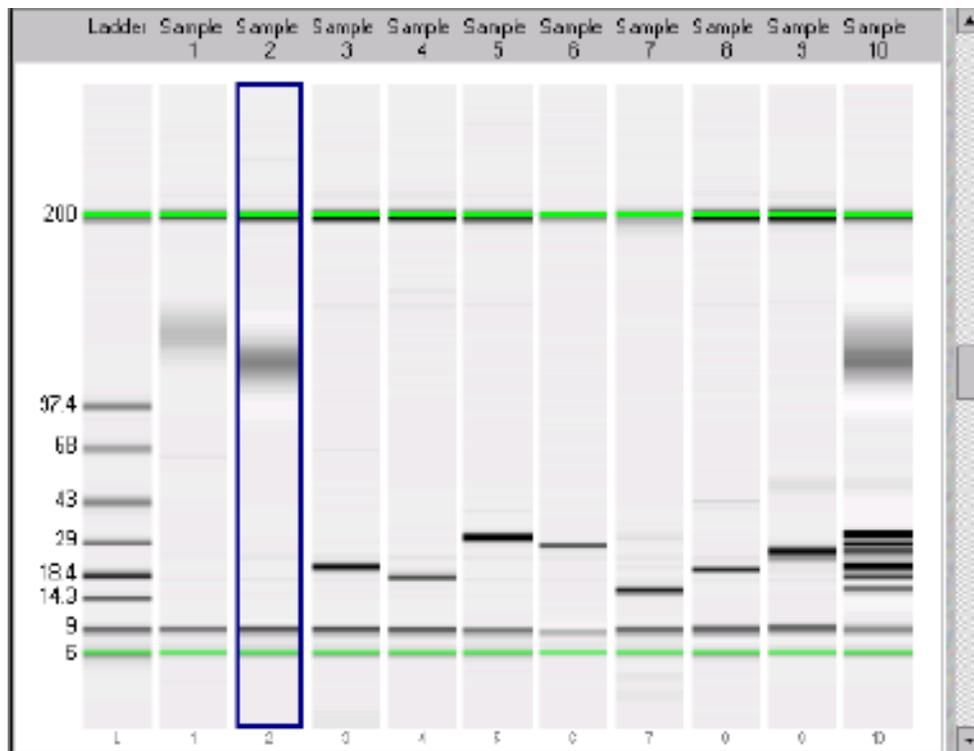
Bounding boxes will appear around the gel lanes signifying which wells are shown overlaid. Each peak graph will be shown in a different color and line style with a legend at the top of the window. You can remove wells from the overlay by CTRL+clicking the corresponding lane in the small gel display (the bounding box will disappear).

A stand-alone program called " Data Evaluation" is also included with the Bio Sizing software and can be accessed by choosing Tools > Compare Results....

This program allows you to compare the results from the same or different runs and even different assays within a single window and provides tools that allow you to manipulate the comparison of the data in different ways. Documentation and help for the Data Evaluation program are available within that program.

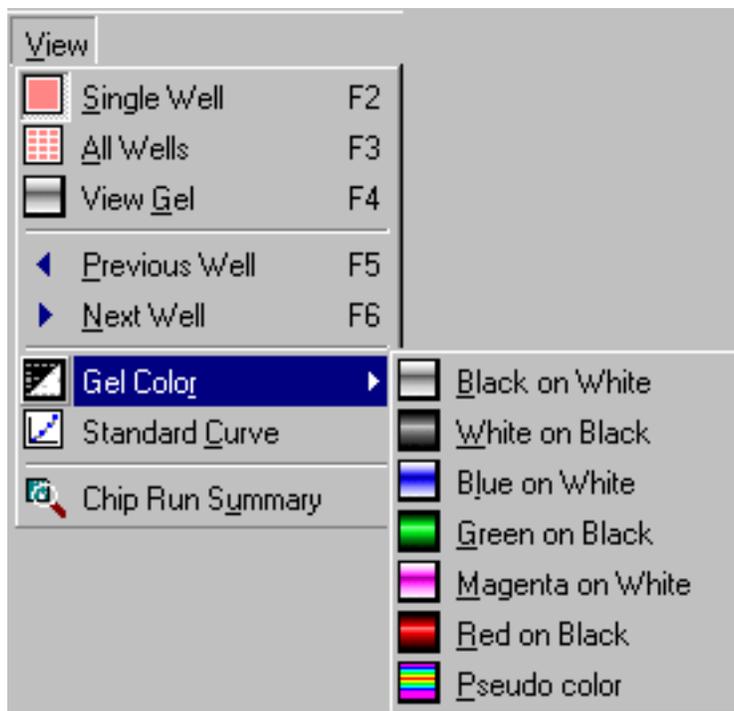
Changing to Gel View

To see an overview of you data in a gel-like image, switch to the Gel view. In the menu bar, click on View > View Gel. The main window will change and display the results in a format as would be generated by a slab gel device.



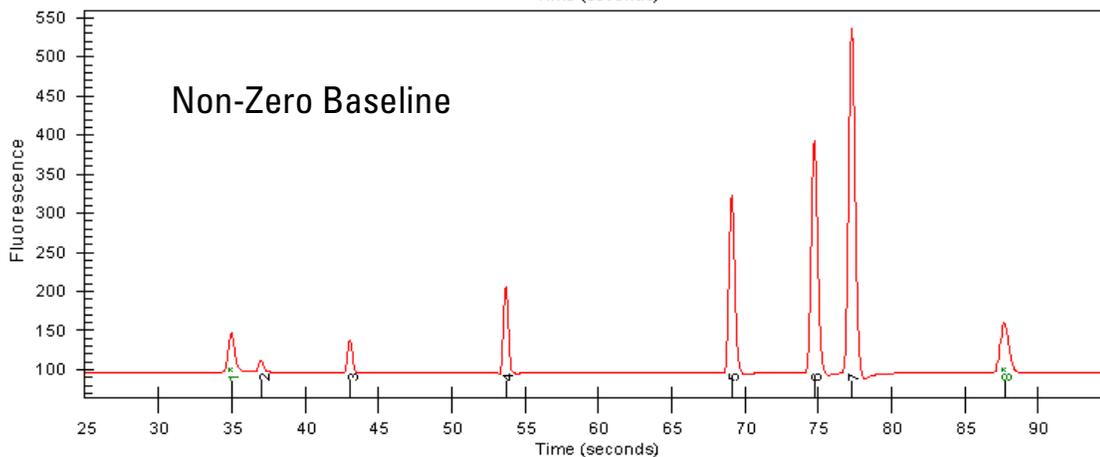
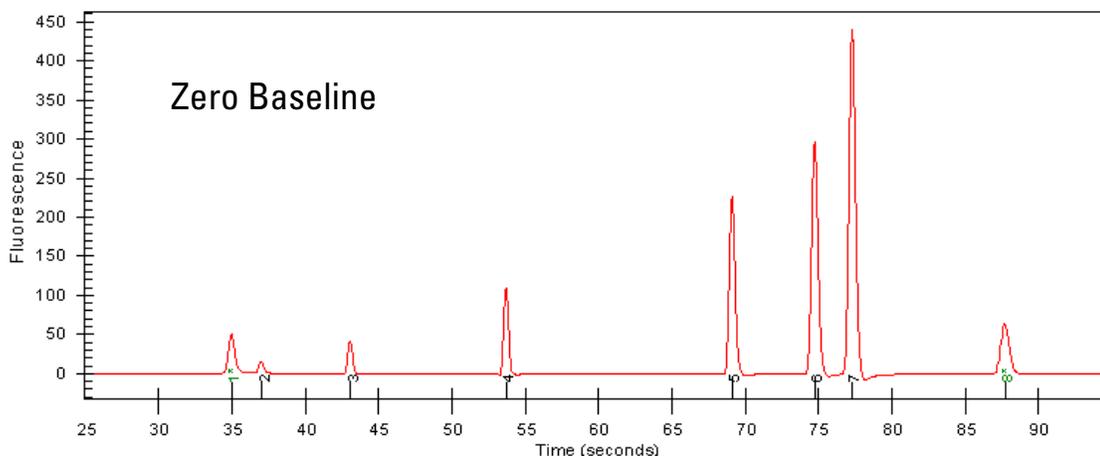
Different gel display colors are available by choosing View > Gel Color and then choosing one of the color schemes from the drop-down menu:

The colors are designed to approximate actual gel staining and imaging techniques. Blue on White, for example, simulates a Coomassie gel often used with proteins. The Pseudo color choice provides more detail (1,280 colors) since it maps the signal into a larger color space than is available with the other monochrome options (256 levels of brightness).



Force Baseline to Zero

Since all electropherograms show some amount of background fluorescence, the Bio Sizing software automatically sets the baseline to zero fluorescence units. To remove the zeroing, select Tools > Options > Advanced and uncheck the Zero Baseline box.



The Results Table

The Results Table appears below the single well view in the large display area. This table provides the following information:

Fragment Number	The order in which the fragments were detected.
Fragment Name	A user-assigned or predefined name for the found fragment. Typically 16S/23S for Prokaryote assays or 18S/28S for Eukaryote assays.
Start Time (secs)	Shows the start time for the peak. The start and end times are also represented on the electropherogram by diamond-shaped points on the peak baseline in the same color as that shown in the RNA tab. Dragging a diamond will change the start or end time and alter the baseline drawn between the diamond markers.
End Time (secs)	Shows the end time for the peak. The start and end times are also represented on the electropherogram by diamond-shaped points on the peak baseline in the same color as that shown in the RNA tab. Dragging a diamond will change the start or end time and alter the baseline drawn between the diamond markers.
Area	The area of the individual fragment measured in base pairs.
% of Total Area	The percentage of the area of the individual fragment compared to the total area or RNA measured above the baseline.
Observations	Additional information about the fragment.

Reanalyzing a Data File

Occasionally you may wish to open and view or reanalyze a data file that was run and saved previously. The raw data values are saved in the data file, along with the analysis settings that were chosen for the run, so that the data can be reanalyzed with different settings.

To do this:

- 1 Click File > Open.
- 2 Choose the filename from the list of data files.
- 3 Click OK. If you have no unsaved data currently open, the chosen file will open, allowing you to view/edit the results. If you have unsaved data open, a dialog box will ask if you want to save the current data first.

The items that can be changed for reanalysis are:

- Global Peak Find settings
- Individual sample peak find settings (chosen in the sample information pane to the right of the Results Table in the single well view window) (see Settings Tab)
- Gel color
- Sample names and comments
- Fragment names and colors associated with labels
- Fragment start/end times, additional peaks (or delete peak)

- Alignment or no alignment with ladder peaks
- Use of ladder run with samples or use of internal assay ladder
- Assay (you can save the changed settings under a new assay name, if desired)

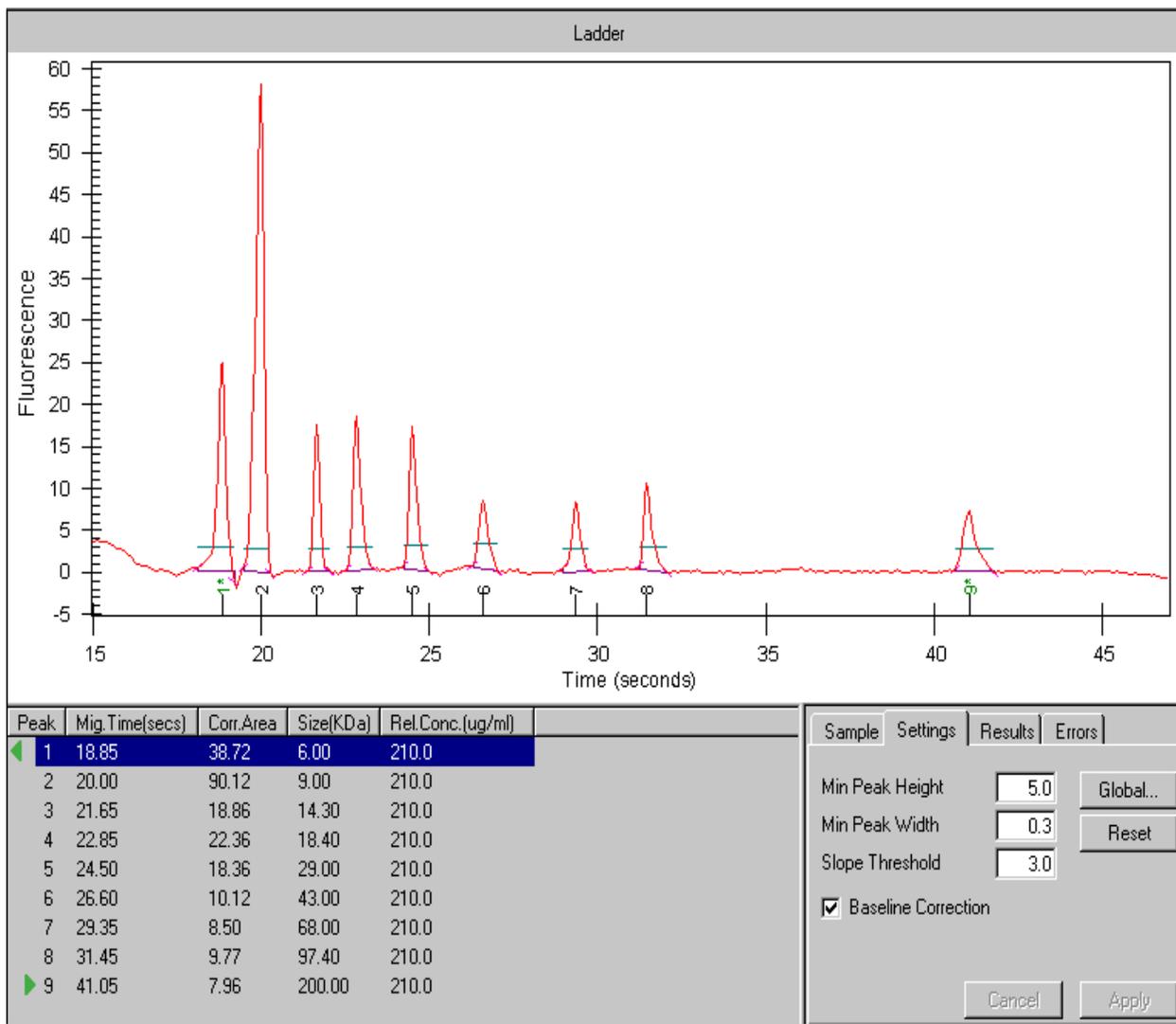
NOTE

If you save the data file after making changes, it will keep a record of the assay in use (if a new assay name has been saved, it will use the settings from this assay the next time the file is opened), gel color, well names, and peak find settings that were in effect at the time the file is resaved. If you don't want to change the original file, choose Save As... and give the file a new name or save it to a different location.

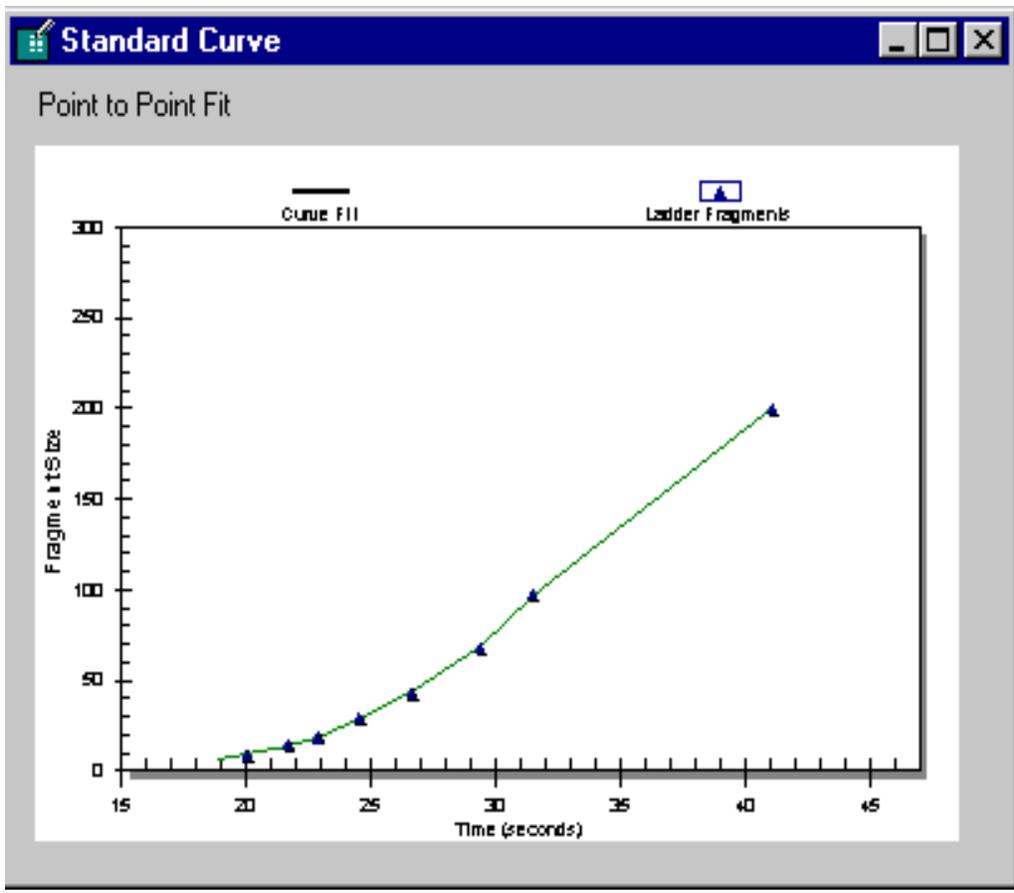
Data Analysis—Protein

The data analysis process for Protein assays consists of the following steps:

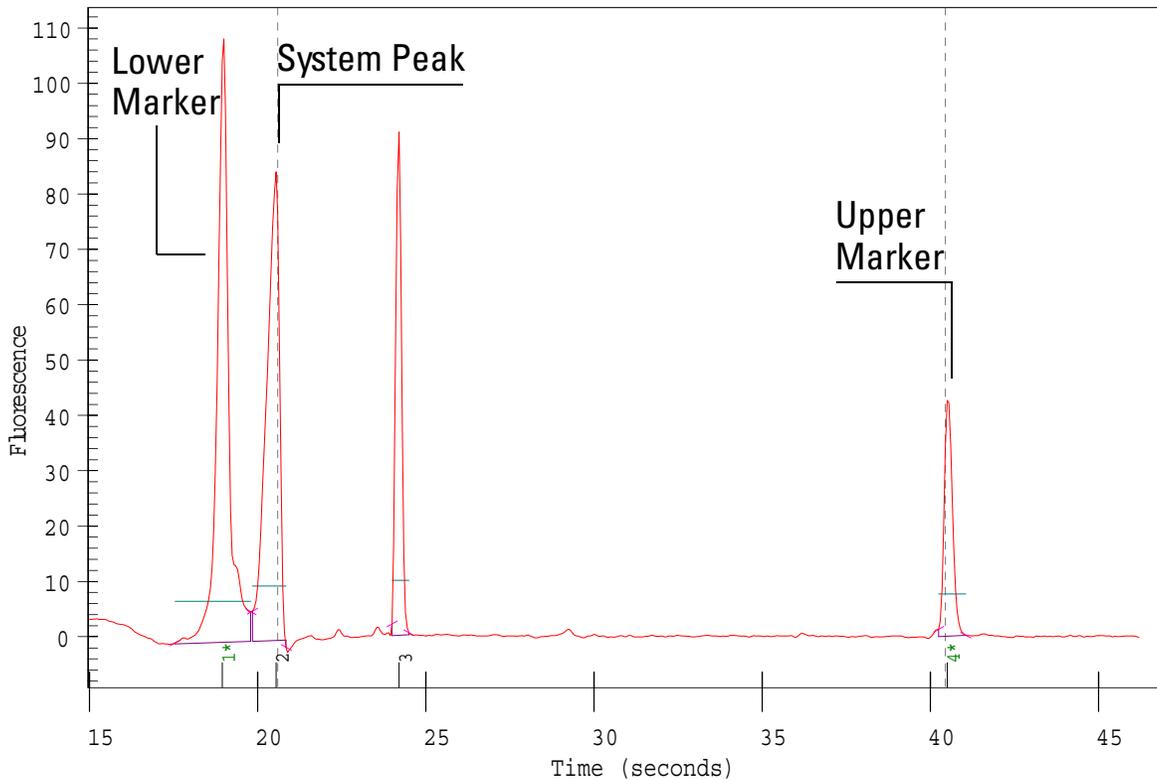
1. Raw data is read and stored by the system for all of the individual wells.
2. The data is filtered and the resulting electropherograms of all wells are plotted. You can change the settings of the filtering algorithm after the run and reanalyze your data.
3. Peaks are identified for all wells and are tabulated by migration time. You can change the settings of the peak find algorithm and reanalyze the data after the run has finished. (Note that peak find settings can be changed for all or only certain wells.)
4. A sizing ladder (see the example electropherogram below), which is a mixture of protein fragments of different sizes, is run first from the ladder well. The concentrations and sizes of the individual base pairs are preset in the assay and cannot be changed



5. A standard curve of migration time versus size is plotted from the sizing ladder by interpolation between the individual fragment size/migration points. The standard curve derived from the data of the ladder well should resemble the one shown below.



6. Two protein fragments are run with each of the samples, bracketing the sizing range. The lower marker peak is followed by the system peak. The "lower marker" and "upper marker" are internal standards used to align the ladder data with data from the sample wells. The figure below shows an example of assigned marker peaks in a sample well.



NOTE

The software performs alignment by default. Turning alignment off suspends data evaluation until you turn it on again.

7. The standard curve, in conjunction with the markers, is used to calculate fragment sizes for each well from the migration times measured.

8. To calculate the concentration of the individual fragments in all sample wells, the upper marker, in conjunction with an assay-specific calibration curve, is applied to the individual sample peaks in all sample wells.

NOTE The software allows you to define upper and lower markers yourself. However, a change in the selection of the markers will lead to quantitative changes of the calibration procedure and will, therefore, alter the entire data evaluation.

Changing Your Data Analysis—Protein

Changing the Settings of the Data Evaluation Algorithm

Different sets of parameters can be changed in the software in order to modify the data evaluation for sample analysis:

- filtering parameters
- peak find parameters for all wells; peak height for individual wells
- time window for analysis
- assigning upper and lower marker peaks
- aligning or unaligning marker peaks

Changes can be made before a new run is started or to reanalyze the data from a previous run.

Filtering Parameters

The first step the software takes in analyzing the data is to apply data filtering. Highlighted in the figure below are the two filtering parameters that can be changed: Polynomial Order and Filter Width.

You can access the Global Peak Find settings in the software by going to: Assay > Assay Properties and choosing the Global Peak Find tab.

The screenshot shows the 'DNA 7500 Properties' dialog box with the 'Global Peak Find' tab selected. The dialog has four tabs: 'Summary', 'Ladder', 'Analysis', and 'Global Peak Find'. The 'Global Peak Find' tab contains the following settings:

- Min Peak Height: 8
- Min Peak Width: 0.5 Secs
- Slope Threshold: 0.8 /Sec
- Start Time: 25 Secs
- End Time: 95 Secs
- Filter Width: 1 Secs
- Baseline Plateau: 0.5 Secs
- Polynomial Order: 6

A 'Reset' button is located to the right of the 'Min Peak Height' field. At the bottom of the dialog, there is a checkbox labeled 'Exclude Ladder (use assay defined default values)' which is checked. The dialog also features 'Save As...', 'OK', and 'Cancel' buttons at the bottom.

Two pink arrows point to the 'Filter Width' and 'Polynomial Order' fields.

The Polynomial Order setting is used to define the power series applied to fit the raw data. The higher the number you set, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.

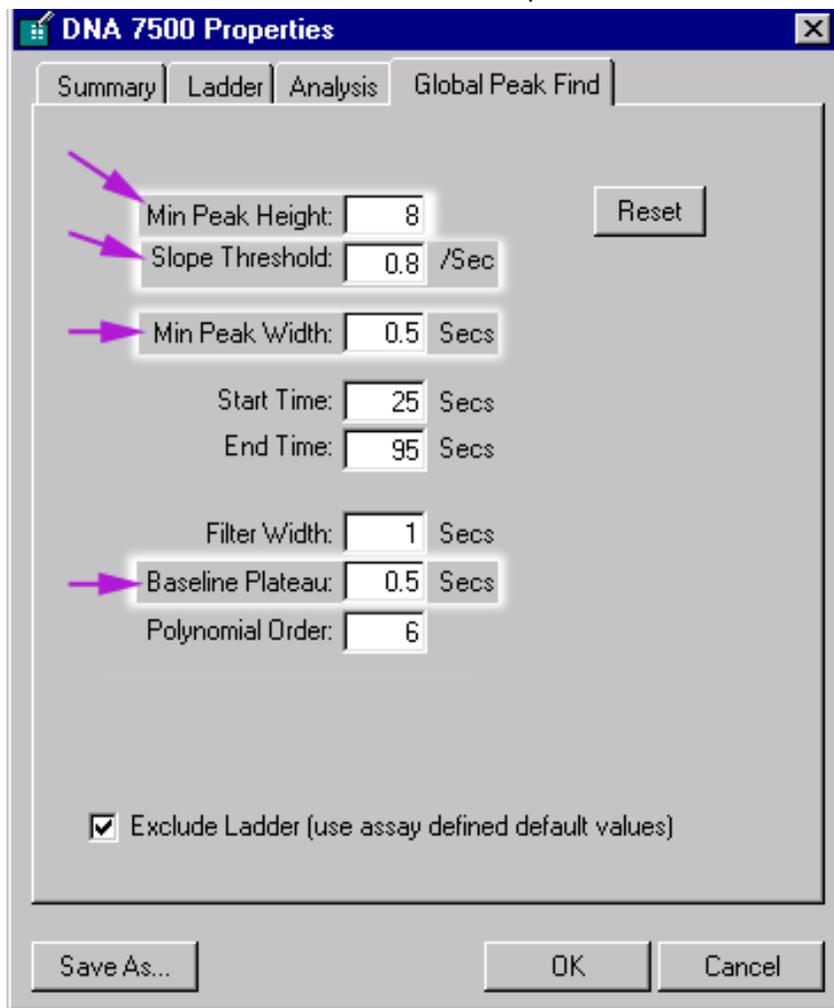
Filter Width defines the data window, given in seconds, used for averaging. The broader the filter width, the more raw data points are used for averaging. As a result, the noise level will decrease but peaks will become lower and broader.

Overall, changing the Filter Width has more effect on the result of the filtering procedure that is applied than does changing the Polynomial Order.

Peak Find Parameters

After data filtering, the Peak Find algorithm locates the peaks and calculates the local peak baselines. The algorithm begins by finding all the peaks above the noise threshold in order to determine the baseline, after which any peaks below the noise threshold are rejected. A local baseline is calculated for each peak to allow for baseline drift.

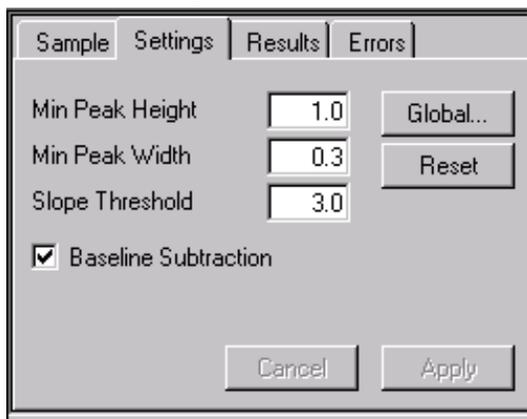
The four peak find parameters that can be changed, Min Peak Height, Slope Threshold, Min Peak Width and Baseline Plateau, are shown below.



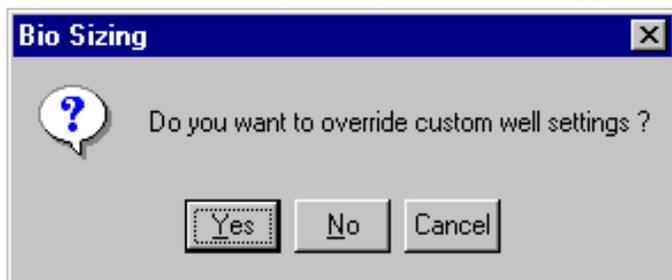
The Reset button sets the Global Peak Find values back to the factory settings.

Min Peak Height	Determines the threshold for the peak find algorithm. For each peak, the difference between the start point value and the center point value (local baseline) must be greater than the Minimum Peak Height value.
Slope Threshold	Determines the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.
Min Peak Width	Determines the minimum amount of time that must have elapsed after threshold was exceeded.
Baseline Plateau	A parameter that assists in finding peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the Slope Threshold setting (either positive or negative) for longer than the time set for the Baseline Plateau. This setting rejects brief, low slope areas such as those found between non-baseline-resolved peaks.
Exclude Ladder	(Default: enabled) This setting causes the Bio Sizing program to use the values defined by the assay for ladder data instead of data obtained from the ladder run with the assay.

You can change all peak find settings except the Baseline Plateau for individual wells. In the lower right pane of the single-well display (to the right of the Results Table) are four tabs. The Settings tab shows the peak find settings that are currently in effect for that well. Changing the settings shown on this tab will affect this well only (to change the settings that affect all wells, click the [Global...] button to open the Assay Properties dialog box and then click the Global Peak Find tab).



If you change the Global peak find settings after making individual well setting changes, the following dialogue box will appear:



Choosing [Yes] causes any changes made to the peak find settings for individual wells to be discarded and applies the global peak find settings to all wells. Choosing [No] allows individual wells to retain changed peak find settings.

NOTE This dialogue appears whenever at least one of the samples has different local settings.

Time Window for Analysis

The Start Time and EndTime parameters on the Global Peak Find tab (see figure below) define the time window within which peaks will be found.

The screenshot shows the 'DNA 7500 Properties' dialog box with the 'Global Peak Find' tab selected. The dialog has four tabs: 'Summary', 'Ladder', 'Analysis', and 'Global Peak Find'. The 'Global Peak Find' tab contains the following settings:

- Min Peak Height: 8
- Min Peak Width: 0.5 Secs
- Slope Threshold: 0.8 /Sec
- Start Time: 25 Secs
- End Time: 95 Secs
- Filter Width: 1 Secs
- Baseline Plateau: 0.5 Secs
- Polynomial Order: 6

A 'Reset' button is located to the right of the Min Peak Height field. At the bottom of the dialog, there is a checkbox labeled 'Exclude Ladder (use assay defined default values)' which is checked. Below the dialog are three buttons: 'Save As...', 'OK', and 'Cancel'.

Assigning Upper and Lower Marker Peaks

For each sample, the upper and lower marker peaks are assigned first and then the data is aligned so that the well markers match the ladder markers in time. This allows the size and concentration of the sample peaks to be determined.

The first peak is assigned to be the lower marker and is then offset to match the lower marker in the ladder. The upper marker is then assigned either to the last peak in the sample well or to the peak nearest to the ladder's upper marker. See **Aligning or Unaligning the Marker Peaks—92** for an example of assigned marker peaks.

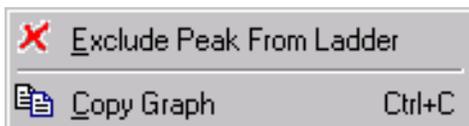
If you see unexpected peaks in the ladder analysis or the markers are set incorrectly, you can exclude peaks manually from the ladder or choose a peak to be used as a marker.

NOTE

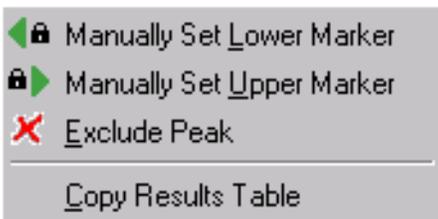
Excluding a peak or manually setting a peak to be an upper or lower marker may cause errors in analysis.

You can move the boundary between the Results Table and the well graph up or down to increase or reduce the amount of space allotted to the Results Table, making it possible to see all of the results at once.

Right-clicking in the result table of a *ladder* well causes this pop-up menu to appear:

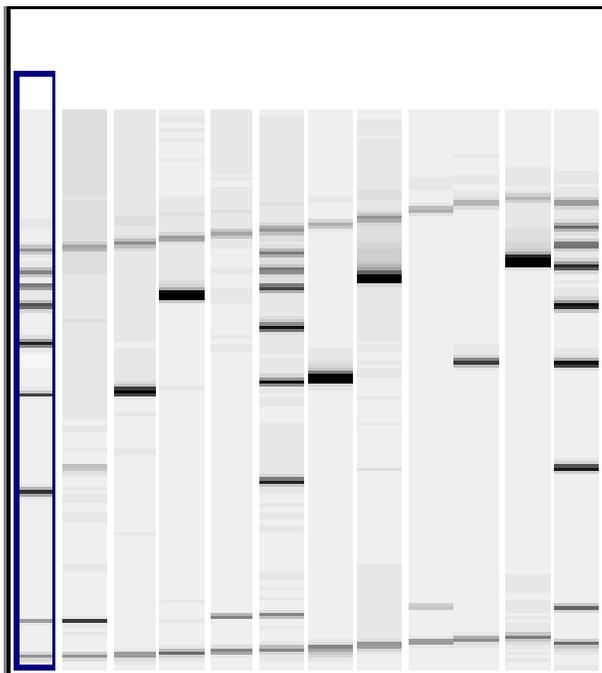


Right-clicking in the result table of a *sample* well causes this pop-up menu to appear:

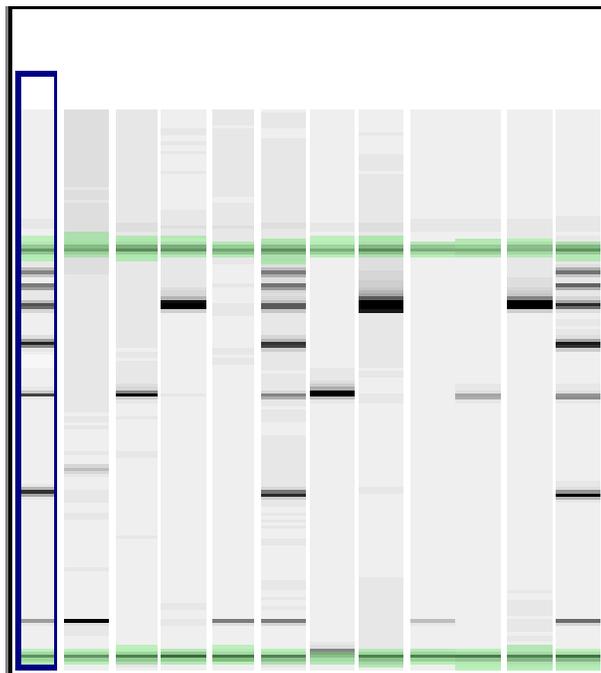


Aligning or Unaligning the Marker Peaks

The upper and lower markers are then aligned to the ladder markers by re-sampling the well data in a linear stretch or compression using a point to point fit.



Data before alignment



Markers aligned to ladder

If the sample marker peaks are either more than twice as far apart or less than half as far apart as the ladder markers, they are assumed to be the wrong peaks and analysis of the well stops, producing the error "Marker peaks not detected."

NOTE

With Protein assays, the height of marker peaks is assay dependent. Ladder peaks are analyzed to calculate a marker peak threshold which is used to locate the marker peaks in the sample wells. If the marker peaks found using this calculated method fail to align with those of a sample, the Bio Sizing software will use the minimum peak height threshold setting instead (if this value is lower than the value for the marker peak).

For example, the calculated threshold might be too high to find the sample's markers if they happen to be very small. Either no markers will be found or the wrong peaks will be assumed to be markers and these may not align with the ladder markers.

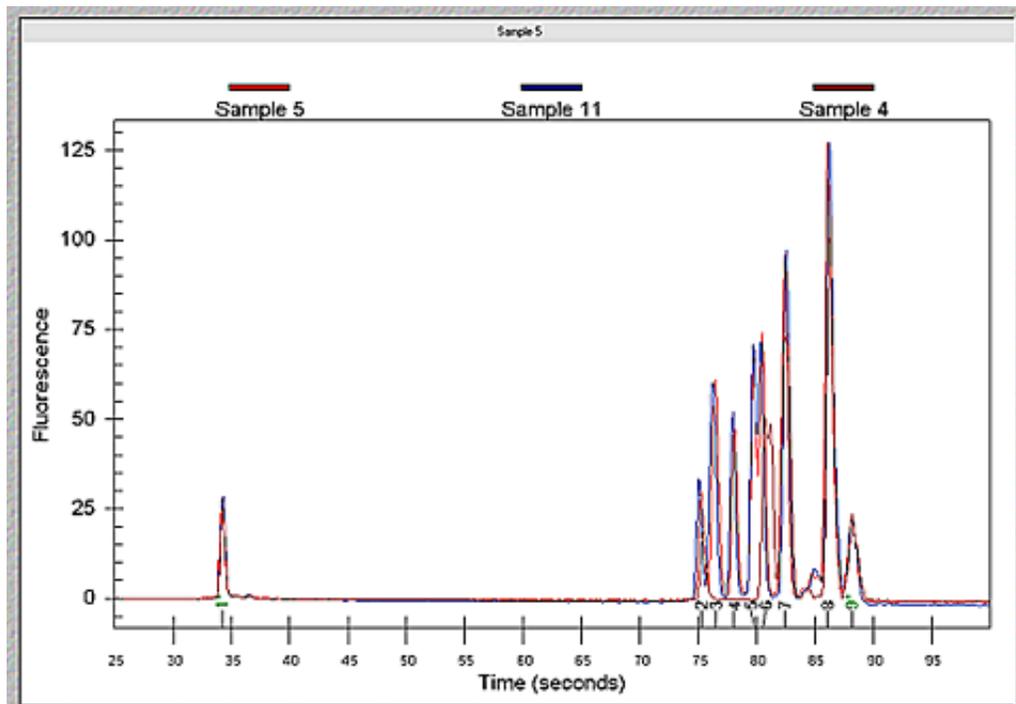
Consequently, the software attempts to use the minimum peak height threshold which, if it is set low enough, will locate the real markers, allowing the sample to align.

Changing the View of the Results

A number of different options are available for viewing the data after it has been acquired by the Agilent 2100 Bioanalyzer. None of these options change the raw data but rather provide different means of viewing the results.

Overlaying Well Graphs

Data from multiple wells can be overlaid within the single-well large display view. Hold down the CTRL key and then click the left mouse button on other lanes in the gel image in the small display.



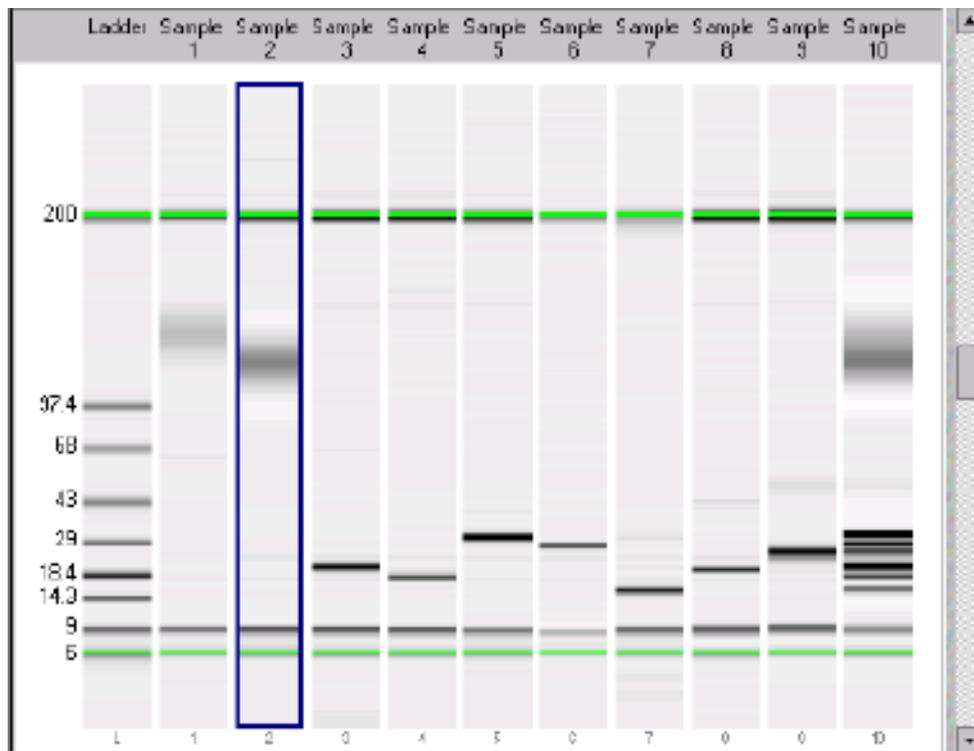
Bounding boxes will appear around the gel lanes signifying which wells are shown overlaid. Each peak graph will be shown in a different color and line style with a legend at the top of the window. You can remove wells from the overlay by CTRL+clicking the corresponding lane in the small gel display (the bounding box will disappear).

A stand-alone program called " Data Evaluation" is also included with the Bio Sizing software and can be accessed by choosing Tools > Compare Results....

This program allows you to compare the results from the same or different runs and even different assays within a single window and provides tools that allow you to manipulate the comparison of the data in different ways. Documentation and help for the Data Evaluation program are available within that program.

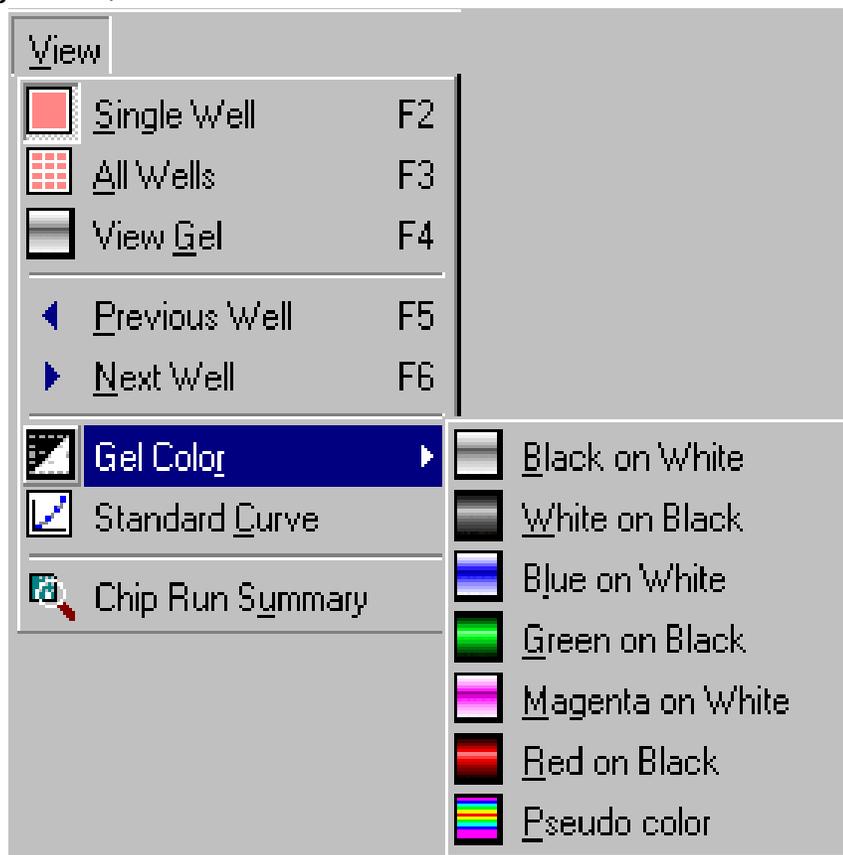
Changing to Gel View

To see an overview of your data in a gel-like image, switch to the Gel view. In the menu bar, click on View > View Gel. The main window will change and display the results in a format as would be generated by a slab gel device.



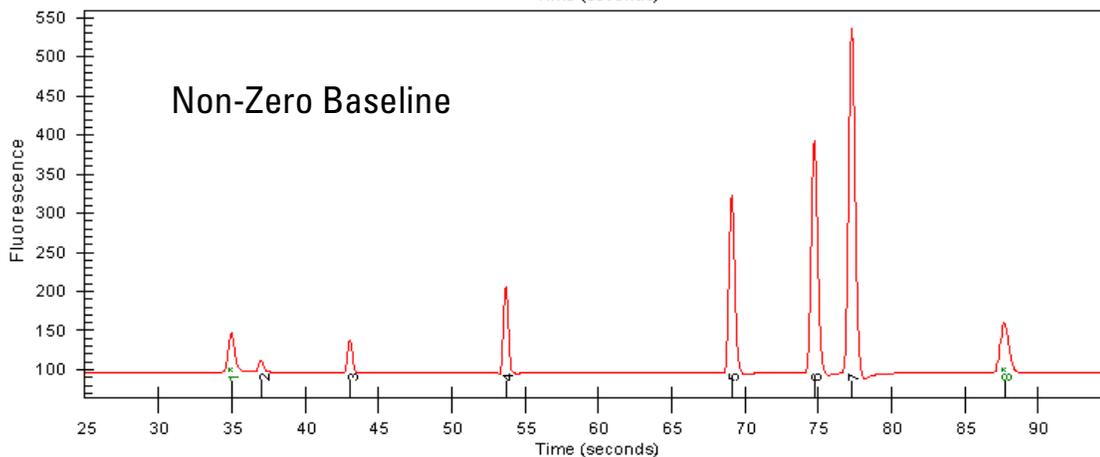
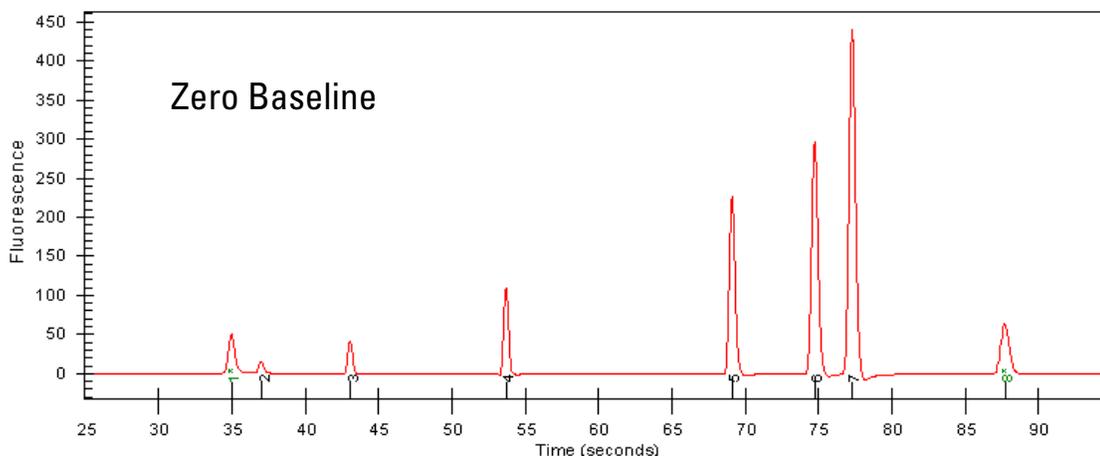
Different gel display colors are available by choosing View > Gel Color and then choosing one of the color schemes from the drop-down menu:

The colors are designed to approximate actual gel staining and imaging techniques. Blue on White, for example, simulates a Coomassie gel often used with proteins. The Pseudo color choice provides more detail (1,280 colors) since it maps the signal into a larger color space than is available with the other monochrome options (256 levels of brightness).



Force Baseline to Zero

Since all electropherograms show some amount of background fluorescence, the Bio Sizing software automatically sets the baseline to zero fluorescence units. To remove the zeroing, select Tools > Options > Advanced and uncheck the Zero Baseline box. .

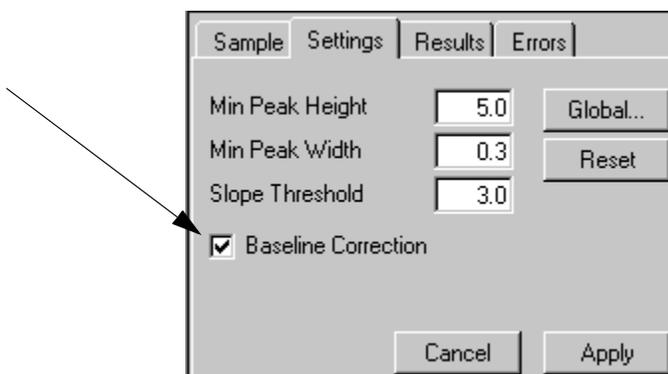


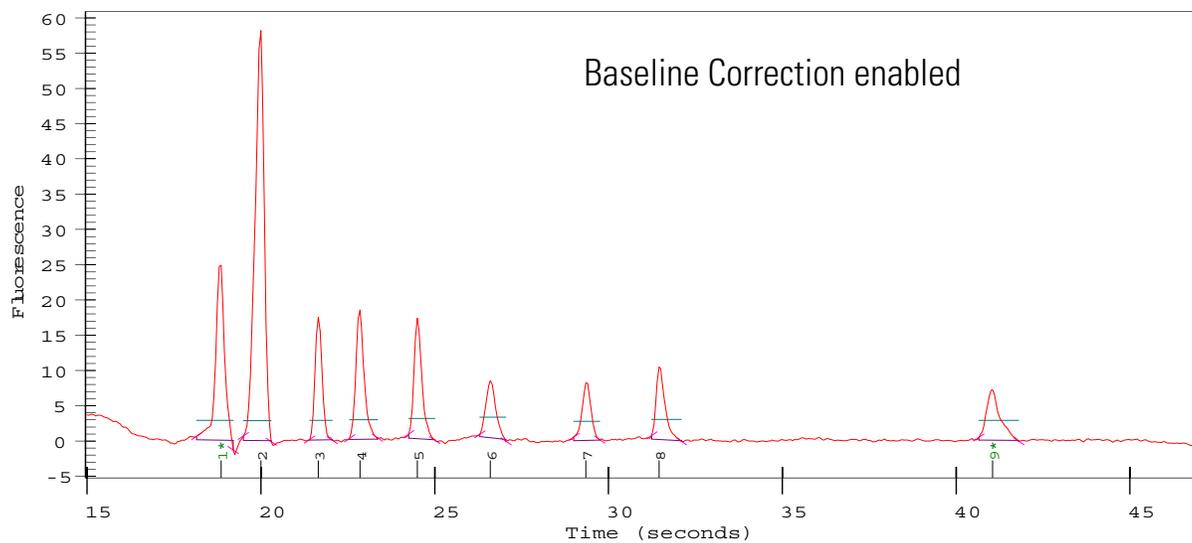
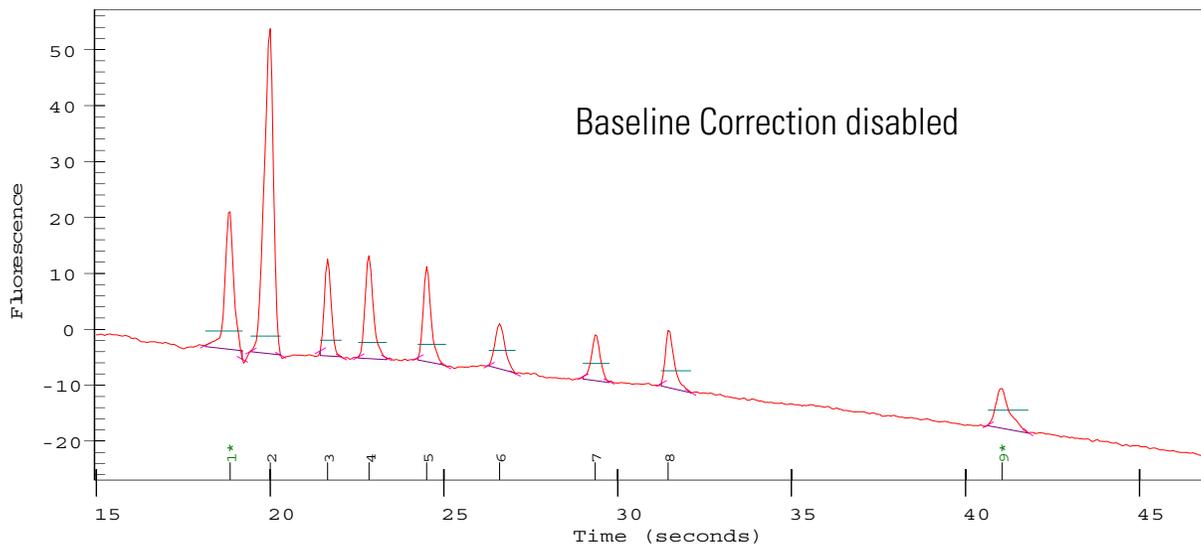
Baseline Correction (Ladder and Samples)

The individual sample settings tab for the ladder and sample wells in a Protein assay shows a checkbox for Baseline Correction (enabled by default).

In case of bend ladder baselines the baseline correction algorithm sets the baseline to zero fluorescence units.

To apply the baseline correction, label the check box and press the Apply button.





The Results Table

The Results Table appears below the single well view in the large display area. This table provides the following information:

Peak Number	The order in which the peaks were detected.
Mig. Time (seconds)	The migration time is the amount of time from sample injection to the detection of a particular nucleic acid fragment.
Corr. Area	The area under the peak is corrected as a result of baseline subtraction.
Size (kDa)	The peak size measured in kiloDaltons.
Relative Conc (ng/mL)	Because of differences in their structure and the manner in which dye binds, the concentration of proteins will vary. Measured in nanograms per milliliter, derived from the area/conc. relationship with the upper marker.
% Total	The percentage of the area of the individual peak compared to the summed total area of all peaks in the sample.
Observations	Additional information about the peak.

Reanalyzing a Data File

Occasionally you may wish to open and view or reanalyze a data file that was run and saved previously. The raw data values are saved in the data file, along with the analysis settings that were chosen for the run, so that the data can be reanalyzed with different settings.

To do this:

- 1 Click File > Open.
- 2 Choose the filename from the list of data files.
- 3 Click OK. If you have no unsaved data currently open, the chosen file will open, allowing you to view/edit the results. If you have unsaved data open, a dialog box will ask if you want to save the current data first.

The items that can be changed for reanalysis are:

- Global Peak Find settings
- Individual sample peak find settings (chosen in the sample information pane to the right of the Results Table in the single well view window) (see Settings Tab)
- Gel color
- Sample names and comments
- Exclude peaks from analysis
- Reassign upper/lower markers

- Alignment or no alignment with ladder peaks
- Use of ladder run with samples or use of internal assay ladder
- Assay (you can save the changed settings under a new assay name, if desired)

NOTE

If you save the data file after making changes, it will keep a record of the assay (if a new assay name has been saved, it will use the settings from this assay the next time the file is opened), gel color, well names, and peak find settings that were in effect at the time the file is resaved. If you don't want to change the original file, choose Save As... and give the file a new name or save it to a different location.

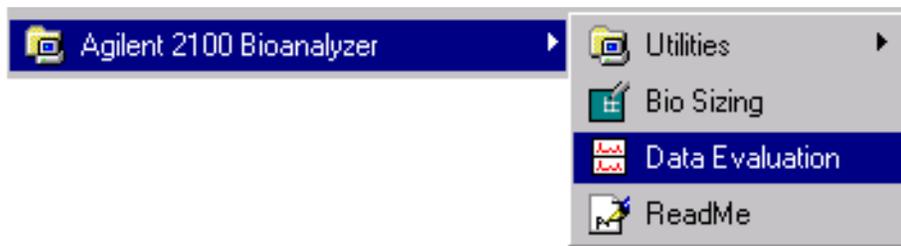
Data Evaluation

The Agilent 2100 Bioanalyzer software allows you to overlay and visually compare two or more electropherograms from the same run, but you cannot adjust these graphs in any way, nor can you compare electropherograms from different runs.

The data evaluation tool extends the capability of the Agilent 2100 Bioanalyzer software by allowing you to compare up to 12 electropherograms recorded by the Agilent 2100 Bioanalyzer. These can be from the same or different chips. You can also adjust the alignment of the curves through either automatic or manual settings, and you can view the graphs in different ways to enhance the presentation of the data.

Starting the Data Evaluation Tool

The data evaluation tool runs as a stand-alone program. It can be started from the software by choosing Tools > Data Evaluation..., or can be chosen from the Agilent 2100 Bioanalyzer program group by selecting Data Evaluation.



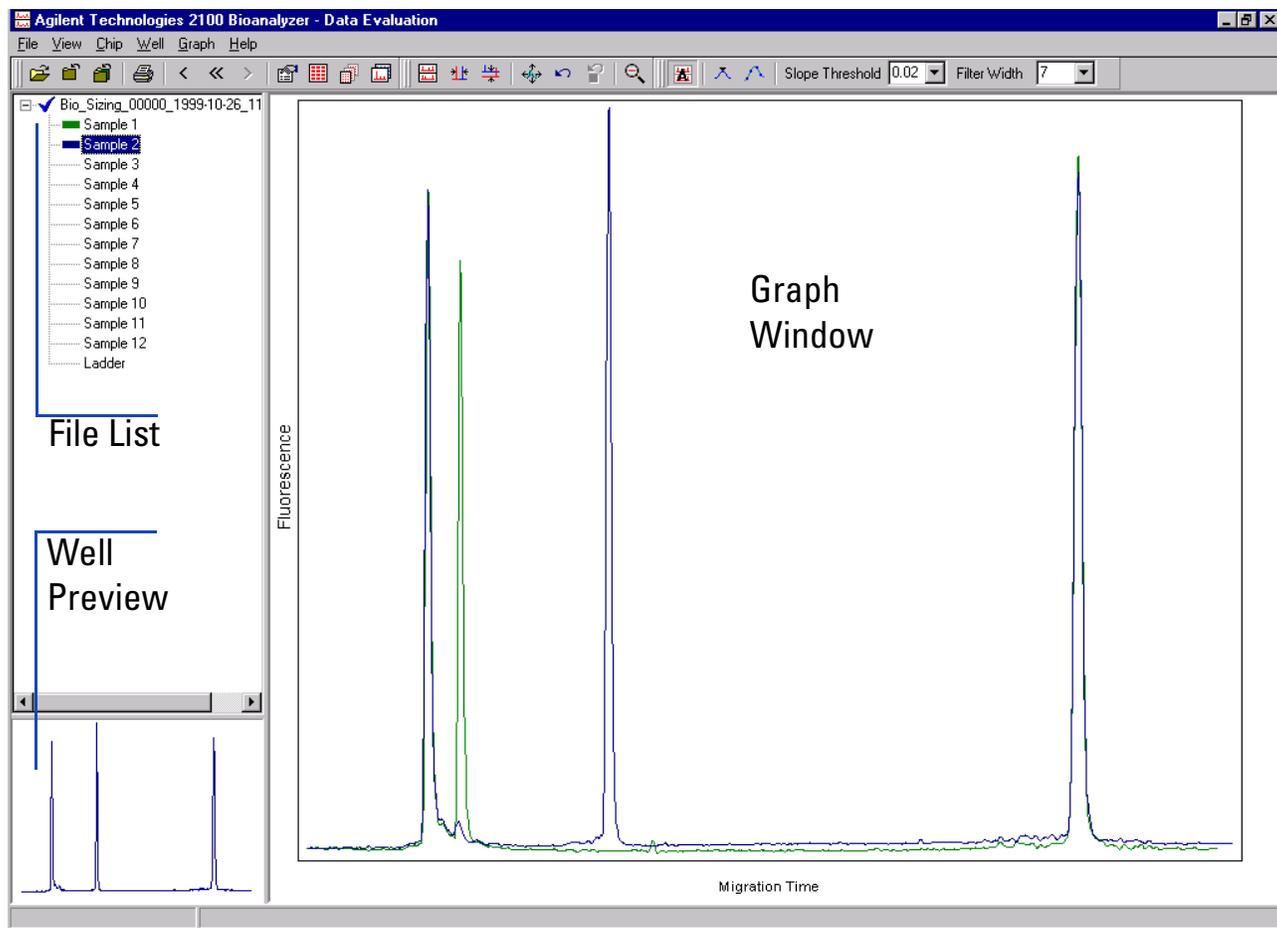
Loading Electropherograms in the Data Evaluation Tool

When you start the data evaluation tool from within the Agilent 2100 Bioanalyzer software, the data file present in the software will be automatically loaded in the data evaluation tool. If you start the data evaluation tool from the program group, no data will be preloaded.

NOTE

You don't have to close the Agilent 2100 Bioanalyzer software before opening the data evaluation tool. The software and the data evaluation tool are coexecutable.

The figure below shows the data evaluation tool interface.



Load all data files holding samples you want compare by going to File > Open in the data evaluation tool interface.

NOTE

No limit for the maximum number of data files you can load is given in the software. The amount of computer RAM available to the data evaluation tool limits the number of data files the software can handle.

In order to compare samples, load the samples in the graph window by double-clicking on the individual sample names in the file list.

NOTE

The maximum number of samples the data evaluation tool allows you to load is 12.

No axis scales (for example, time in seconds for the x-axis) are shown in the display.

Alignment of Electropherograms in the Data Evaluation Tool

Principles of Alignment

Within the data evaluation tool, the term *alignment* describes the process of making two or more electropherograms more comparable by stretching or compacting them in either or both the x- and y-axis directions. The data evaluation tool does this by using two reference points on each sample trace and then aligning these points on the traces.

When aligning along either the X- or Y-axis, the first point defines where the alignment starts. The distance between the first and the second reference point defines the scaling factor by which each curve will be stretched or compressed.

While it is possible to overlay and align electropherograms from any two (or more) runs, the data evaluation tool works best with similar or identical samples. It is useful for comparing RNA preps.

You may choose to use Automatic Alignment in which the data evaluation tool chooses the reference points and aligns both axes automatically. Or, if this is unsatisfactory, you may use Manual Alignment instead which requires that you set the reference points for X- and/or Y-axis alignment.

Automatic Alignment

A sample can be aligned in three ways: along the X-axis, along the Y-axis, or along both axes simultaneously. The X-axis uses reference points defined at the center points of the first and last peak on an electropherogram. With most DNA and Protein samples, these are usually the marker peaks; with total RNA samples, these correspond to the 18S and 28S ribosomal peaks for eukaryotic samples and the 16S and 23S ribosomal peaks for prokaryotic samples. The Y-axis uses reference points defined by the baseline and the apex of the first peak. By modifying the Filter Width and Slope Threshold settings, you can alter the results shown.

Manual Alignment

In some cases, the reference points that are defined automatically may not be adequate. In this case, you may define your own points using Manual Alignment. Manual Alignment consists of first placing new reference points on a graph, which can be done by clicking on any data point, and then choosing alignment in the X-axis or the Y-axis.

You may define only two new reference points on each electropherogram you wish to align: a starting point and an ending point. If you place only one point, another reference point will be defined automatically by the data evaluation tool. Where the software places the second depends on whether you are aligning the X-or Y-axis and where you define the first point:

- If you are aligning in X and you place a single marker in the first half of the electropherogram (measured from the start of the first peak to the end of the last peak), the data evaluation tool assumes the point to be the starting reference point and automatically assigns the ending reference point. If you are aligning in X and you place the marker in the second half of the electropherogram, the data evaluation tool assumes this point to be the ending point and automatically assigns the starting reference point.
- If you are aligning in Y and you place a single marker less than halfway up the first found peak, the data evaluation tool assumes you are choosing a point for the baseline and automatically assigns the second point at the apex of the first peak. If you are aligning in Y and you place the marker more than halfway up the first found peak, the data evaluation tool assumes the marker to be the apex of the peak and assigns the baseline automatically.

After defining all reference points, you may start the alignment process in either direction. After alignment, all manually defined markers are automatically deleted.

Placing markers for manual alignment often is easier if you can see the data points along the electropherogram.

Click the Show Data Points button, located on the Options toolbar.



Or enable Show Data Points on the View > Options dialog box.

Any of the data points now showing may be selected as a new reference point. The mouse cursor will change to a pointing hand when a data point may be selected. Choose either one or two points on each graph you wish to align.

To align manually along the X-axis, click the Align in X button on the Alignment toolbar.



Or choose Graph > Align in X.

To manually align the Y-axis, click the Align in Y button, located on the Alignment toolbar.



Or choose Graph > Align in Y.

Peak Find Settings

Two Peak Find Settings are available in the data evaluation tool.



Slope Threshold 0.02 ▼

Slope Threshold	Determines the sensitivity of the data evaluation tool to the start of a new peak. Setting the Slope Threshold to a smaller number increases the sensitivity, causing more peaks to be found. Setting the Slope Threshold to a larger number reduces the sensitivity, causing fewer peaks to be found. The default setting for Slope Threshold is 0.02.
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Filter Width 7 ▼

Filter Width	Determines the number of data points used for calculating the peaks. This value determines the width of a floating <i>window</i> that is <i>laid over</i> the data points. Within this window, the data evaluation tool calculates a derivation to determine if there is a peak.
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Baseline Subtraction	Can help when signal-to-noise ratios are very low and is enabled by default for all wells. Baseline subtraction can be changed globally or for an individual well by clearing the checkmark on the Settings tab in the Sample Information area.
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Data Handling and Printing

Organizing, Retrieving, and Backing Up Data Files

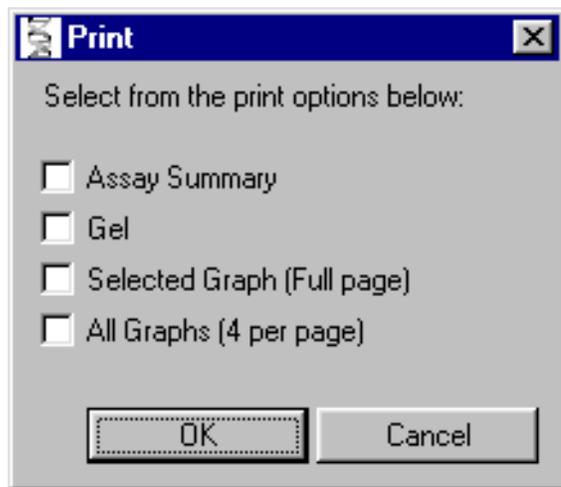
As you begin to work with the Agilent 2100 Bioanalyzer software, it is good practice to organize your files. If you are not the only user of the Agilent 2100 Bioanalyzer, creating a directory within which to save your files is recommended; having all users save their files to their own directories will speed the process of finding a particular file at some point in the future. Even if only one person uses the Agilent 2100 Bioanalyzer software, it is still wise to review your files periodically, archive files you are no longer using but wish to save, and discard unneeded files.

Each user in your laboratory may want to specify a particular file prefix which will easily differentiate their data files from any others. Additionally, you may specify that a new directory be created each day for storage of that day's runs.

It is a good idea to archive files to a backup disk for safekeeping and/or to remove files from your hard disk periodically. Depending on the amount of hard disk space available to the Agilent 2100 Bioanalyzer software, you may need to clear space on your hard drive to ensure that you have enough room to save upcoming assay data.

Printing a Report

Choosing File > Print opens a dialog box which allows you to print up to four different types of information in a report from a data file:



You may choose one or more of the items shown by clicking in the checkbox next to the desired item. Clicking OK in this dialog box causes another printer-specific dialog box to appear, allowing you to set the number of copies you would like to print, along with other settings that determine the appearance of the printed document.

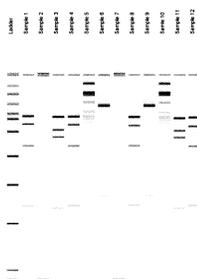
For more information, see **Setting Up Your Printer—118**

Printing an Assay Summary Report

CL75_10_00022_1999-06-10_16-35-36

Read: 06/10/99,04:35 PM Page 1

Data path: C:\Program Files\HP 2100 Bio Analyzer\Bio Sizing\Data
Assay: McPlus DNA 7500



Assay: C:\Program Files\Caliper\Bio Sizing\Assays\dsDNA\McPlus DNA 7500.ass
Title: 100 to 7500 dsDNA Sizing
Version: 2.9 Beta
Comments: Copyright 1999 Caliper Technologies Corp.

2.3 - 12.5uM dye script, 785V, 180sec pre-run
2.2 - Improved bubble detect and 95 sec runs
2.1 - Add bubble detection and focus check

Upper Marker: 10380 BP Sizing: Upper Marker
Lower Marker: 50 BP Baseline: 25 Seconds

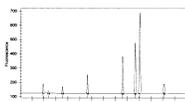
Ladder Concentration: 4 ng/ul Marker Peaks: Aligned upper and lower
Upper Marker Conc: 4.2 ng/ul Filter Width: 1 Seconds
Lower Marker Conc: 8.3 ng/ul Baseline Plateau: 0.9 Seconds
Poly order: 6
Standard Curve: Point to point

Min Peak Height: 8 (above baseline)
Slope Threshold: 0.9 Second
Min Peak Width: 0.5 Seconds

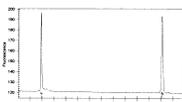
First Peak Time: 25 Seconds
Last Peak Time: 95 Seconds

Notes: D26BBP
F04BBU

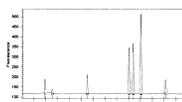
Sample 1



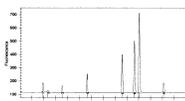
Sample 2



Sample 3



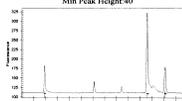
Sample 4



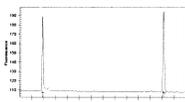
Sample 5



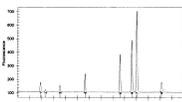
Sample 6



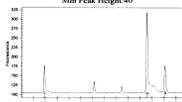
Sample 7



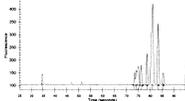
Sample 8



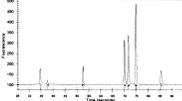
Sample 9



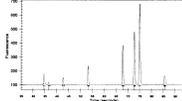
Sample 10
Min Peak Height: 15



Sample 11



Sample 12

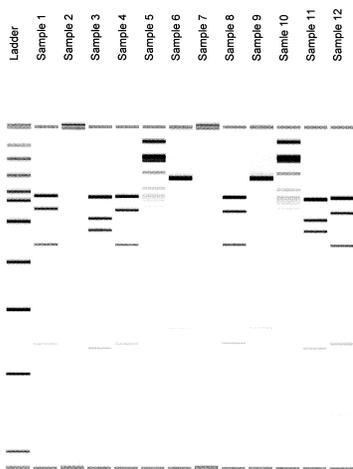


Printing a Gel Report

CL75_10_00022_1999-06-10_16-35-36

Read: 06/10/99,04:35 PM Page 2

Data path: C:\Program Files\HP 2100 Bio Analyzer\Bio Sizing\Data
Assay: McPlus DNA 7500



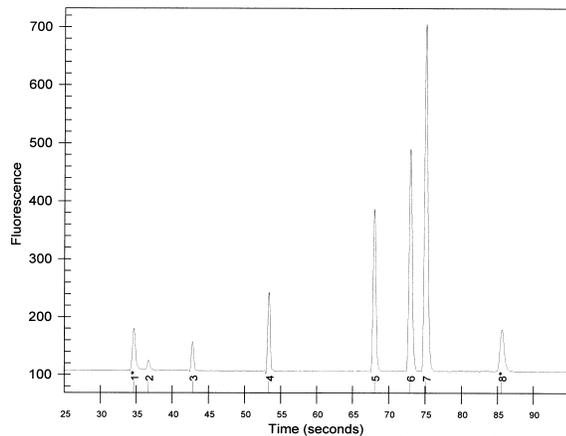
Printing a Selected Graph Report

CL75_10_00022_1999-06-10_16-35-36

Read: 06/10/99,04:35 PM Page 3

Data path: C:\Program Files\HP 2100 Bio Analyzer\Bio Sizing\Data
Assay: McPlus DNA 7500

Sample 8



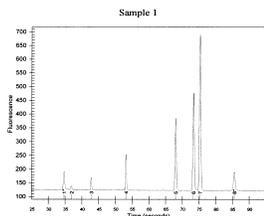
Peak	Ret. Time(sec)	Area	Size(BP)	Conc.(ng/ul)	Molarity(nM)
1 - Lower	34.65	33.11	50	8.30	251.51
2	36.70	7.13	90	1.46	24.58
3	42.80	16.36	197	2.44	18.74
4	53.35	45.57	397	4.83	18.46
5	68.05	111.57	832	9.67	17.60
6	72.95	167.35	1246	14.50	17.63
7	75.10	267.51	1712	23.18	20.52
8 - Upper	85.55	44.20	10380	4.20	0.6131

Printing an All Graphs Report

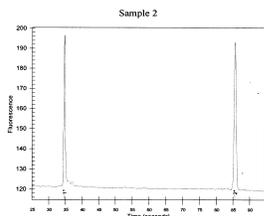
CL75_10_00022_1999-06-10_16-35-36

Read: 06/10/99,04:35 PM Page 4

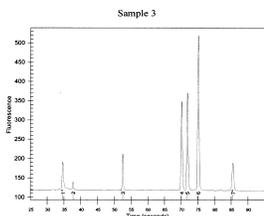
Data path: C:\Program Files\HP 2100 Bio Analyzer\Bio Sizing\Data
Assay: MCP/Program Files\HP 2100 Bio Analyzer\Bio Sizing\Data



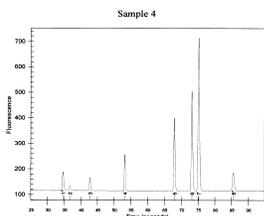
Peak	Mig.	Time(sec)	Area	StdDev	Conc (ng/ml)	MolWeight
1 - Lower	34.05	32.12	50	6.36	251.51	
2	36.65	6.48	18	1.26	22.00	
4	53.15	45.23	393	4.97	19.19	
5	62.76	18.87	147	2.45	16.90	
6	73.30	165.77	1302	15.13	17.69	
7	75.25	276.57	1769	24.75	21.00	
8 - Upper	95.95	42.79	10360	4.20	0.6131	



Peak	Mig.	Time(sec)	Area	StdDev	Conc (ng/ml)	MolWeight
1 - Lower	34.05	32.12	50	6.36	251.51	
2 - Upper	95.95	42.79	10360	4.20	0.6131	

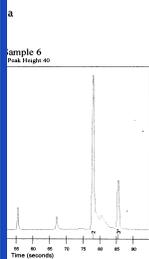


Peak	Mig.	Time(sec)	Area	StdDev	Conc (ng/ml)	MolWeight
1 - Lower	34.05	32.12	50	6.36	251.51	
2	37.70	7.73	109	1.43	20.78	
3	52.00	32.77	290	3.45	12.95	
4	70.10	102.95	5395	6.73	14.13	
5	71.85	118.71	11971	110.49	14.22	
6	75.10	104.82	131.2	16.43	8.85	
7 - Upper	95.95	42.79	10360	4.20	0.6131	

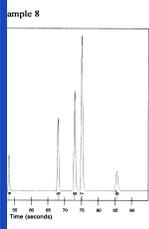


Peak	Mig.	Time(sec)	Area	StdDev	Conc (ng/ml)	MolWeight
1 - Lower	34.05	32.12	50	6.36	251.51	
2	26.50	7.32	90	1.43	25.95	
3	62.76	17.16	197	2.44	13.92	
4	63.20	46.40	354	5.10	16.63	
5	67.80	118.42	828	10.17	16.63	
6	73.10	17.79	173	11.22	16.00	
7	75.10	295.00	1731	24.42	21.41	
8 - Upper	95.95	42.79	10360	4.20	0.6131	

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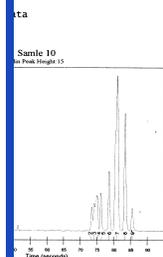


Area	StdDev	Conc (ng/ml)	MolWeight
127.46	2657	10.44	6.54
46.76	10360	4.20	0.6131

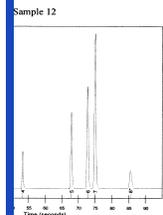


Area	StdDev	Conc (ng/ml)	MolWeight
23.13	92	3.42	24.98
16.36	197	2.44	10.74
45.97	2967	6.82	19.69
11.10	63	0.82	17.69
117.79	1245	3.45	17.62
267.75	1713	23.18	33.62
44.20	10360	4.20	0.6131

06/10/99,04:35 PM Page 6



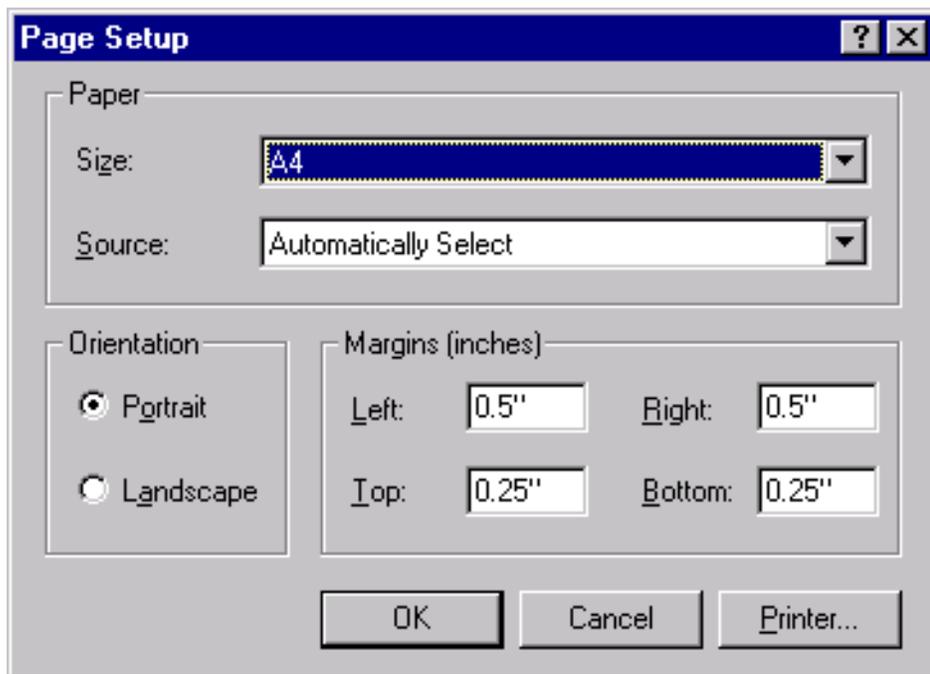
Area	StdDev	Conc (ng/ml)	MolWeight
177.05	52	6.20	251.51
21.19	1933	5.97	1.36
23.09	1444	3.03	3.56
51.21	1622	7.19	6.44
64.76	2143	4.00	3.29
86.77	3206	9.77	3.27
259.06	5244	35.52	10.26
127.05	3076	18.18	3.29
27.29	10360	4.20	0.6131



Area	StdDev	Conc (ng/ml)	MolWeight
25.81	92	6.30	251.51
9.10	89	1.69	26.45
17.47	136	2.64	26.43
45.14	281	4.85	19.04
113.09	822	5.97	19.15
109.16	1522	14.69	16.39
262.19	1673	22.90	20.74
43.09	10360	4.20	0.6131

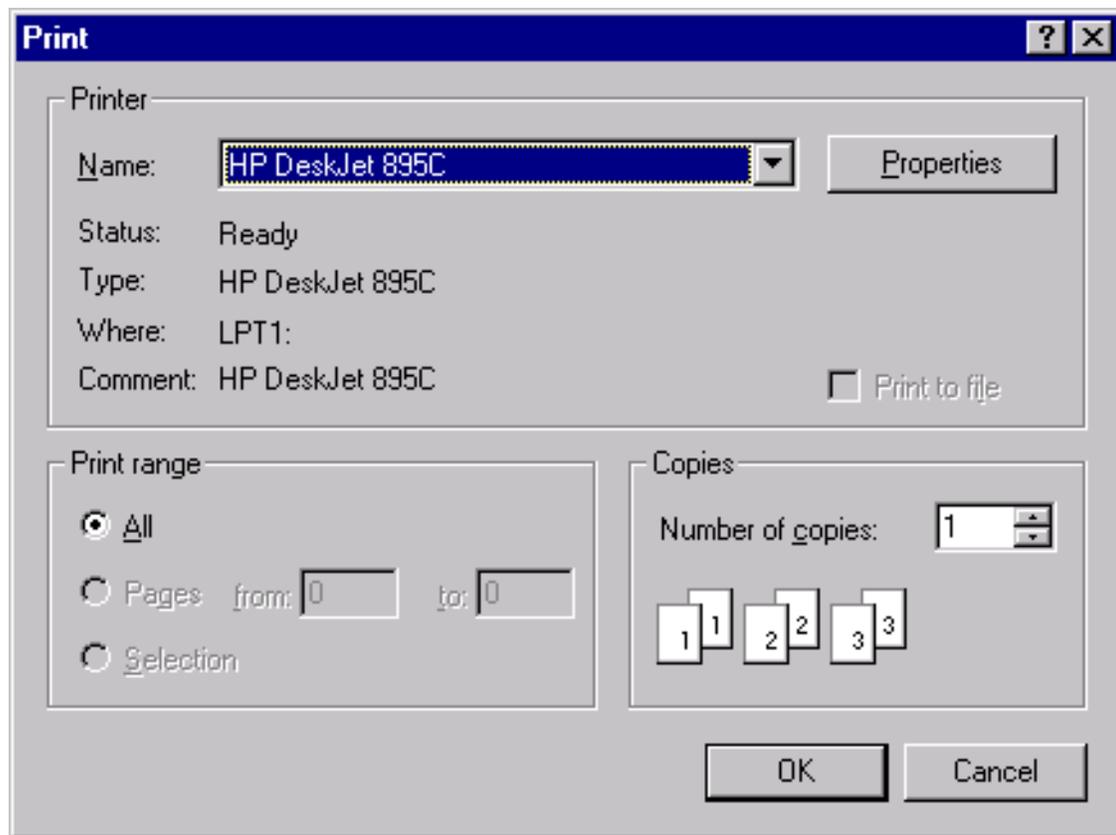
Setting Up Your Printer

If your printer is not functioning correctly, you must select and properly configure the appropriate printer driver. Consult the printer manufacturer's instructions or the Windows documentation to find out how best to set up the printer driver. From the File menu, choose Page Setup.



Make selections that are appropriate for your particular printer. Clicking the arrows to the right of selection boxes will provide you with different options.

Clicking the Printer button opens another dialog box, allowing you to select options for the default printer or to choose a different printer:



When you are finished, click OK to close the dialog box.

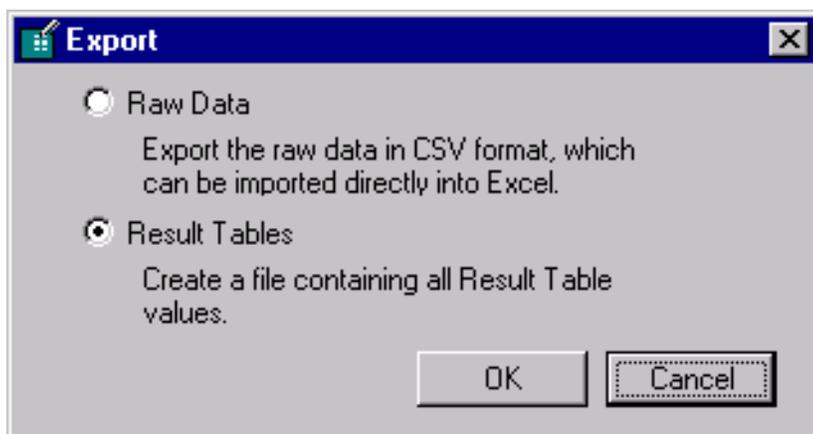
Exporting Data

Data can be exported in two different formats:

- **Raw Data:** ASCII format file containing the raw data of the electropherograms. Raw data can be analyzed using different software applications..
- **Results Tables:** ASCII text file that contains the results from all wells in the same format as would be copied to the clipboard.

To Export Files

- 1 Open the file you want to export.
- 2 Click File > Export. The Export dialog box opens:



3 Choose an export format from the Export dialog box:

Raw Data	Export the raw data in CSV (comma-separated values) format which is suitable for pasting directly into Microsoft Excel or another spreadsheet application.
Result Tables	Create an ASCII text file containing all Result Table values.

- 4 Clicking the OK button in the Export dialog box causes the Export Data Dialog box to appear. Accept the default filename shown in this dialog box or enter a different one. The file extension will automatically be .csv for raw data and .txt. for result tables. Accept the default location for saving this file or choose a different location, if desired.
- 5 Click OK and the data will be exported to that filename in that location.

Copying Information

The Edit menu offers three choices for copying information from the Agilent 2100 Bioanalyzer software for use with other applications:

- Copy Gel
- Copy Graph
- Copy Results Table

Choosing any of these copy commands places a copy of the chosen item to the Windows clipboard. You may then paste the item into a word processing, graphics, or other application.

Choosing Copy Gel from the Edit menu always copies a large gel picture (such as would be seen if you viewed a large gel display) with the lane labels as part of the graphic.

The size of the image that is placed on the clipboard when copying a graph depends on the display mode at the time you choose Edit > Copy Graph or right-click the mouse and choose the copy graph command from the pop-up menu. If you right-click and choose to copy while the cursor is over the large well graph or if you choose Copy Graph from the Edit menu while viewing a single well display, a large-sized graphic (the same size as that shown in the large display) of the well graph will be placed on the clipboard.

Copying the result table, either by choosing this command from the Edit menu or by right clicking in the result table area and choosing the copy command from the pop-up menu, causes ASCII information to be placed on the Clipboard. The following text is an example of a DNA result table data that was copied from a sample run:

Bio Sizing_00589_2000-05-06_09-19-49					Sample 1
Peak	Mig. Time(secs)	Area	Size(BP)	Conc.(ng/ul)	Molarity(nmol/l)
1	30.30	2.57	50	4.0	121.21
2	32.80	3.24	100	7.5	113.85
3	63.60	2.96	3000	4.0	2.02

The first line shows the name of the saved data file followed by the name of the sample from which the result table data came. The second line provides the headers for the rest of the information which includes the peak number, the migration time in seconds, the peak area, the size of the peaks in base pairs, the concentration in nanograms per microliter, and the molarity in nanomoles.

Using Help

The Help system enables you to retrieve the information you need quickly and then return to your work. Help appears in a separate window on your screen. For quick access, you may keep the Help window displayed on top of or behind the application. You can also print specific topics from the online Help system. Context-sensitive help is also available.

Contents and Index

When you click Contents and Index from the Help menu, the Help window opens, allowing you to do one of the following things:

- Click the Contents tab to display conceptual and *how-to* information.
- Click Index to search by a name or concept.

Context-Sensitive Help

The context-sensitive Help displays information that is relevant to the current window or dialog box displayed by the Agilent 2100 Bioanalyzer software. To access context-sensitive help, click the Help button or press F1.

NOTE

Positioning the cursor over a tool on the tool bar or over other items in the workspace and leaving it there momentarily will cause a Tool Tip to appear, listing the name of the tool. Often this will be enough to describe the item's function.

Printing Help

You can print specific help topics or print entire sections of online Help.

To print an entire section, from the Contents page, click the Print button that appears along the bottom-right side of the window.

To print a single help topic, go to that topic and click the Print button that appears at the top of the window.

Types of Help Available

Three types of help are available within the Agilent 2100 Bioanalyzer software:

- **General Help** — By clicking the Help menu and choosing Contents and Index, you can view the Help topics that are grouped by subject matter into books or search for a keyword and jump to a topic.
- **Context-Sensitive Help** — Pressing F1 will provide help that is specific to the active window.
- **Hotspots and Links** — Certain items in the help files are links to other types of help. Clicking items that are underlined or over which the cursor changes to a pointing hand will cause additional help to pop up or will take you to another help screen containing more information.

For more information, see [Using the Help Function](#).

Mouse Notation Conventions in Help

When you see this ...	Do this ...
Click File > Open	Click the File menu with the mouse, and click the Open item in the menu.
Click Assay > DNA > DNA7500	Click the Assay menu, click DNA, and click DNA7500 from the sub-menu that appears.
Enable a check box	Click the check box to place a check mark or an X inside the box.
Disable a check box	Click the check box to remove the check mark or X.
Select	Click to highlight/bound a well/peak/lane.
Choose Print from the File menu	Click the File menu, and click the mouse button on the Print command.
Right-click and click Copy	Click the right mouse button, and click the Copy command from the submenu that appears.

Keyboard Notation Conventions in Help

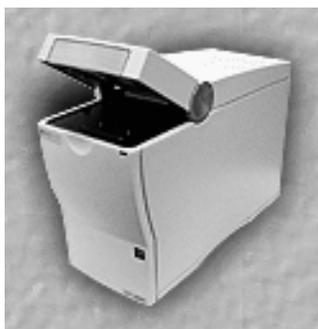
When you see this ...	Do this ...
Press Enter	Press the ENTER key on your keyboard.
Ctrl + Shift	Press the Ctrl key and Shift key at the same time.
Ctrl + A	Press the Ctrl key and the A key at the same time.

Troubleshooting and Diagnostics

Communication

To check whether your PC communicates with the Agilent 2100 Bioanalyzer:

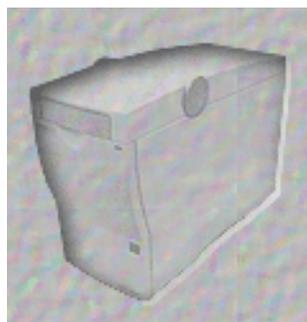
- 1 Start the Agilent 2100 Bioanalyzer software.
- 2 Open and close the lid—the icon in the main screen should change from open to closed:



Lid open



Lid closed



Not communicating properly

If the icon doesn't change:

- ❑ Check whether the status indicator is on. If it is off, replace the fuses as described in the *Site Preparation and Safety Manual*.
- ❑ Check whether the status indicator is red. If it is red, turn off line power to the Agilent 2100 Bioanalyzer and turn on again. If the problem persists, call Agilent Technologies.
- ❑ Check that the RS232 communication cable is connected correctly.
- ❑ Check if another hardware device is connected to your computer via RS232 cable
- ❑ Check the Com port settings in the Agilent 2100 Bioanalyzer software, see **Changing the COM-Port Settings—130**
- ❑ Replace the RS232 cable.
- ❑ Reinstall the Agilent 2100 Bioanalyzer software.

If the Agilent 2100 Bioanalyzer will still not communicate, call Agilent Technologies.

Changing the COM-Port Settings

NOTE The number of COM-Ports available depends on your bundle PC:

Laptop system: only one COM-Port is available.

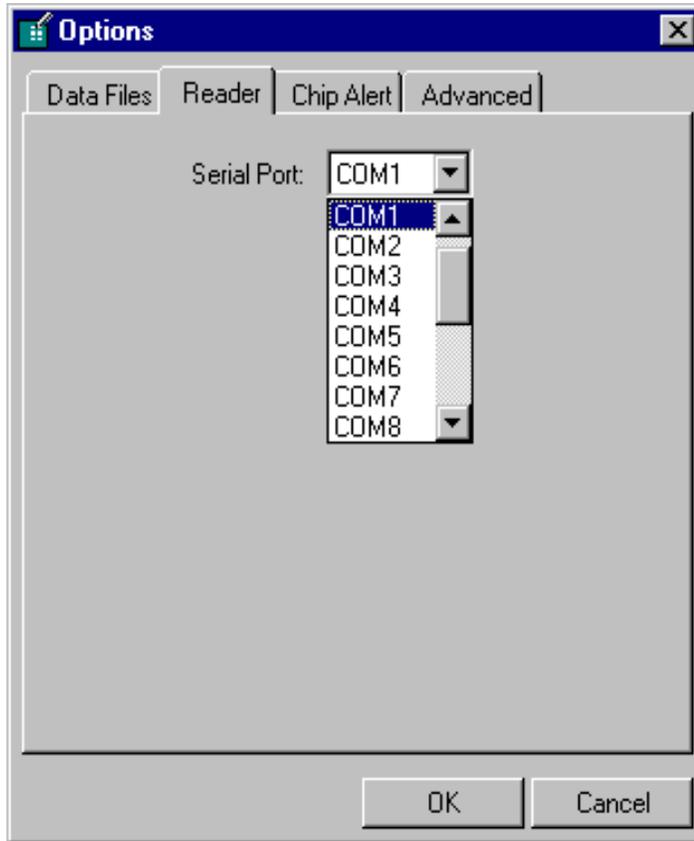
Single instrument system: two COM-Ports (A and B) are available.

Multi-instrument system: up to 4 COM-Ports (1 to 4) are available.

In case of a Multi-instrument system a Multi Port Card (e.g. RocketPort PCI/Quad DB9) is installed in your PC. Only connect the Agilent 2100 Bioanalyzer via the Multi Port Card/Multi Port cable with your computer. Do not use the default COM-Ports of your PC (Port A or B). Setting-up the Multi Port Card might be necessary after PC-repair or reinstallation of the operation system.

Laptop and single instrument system

- 1 Choose Options from the Tools menu. The dialog box that appears contains four tabbed sections, labeled Data Files, Reader, Chip Alert, and Advanced. Click the Reader tab. The dialog box should look like this (your COM setting may be different):



- 2 Try choosing a COM-Port setting that is different from the one that is currently selected. If you know which port is in use on the PC, choose that port.

NOTE

If you have a laptop connected to your instrument you must choose COM1.

- 3 Check the icon of the Agilent 2100 Bioanalyzer on the screen. If it is no longer dimmed, communication between the Agilent 2100 Bioanalyzer and PC is working properly. In addition hardware information is displayed.

NOTE

If you have a PC connected to your instrument and the icon is still dimmed, repeat step 2, choosing a different Com Port each time, until it is not dimmed anymore.

If you cannot resolve the communication problem in this way, check the troubleshooting help for more information.

- 4 When you are finished with the Options dialog box, click the OK button to close it.

Multi-instrument system

Setting up the Serial Interface of your Agilent 2100 Bioanalyzer Multi-Instrument System (G2942AA)

General

In case you have to re-install your operating system using the recovery CD provided, you also need to setup the RocketPort PCI/Quad DB9 Multi Port Serial card in your PC. This will enable multi-instrument support for the Agilent Technologies Bioanalyzer 2100 again.

NOTE

Reloading the software might be necessary after severe PC system crashes or in case part of the software installation has been corrupted.

NOTE

Please make sure to have the RocketPort PCI/Quad DB9 Multi Port Serial card properly installed before trying to configure the serial ports as described in this document.

Multi Port Card Setup Process

The setup process of the RocketPort PCI/Quad DB9 Multi Port Serial card requires 2 steps:

- disabling the standard serial ports
- installing the RocketPort driver.

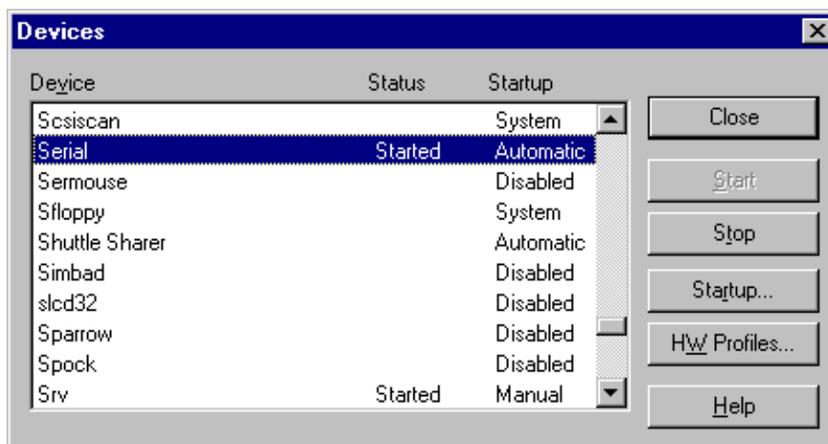
The setup process is described on the following pages. For support on configuring the RocketPort PCI/Quad DB9 Multi Port Serial card, please contact Agilent service personnel.

Disabling the Standard PC Serial Ports (COM1 and COM2) of your PC

The embedded serial ports of your PC must be disabled before you can use your RocketPort card. To do so,

- 1 open the *Control Panel* from the *Settings* menu.
- 2 Double click the symbol called "Devices" and look for the Device named "Serial". This is the standard device driver for the embedded serial ports.

- 3 To disable these serial ports press the Stop button to halt the active process (answer the following dialog with "Yes") and then change the startup mode from "Automatic" to "Disabled" (follow the instructions of the dialog presented after you have pressed the button "Startup...").
- 4 Close this dialog.

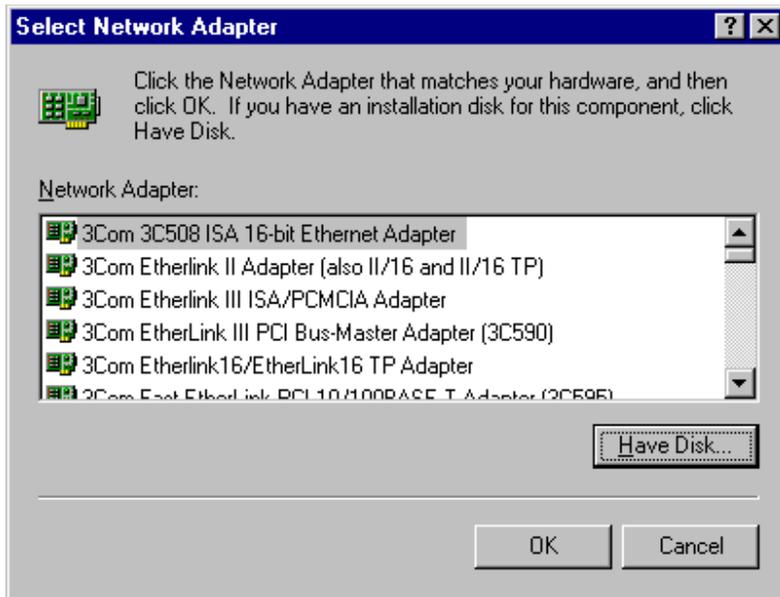


Installing the RocketPort PCI Driver

Installing the PCI Card as Network Adapter

The RocketPort card will be installed as an additional network adapter.

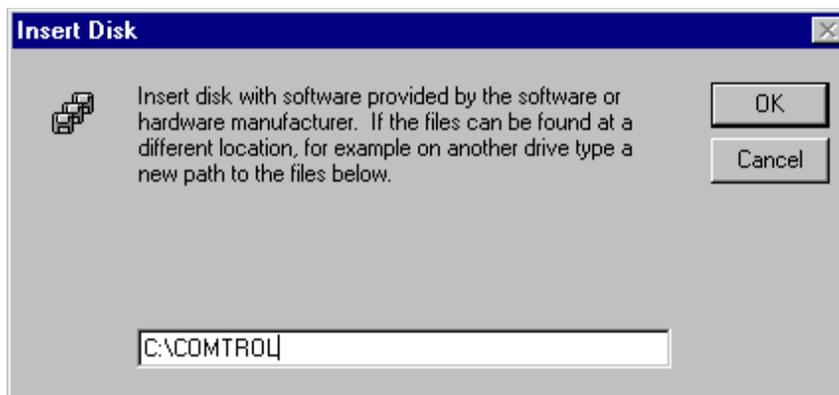
- 1 From the Control Panel open the "Network" dialog and click on the "Adapters" tab.
- 2 Press the "Add..." button to add a new Network Adapter.
- 3 In the following dialog choose the "Have Disk..." - Button to load the RocketPort PCI/Quad driver.



4 Enter the path to the RocketPort driver.

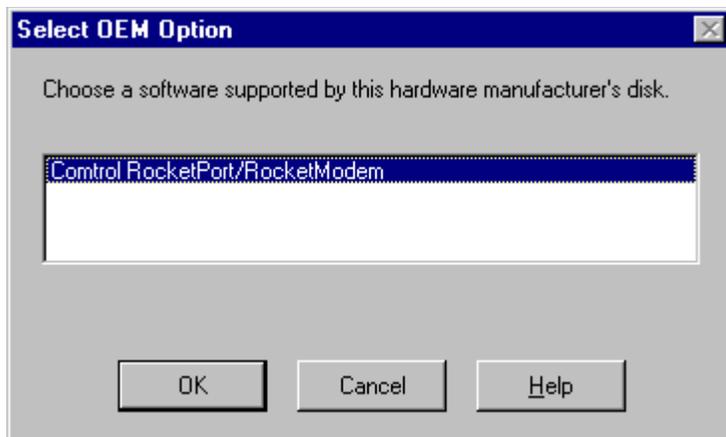
NOTE

Please do NOT use the driver located on the Bioanalyzer software CD. Please use the driver that is already copied to your system drive in directory C:\COMTROL.

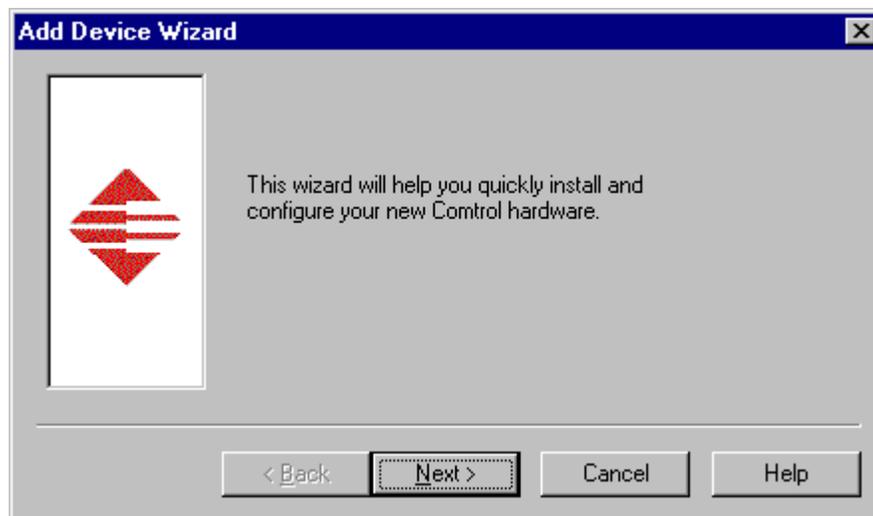


In the next dialog you are able to choose the driver that should be installed (there should only be one choice, already pre-selected: *Control RocketPort/RocketModem*).

5 Press *Ok* to start the installation.



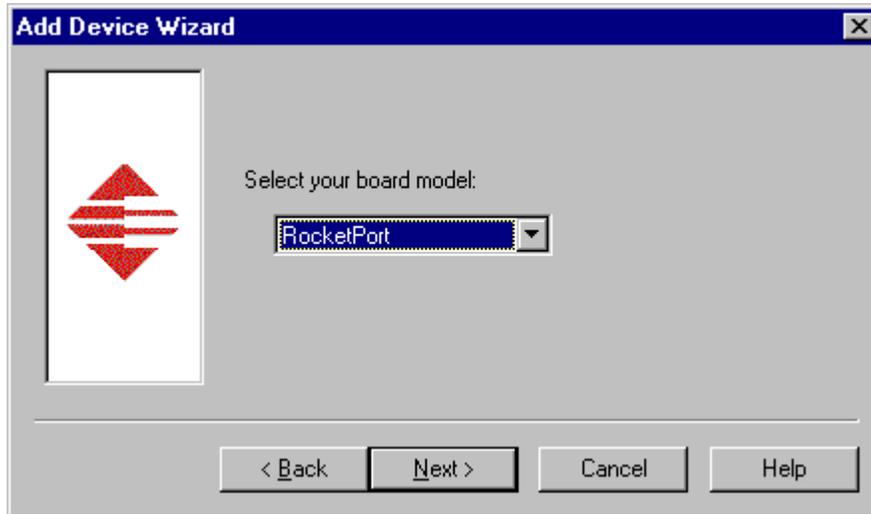
- 6 The device wizard will guide you through the rest of the setup. You can navigate between the different dialogs by clicking on the Back and Next buttons.



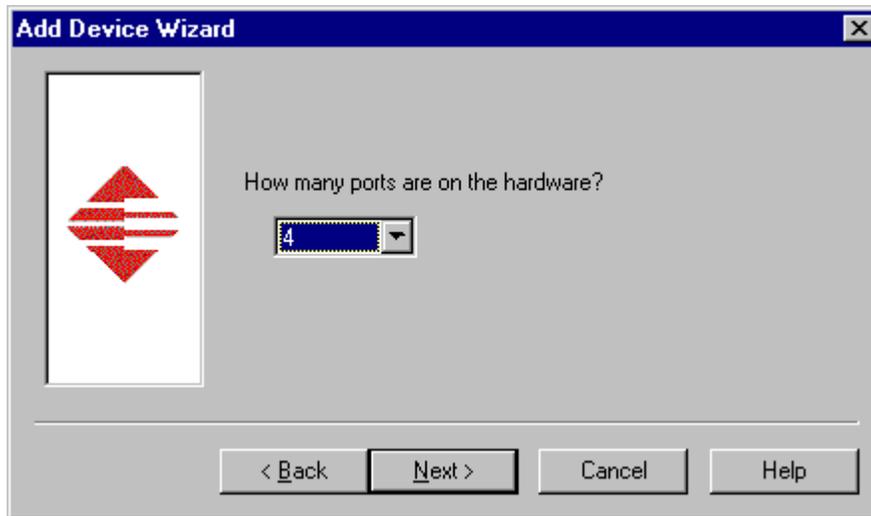
- 7 On the second window of the Add Device Wizard you must specify the bus type of the installed RocketPort card. Choose the option PCI bus.



- 8 Then you must specify the board model. You must keep the proposed setting: RocketPort.
- 9 Press Next to continue to the following dialogue.



- 10** The next step is to specify the number of ports supported by the installed card. Change the number of supported ports from 8 (default value) to 4 as this is the number of physical ports of the installed card.
- 11** Press Next to continue to the following dialogue.



12 Click the "Finish" button to finish the installation.

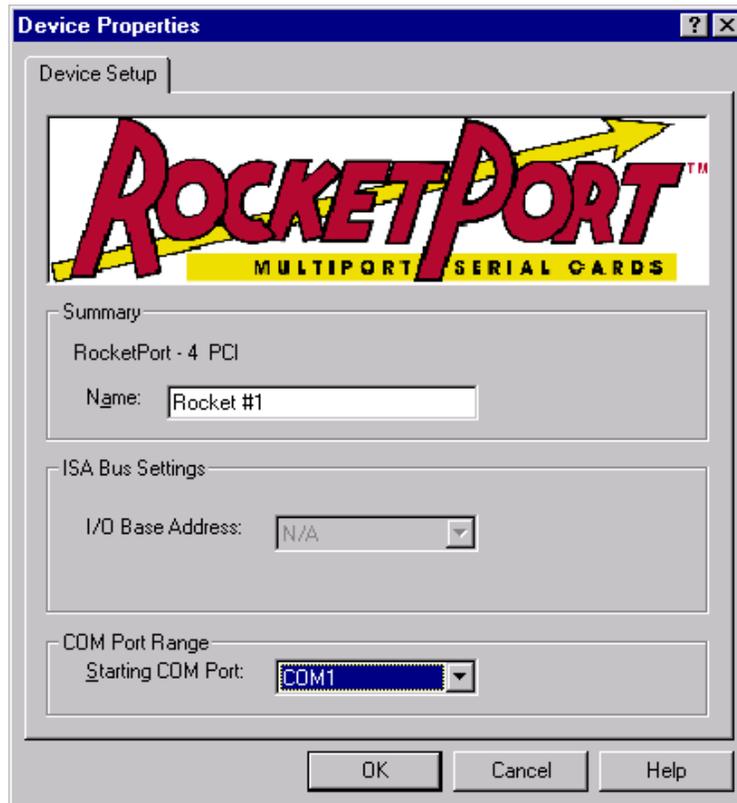


Now you have successfully installed the device driver for the RocketPort PCI/Quad DB9 card.

Setup of the Device Properties

The installation process continues with the setup of the device properties.

- 1 You need to change the range of COM ports. Please change the *Starting COM Port* from *COM3* (default) to *COM1*.



2 Press *Ok* to save the settings. You can verify your specifications in the next dialog, which shows a summary of the RocketPort Setup.

NOTE Later on you can access this dialog by starting the Setup program of the RocketPort card from the Start menu.



3 Press OK to close the following window.



4 After closing the network setup window you will be requested to restart your computer.

5 Close any other open application and press *Yes* to reboot your computer.



After the reboot the RocketPort card is set up properly to work with the Agilent Bioanalyzer 2100 Software. The instruments connected to the card can be referred as COM1 to COM4.

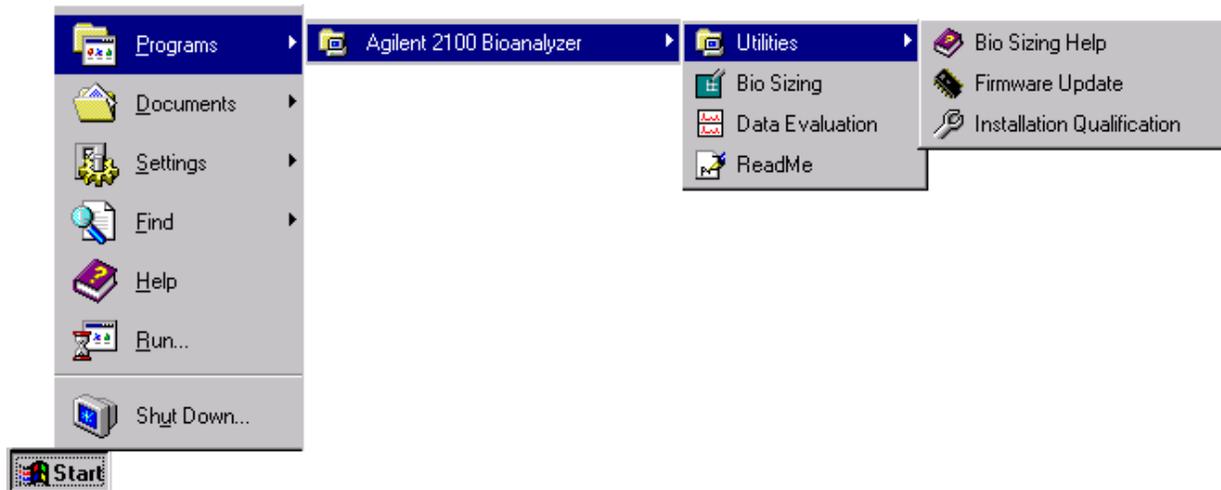
NOTE

The warning message "Warning, PCI num-ports mismatch" may appear in the event logbook of your PC. This is NOT an error and can be ignored. It happens only if you have no instrument connected to the RocketPort card. After connecting an instrument no event is logged after startup.

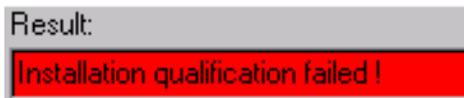
Agilent 2100 Bioanalyzer Software Troubleshooting

If your Agilent 2100 Bioanalyzer software is no longer working properly, you can check for corrupted or missing files.

- 1 If the Bio sizing is running, close it.
- 2 Start the software test tool by clicking Installation Qualification in the Agilent 2100 Bioanalyzer program group.



- 3 The Installation Qualification interface appears. Click Start Validation to start the software test tool. The result of the installation qualification test depends on whether the software installation is complete and no files are corrupted.



- 4 If the test passes and the Agilent 2100 Bioanalyzer system still does not function correctly, see **Communication—2** and **Hardware Diagnostics (User's Guide)** for further troubleshooting procedures. Finally, check your application, see **Troubleshooting the DNA, RNA or Protein-Application**.
- 5 If the test fails, reinstall the Agilent 2100 Bioanalyzer software using the Agilent 2100 Bioanalyzer software CD-ROM that is supplied with the system.
- Insert the Agilent 2100 Bioanalyzer software CD-ROM in the CD-ROM drive of your PC.
 - Start Windows NT Explorer and go to the CD-ROM drive.
 - Double-click on the SETUP.EXE file and follow the instructions on the screen.
 - Repeat steps 1 through 4 to verify proper installation.

6 If the test continues to fail, save the result of the test by choosing File > Save log file as... in the Installation Qualification interface and call Agilent Technologies.

Hardware Diagnostics

Built in Tests—Firmware Error Messages

Whenever you start a run, the firmware of the Agilent 2100 Bioanalyzer checks for errors such as, for example, defective high voltage supplies, or missing conductivity between wells. If an error is detected, the firmware pops up a message box and aborts the run. Further, the message box contains hints on how to resolve the problem, or tells you to call Agilent Technologies.

Manual Tests

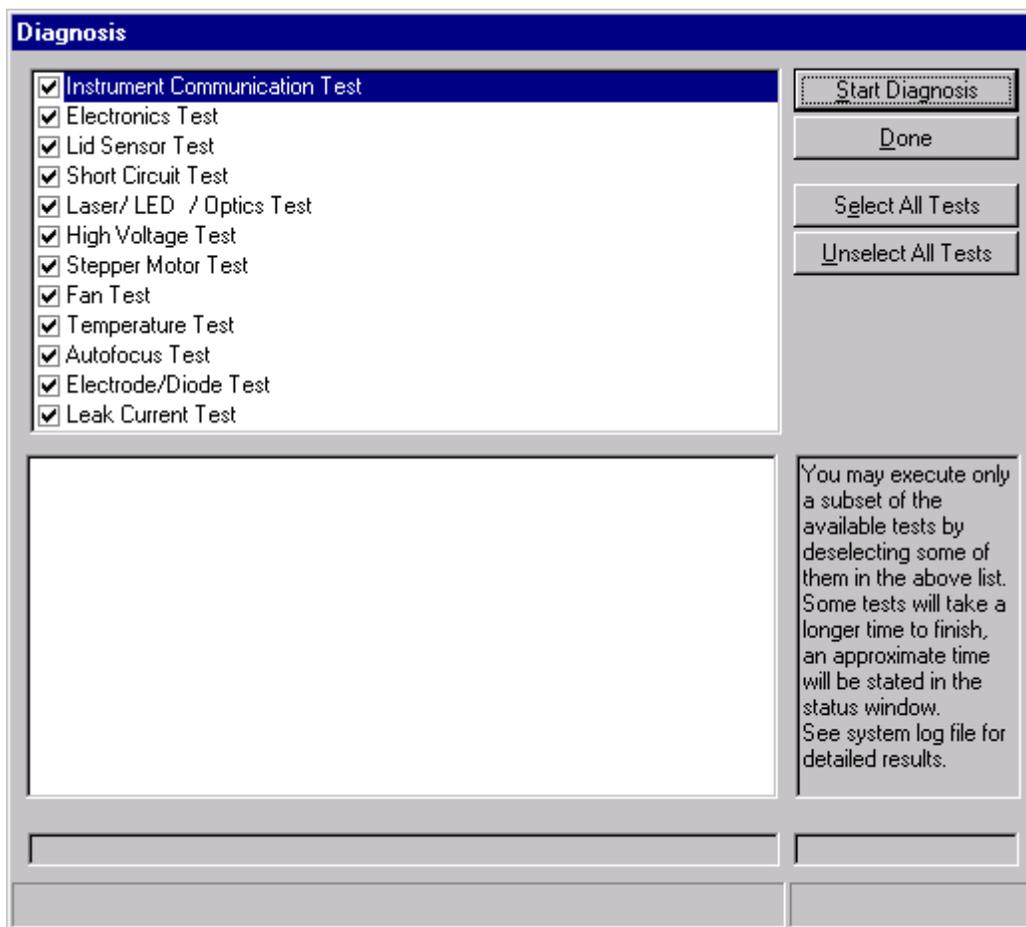
To successfully perform the complete set of hardware diagnostic tests, you need a new chip. With the chip and a diagnosis software interface, you can run system component tests and check all hardware components of the Agilent 2100 Bioanalyzer. Here is a complete list of hardware diagnostic tests.

Test	Description
Instrument communication test	Checks for proper communication between PC and Agilent 2100 Bioanalyzer.
Electronic test	Verifies proper functioning of all electronic boards in the Agilent 2100 Bioanalyzer.
Lid sensor test	Checks for the devices sensing for open or closed lid, and for laser and LED off when lid is being closed.
Short circuit test	Checks for instrument current leaks.

Test	Description
Laser / LED / Optics.	Checks for proper alignment of internal optics and proper function of the laser and LED
High voltage test	Checks the calibration of all 16 high voltage power supplies in the Agilent 2100 Bioanalyzer.
Stepper motor test	Checks for proper movement of stepper motor.
Fan test	Checks that the fan is running at the appropriate speed.
Temperature test	Checks that the temperature ramp up speed of the heater plate is within specifications.
Autofocus test	Checks focusing capability of optical system.
Electrode/diode test	Checks photodiode and current versus voltage performance of Agilent 2100 Bioanalyzer system.
Leak current test	Measures electrode cartridge leak current(s) between pins.
Stepper Motor	Checks for proper movement of the stepper motor.
Fan	Checks that the fan is running at the appropriate speed.
Temperature	Checks that the temperature ramp-up speed of the heater plate is within specifications
Autofocus	Checks focusing capability of the optical system.
Electrode / Diode	Checks the photodiode and current-versus-voltage performance of the Agilent Technologies 2100 Bioanalyzer.
Leak Current	Measures electrode cartridge leak current(s) between pins.

Test Procedure:

- 1 Access the hardware diagnostic tests by selecting Tools and then Diagnose Instrument in the Agilent 2100 Bioanalyzer software.
- 2 Select any of the hardware tests you want to apply from the list given in the interface. The recommendation is to apply all tests.



3 Click the Start Diagnosis button to execute the tests.

NOTE

If there is no communication between the Agilent 2100 Bioanalyzer and the PC, the software will prompt you. See **Communication— 2** for troubleshooting hints.

4 Follow the instructions as given by the Agilent 2100 Bioanalyzer software.

5 At the end of the procedure all tests must be passed.

6 If the tests are not passed, redo the tests.

7 If failures still persist, call Agilent Technologies.

Troubleshooting the DNA Application

Good Measurement Practices

For hints on how to handle chips and chemicals, see **Good Measurement Practices—22**

Troubleshooting the Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms—157**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause
- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Too High Quantification Results—159

Too Low Quantification Results—160

Wrong Sizing Result—161

Chip Not Detected—163



Additional Sample or Ladder Peaks—165



Spikes/Glitches—168



Poor Sensitivity—170



Noisy Electropherogram—173



Broad Peaks—175



Missing Peaks or Marker Peaks—177



Poor Baseline: Dips—181



Poor Baseline: Drift—183



Poor Baseline: Noise—186



Poor Baseline: Bend—189



No Peaks and High Background—191



No Peaks and Low Background—194



Cross Contamination—196



Peaks Migrating Late—198



Bend Ladder Baseline—200

Too High Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Too Low Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the DNA Reagent Kit Guide.

Wrong Sizing Result

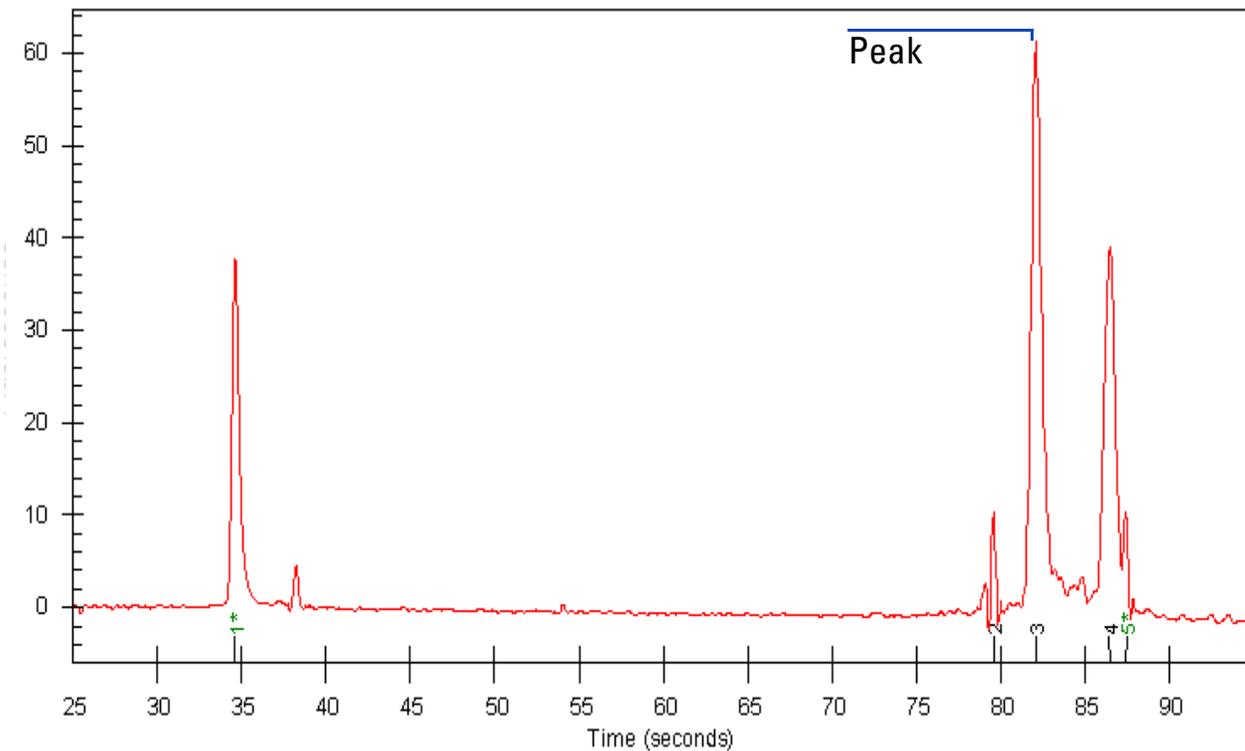
Most Probable Causes	Solution
dsDNA ladder degraded.	Check expiration date of chemicals.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
No ladder in ladder well.	Use a new chip.

Least Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Chip Not Detected

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted.
Lid sensor broken.	Check lid sensor using the hardware diagnostic tools.
Probable Causes	Solution
No communication between Agilent 2100 Bioanalyzer and PC.	Check whether serial cable is connected. Check status control image of Agilent 2100 Bioanalyzer (open and close the lid.)
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Least Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
No sample or buffer in well.	Pipette sample or buffer in all wells.

Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks—165**

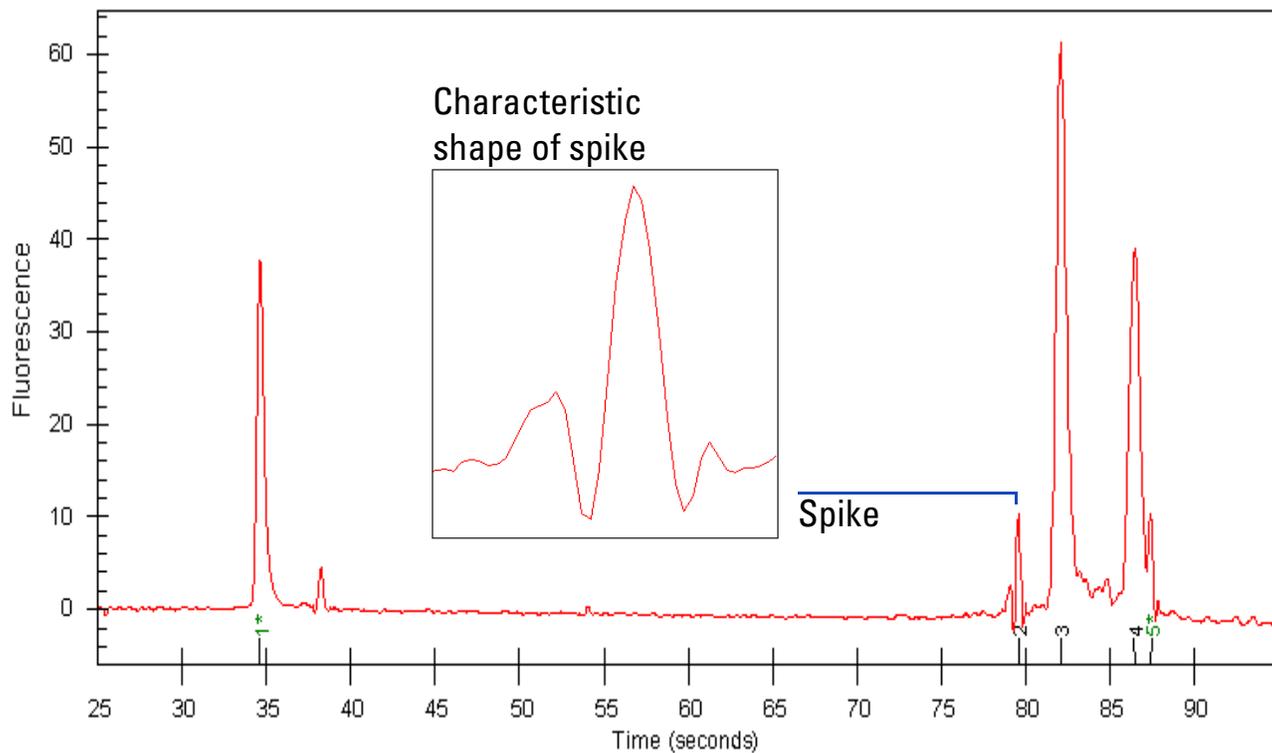
Back to **Symptoms—157**

Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Least Probable Causes	Solution
dsDNA ladder degraded.	Check expiration date of chemicals
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Spikes/Glitches



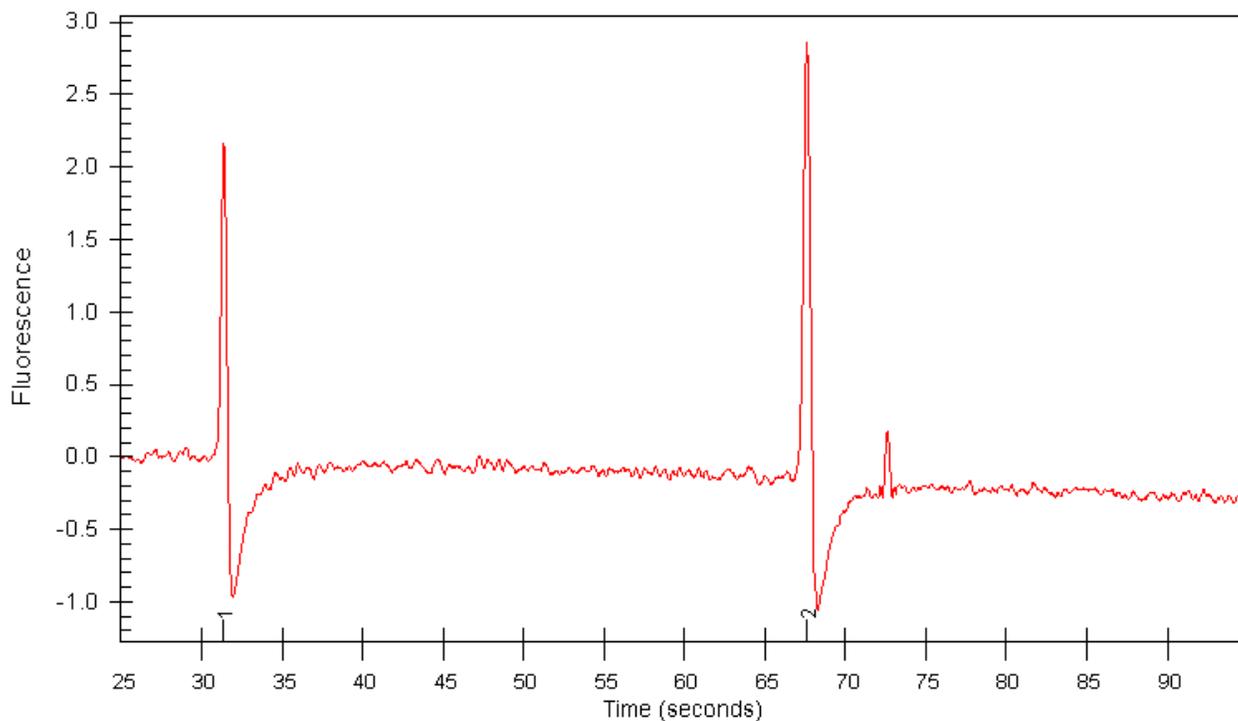
Show me how to solve **Spikes/Glitches—168**

Back to **Symptoms—157**

Spikes/Glitches

Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Power outlett	Install power filter.

Poor Sensitivity



Show me how to solve **Poor Sensitivity—170**

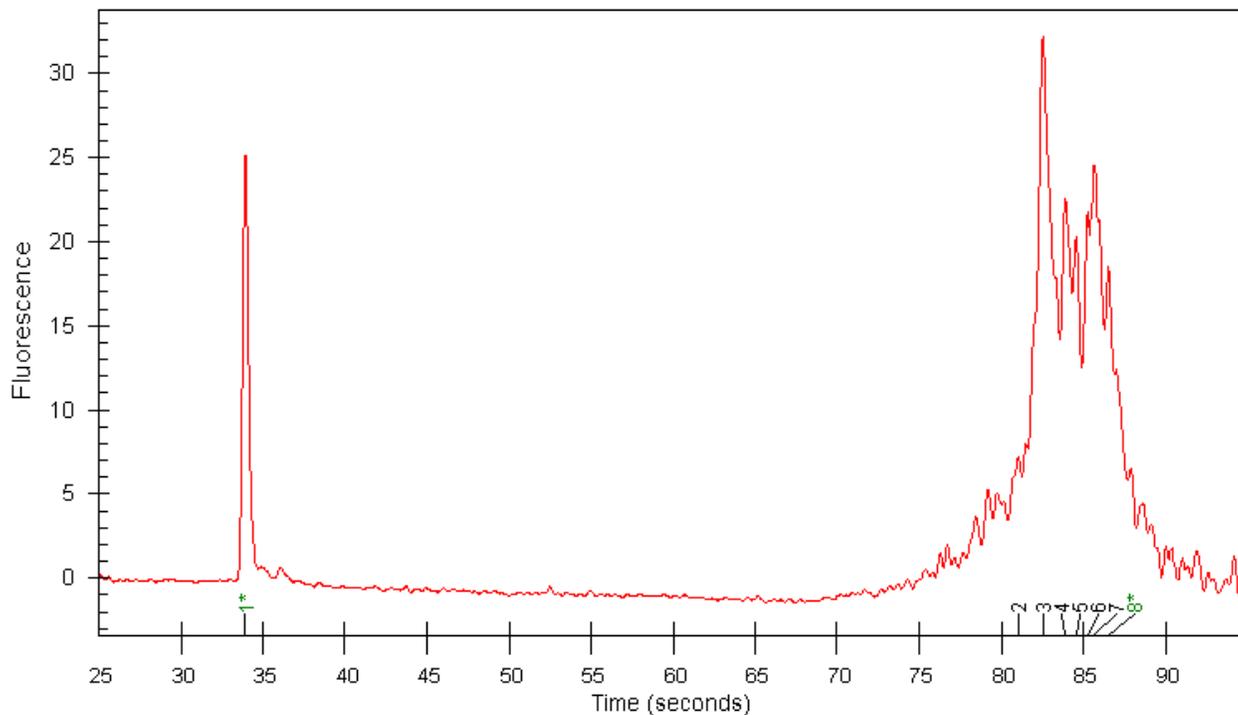
Back to **Symptoms—157**

Poor Sensitivity

Most Probable Causes	Solution
Insufficient vortexing of chip.	Vortex chip at medium setting for 1 minute.
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Fingerprint on focusing lens.	Clean lens using a dry cloth and isopropanol.
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vacuum pumps, from bench.</p>

Noisy Electropherogram



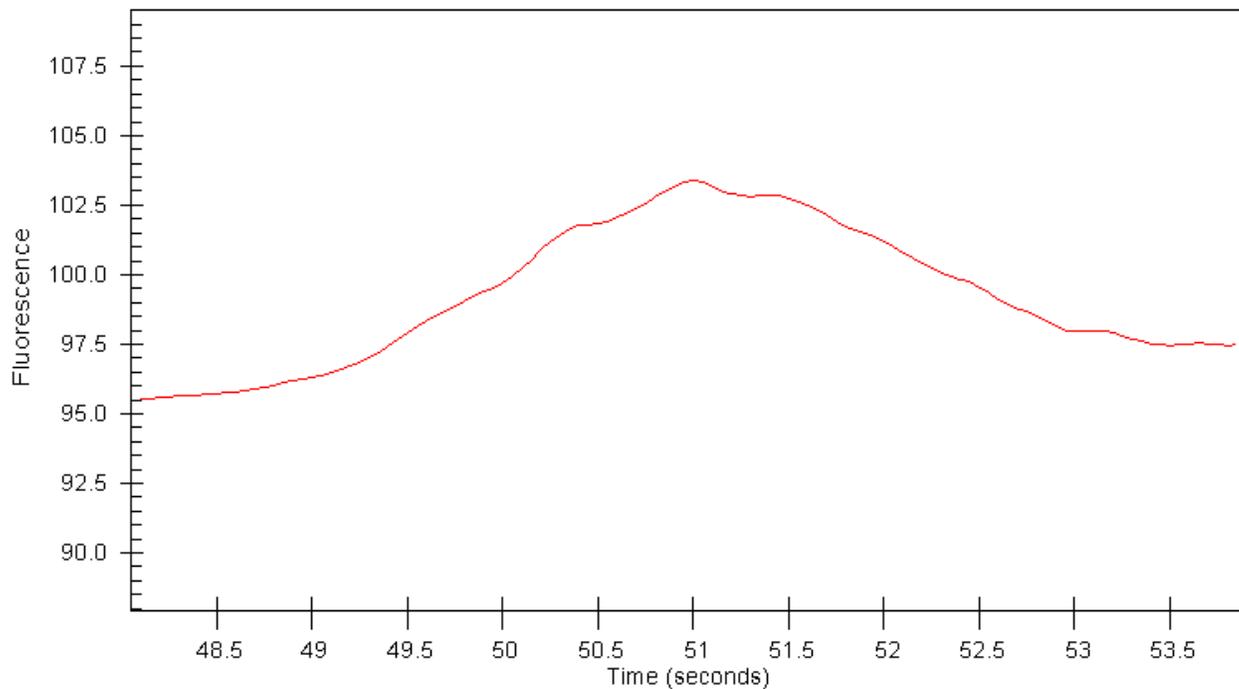
Show me how to solve **Noisy Electropherogram—173**

Back to **Symptoms—157**

Noisy Electropherogram

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable Causes	Solution
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Broad Peaks



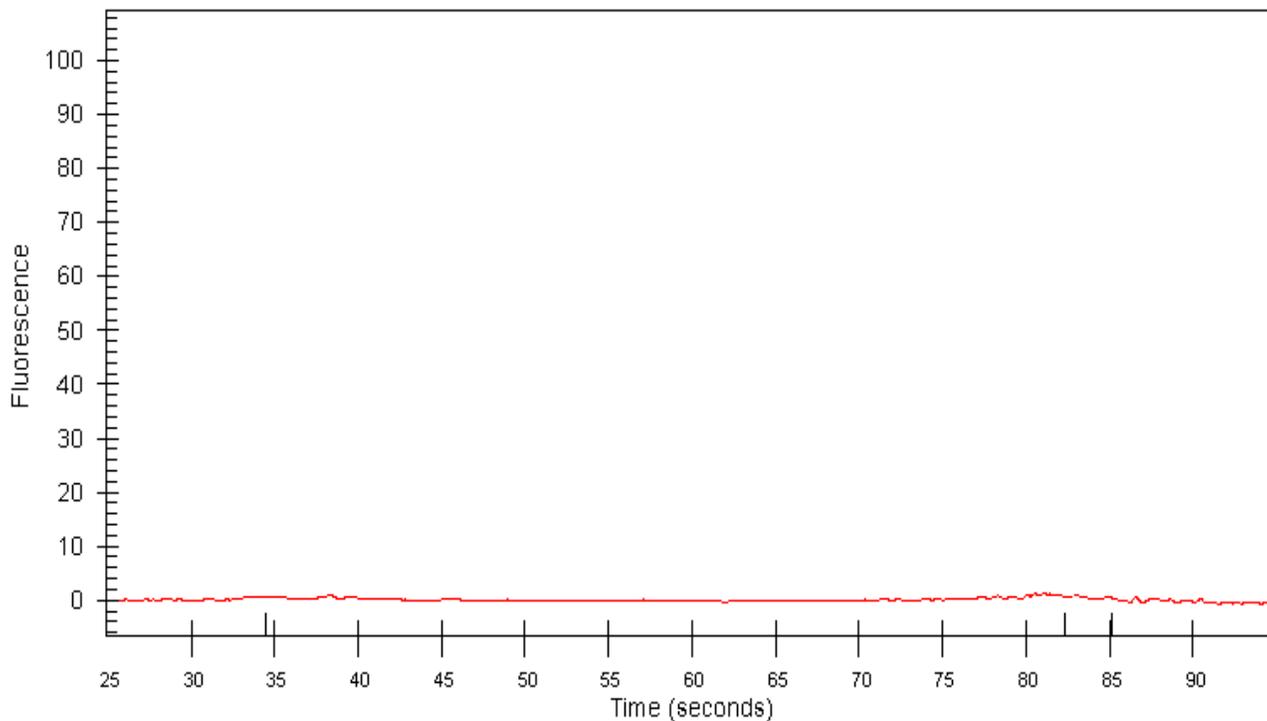
Show me how to solve **Broad Peaks—175**

Back to **Symptoms—157**

Broad Peaks

Most Probable Causes	Solution
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Replace electrode cartridge.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too high.	Use dye concentration according to the DNA Reagent Kit Guide.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Missing Peaks or Marker Peaks



Show me how to solve **Missing Peaks or Marker Peaks—177**

Back to **Symptoms—157**

Missing Peaks or Marker Peaks

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
Insufficient vortexing of chip.	Vortex chip at lower (medium) setting for 1 minute.
No ladder in ladder well.	Use a new chip.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
No sample in well.	Pipette sample in all wells.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 Replace electrode cartridge.

Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.

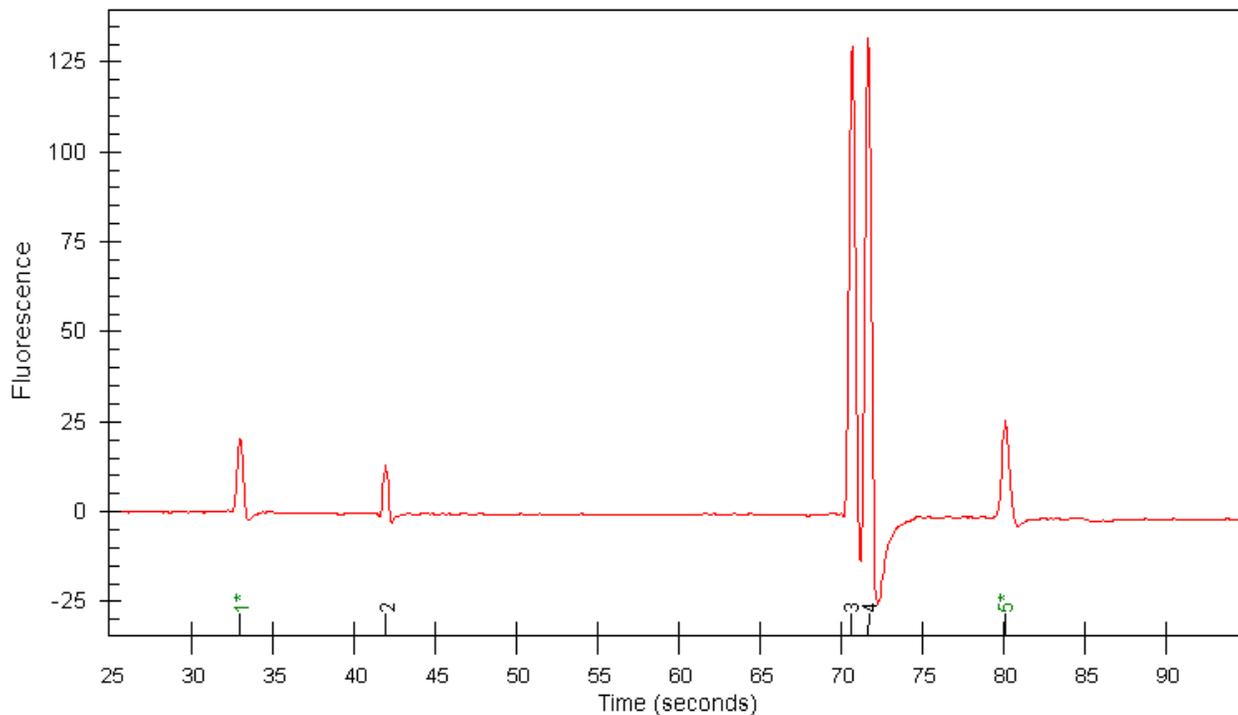
Laser defective.

Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

dsDNA ladder degraded.

Check expiration date of chemicals.

Poor Baseline: Dips



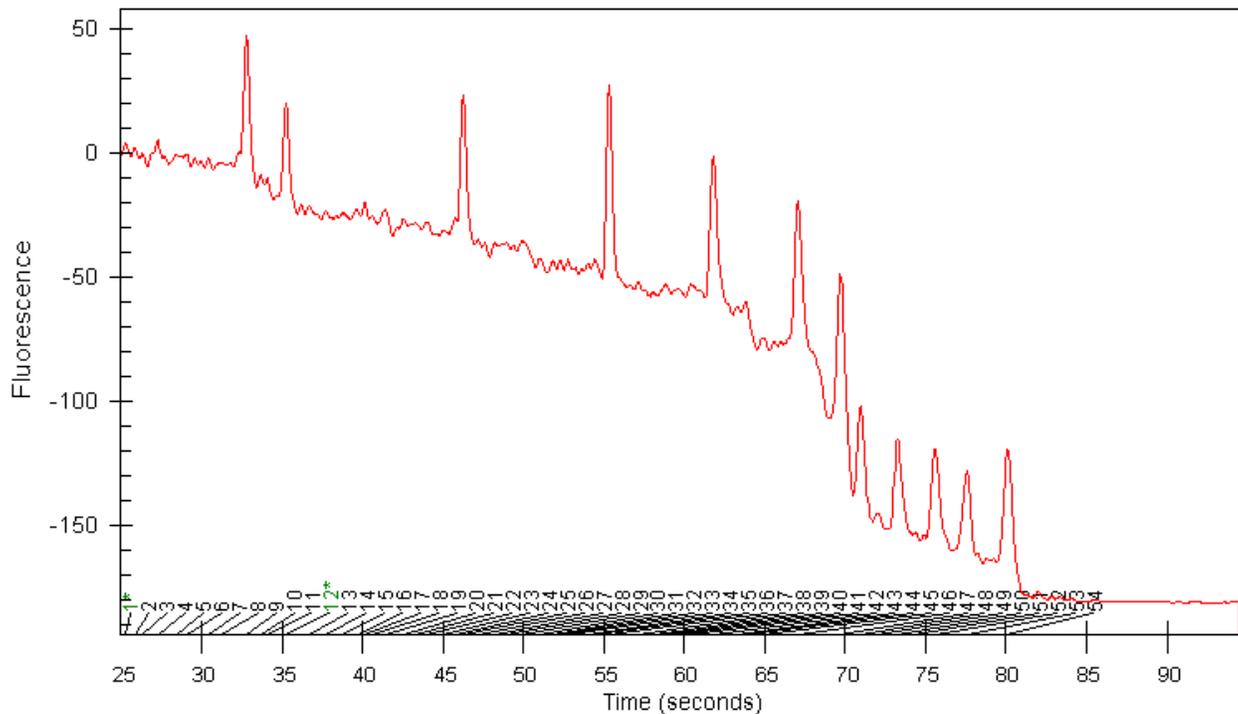
Show me how to solve **Poor Baseline: Dips—181**

Back to **Symptoms—157**

Poor Baseline: Dips

Most Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Too high sample concentration.	Use sample concentration according to the DNA Reagent Kit Guide.

Poor Baseline: Drift

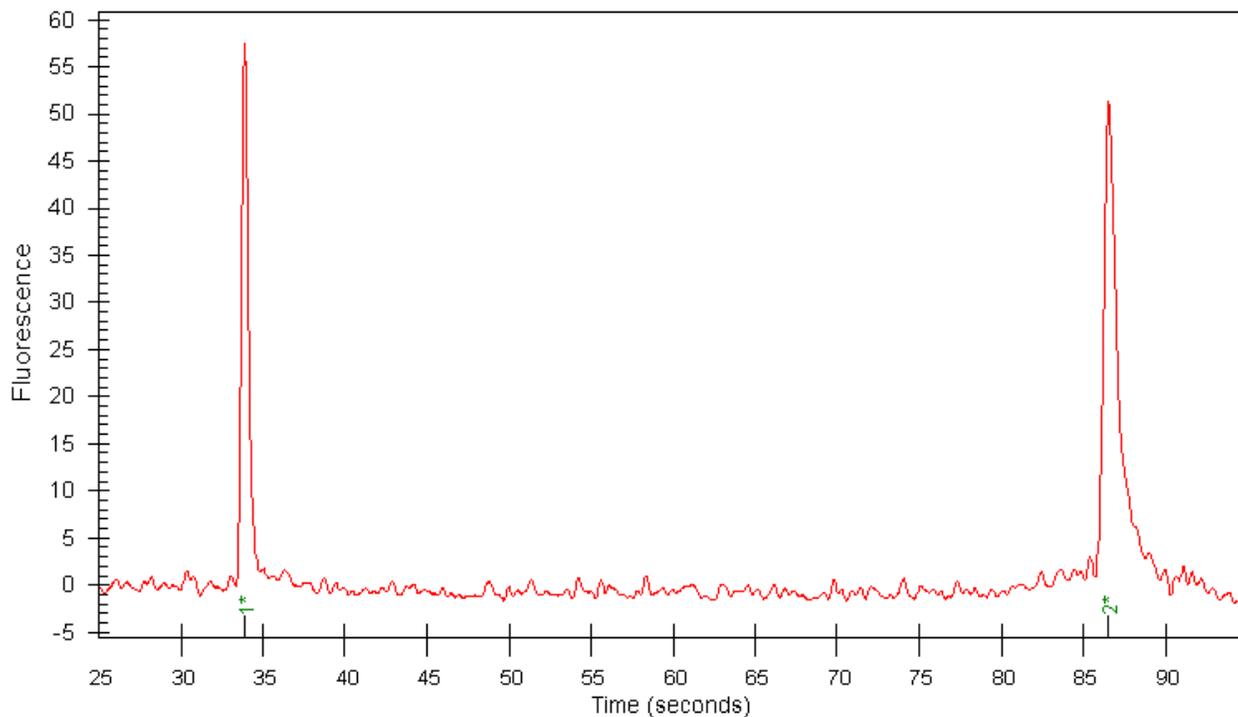


Poor Baseline: Drift

Most Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Leak current due to dirty electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Replace electrode cartridge.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
Laser defective.	Check Laser by using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Poor Baseline: Noise



Show me how to solve **Poor Baseline: Noise—186**

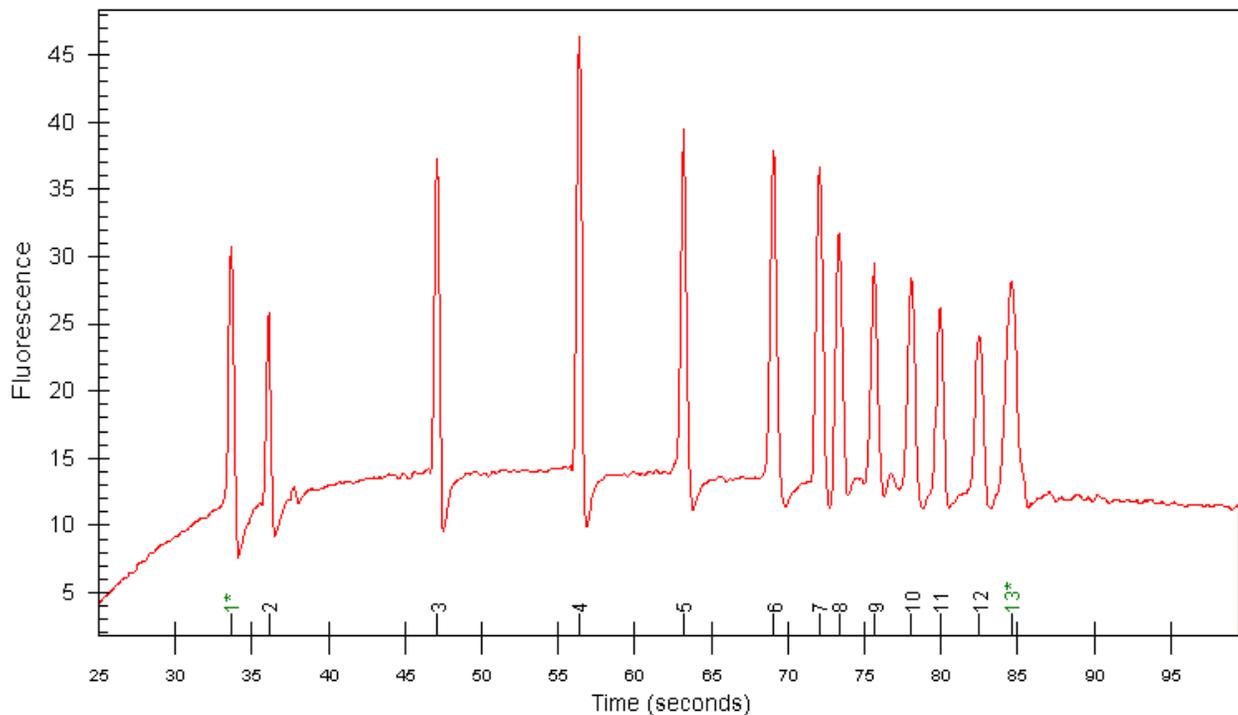
Back to **Symptoms—157**

Poor Baseline: Noise

Most Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.

Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Laser defective.	Check Laser by using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Poor Baseline: Bend



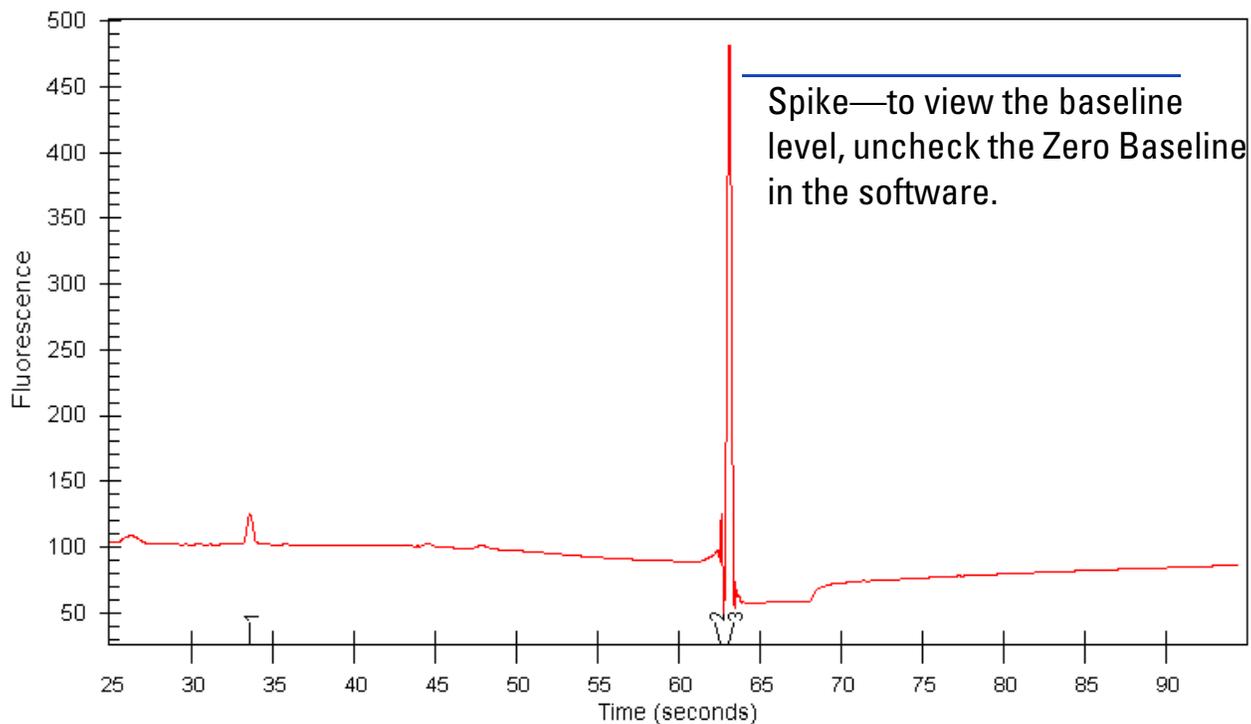
Show me how to solve **Poor Baseline: Bend—189**

Back to **Symptoms—157**

Poor Baseline: Bend

Most Probable Causes	Solution
Too cold reagents and supplies.	Allow all reagents to equilibrate to room temperature before use. Apply baseline correction algorithm. (software revision A.01.20 or later)

No Peaks and High Background



Show me how to solve **No Peaks and High Background—191**

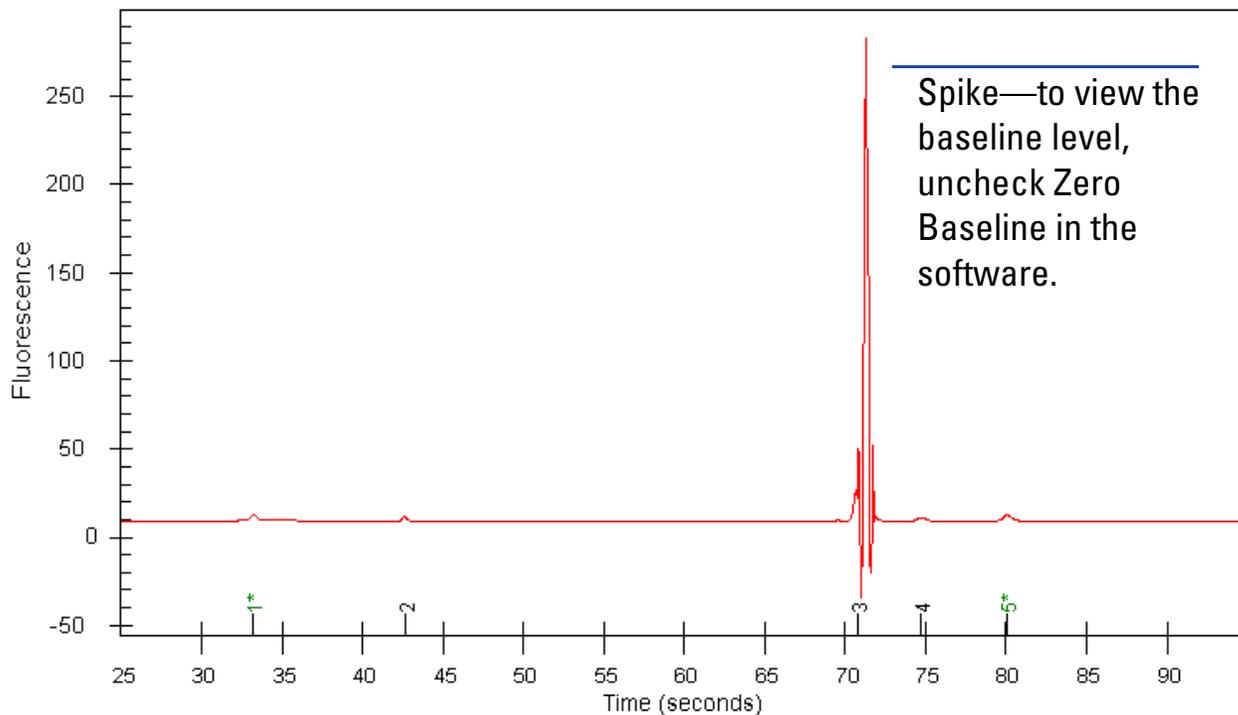
Back to **Symptoms—157**

No Peaks and High Background

Most Probable Causes	Solution
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 Replace electrode cartridge.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
No sample in well.	Pipette sample in all wells.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
High voltage power supply defective.	<p>Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.</p>
Broken chip.	<p>Don't use a chip that has dropped on the floor, always use a new one.</p>
Fingerprint on focusing lens.	<p>Clean lens using a dry cloth and isopropanol.</p>

No Peaks and Low Background



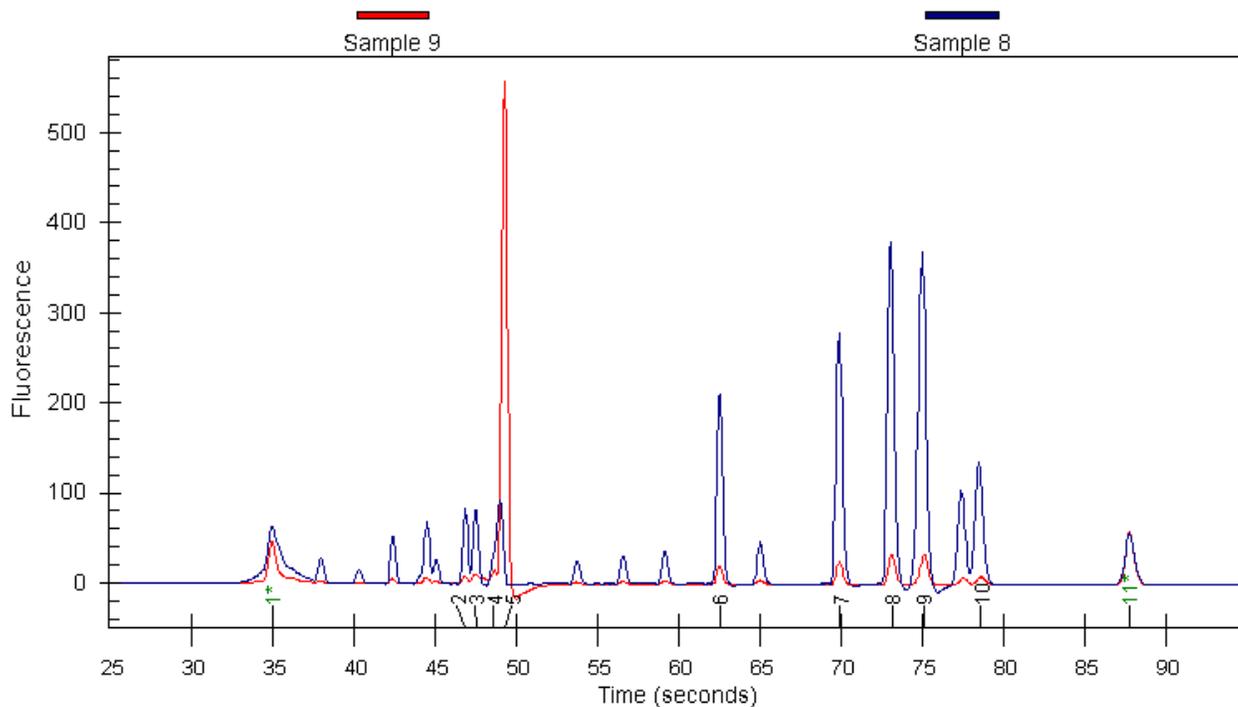
Show me how to solve **No Peaks and Low Background—194**

Back to **Symptoms—157**

No Peaks and Low Background

Most Probable Causes	Solution
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide.
Probable Causes	Solution
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).

Cross Contamination



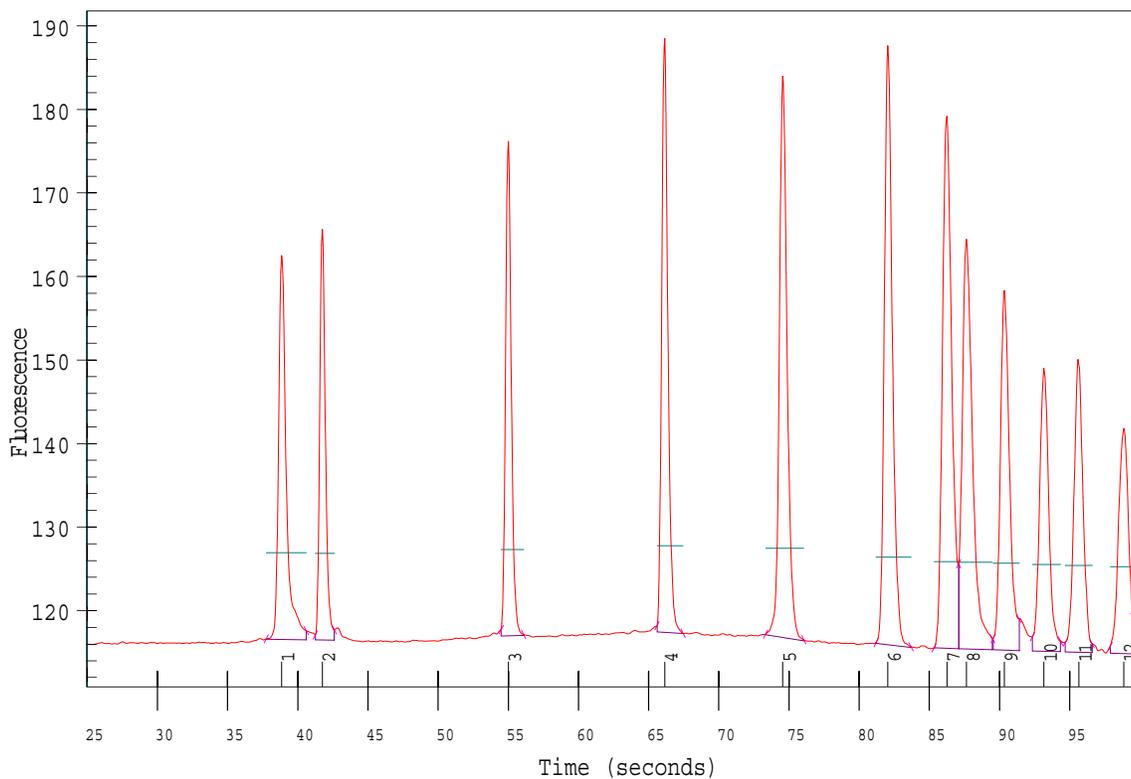
Show me how to solve **Cross Contamination—196**

Back to **Symptoms—157**

Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Replace electrode cartridge.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Peaks Migrating Late



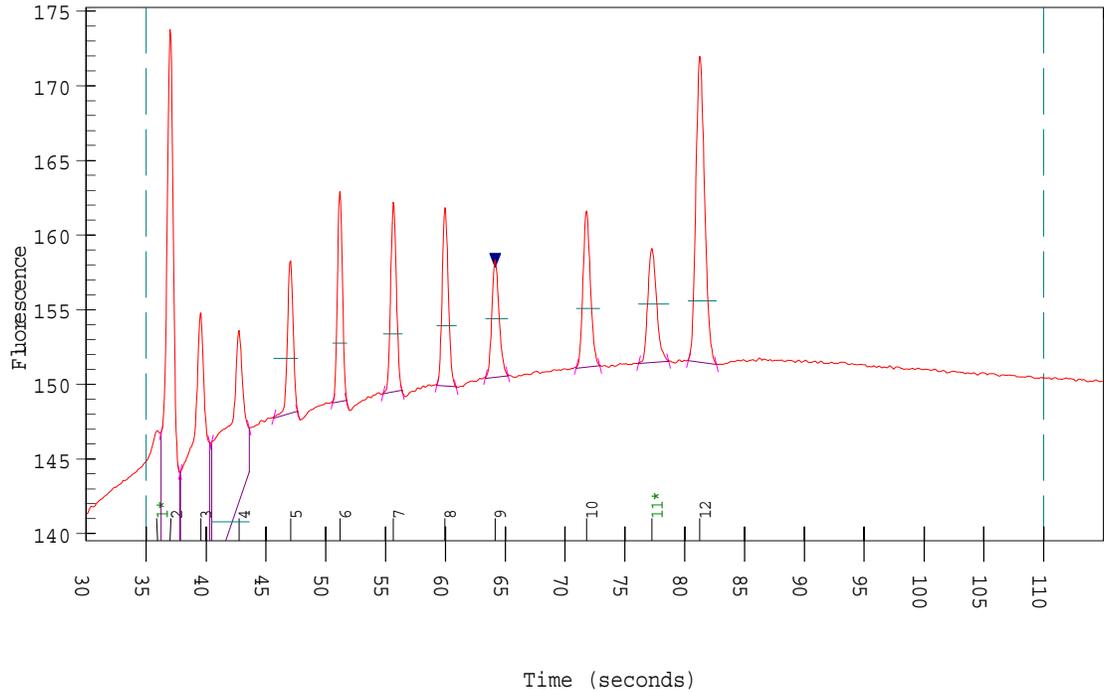
Show me how to solve **Peaks Migrating Late—198**

Back to **Symptoms—157**

Peaks Migrating Late

Most Probable Causes	Solution
Vortex speed too high.	Vortex at lower (medium) speed.
Probable Causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock).

Bend Ladder Baseline



Show me how to solve **Bend Ladder Baseline—200**

Back to **Symptoms—157**

Bend Ladder Baseline

Most Probable Causes	Solution
Temperature of gel/dye mix too low.	Allow gel/dye mix to equilibrate to room temperature for 30 min before use. Apply baseline correction algorithm. (software revision A.01.20 or later)

Troubleshooting the RNA Application

Good Measurement Practices

For hints on how to handle chips and chemicals, see **Good Measurement Practices**.

Troubleshooting the Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause
- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Too High Quantification Results

Too Low Quantification Results

Wrong Quantification Result

Chip Not Detected



Additional Sample or Ladder Peaks



Spikes/Glitches



Poor Sensitivity



Noisy Electropherogram



Broad Peaks



Missing Peaks



Poor Baseline: Dips



Poor Baseline: Drift



Poor Baseline: Noise



No Peaks and High Background



No Peaks and Low Background



Cross Contamination

Too High Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Too Low Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip at medium setting for 1 minute.
Inaccurate reference measurement (e.g. UV-absorption) due to remaining UV absorbing solvent in the sample.	Purify sample for UV measurement.
Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.
Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the RNA Reagent Kit Guide.

Wrong Quantification Result

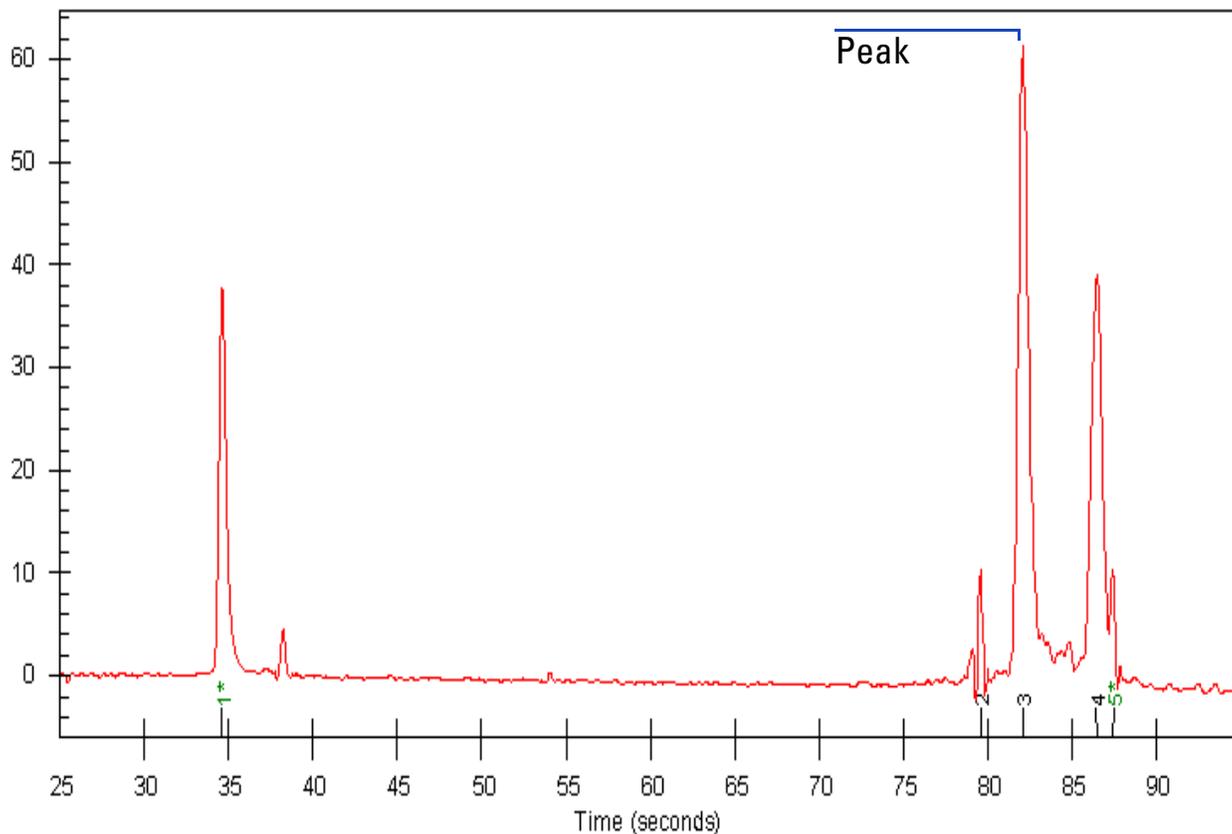
Most Probable Causes	Solution
RNA ladder degraded.	Check expiration date of chemicals.
Electrodes contaminated with RNAses.	Clean electrodes with RNaseZAP.
Wrong time window in ribosomal peak assignment selected.	Select correct time window.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
No ladder in ladder well.	Use a new chip.
Probable Causes	Solution
Insufficient vortexing.	Vortex at higher (medium) speed.

Least Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Chip Not Detected

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted.
Lid sensor broken.	Check lid sensor using the hardware diagnostic tools.
Probable Causes	Solution
No communication between Agilent 2100 Bioanalyzer and PC.	Check whether serial cable is connected. Check status control image of Agilent 2100 Bioanalyzer (open and close the lid.)
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Least Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
No sample or buffer in well.	Pipette sample or buffer in all wells.

Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

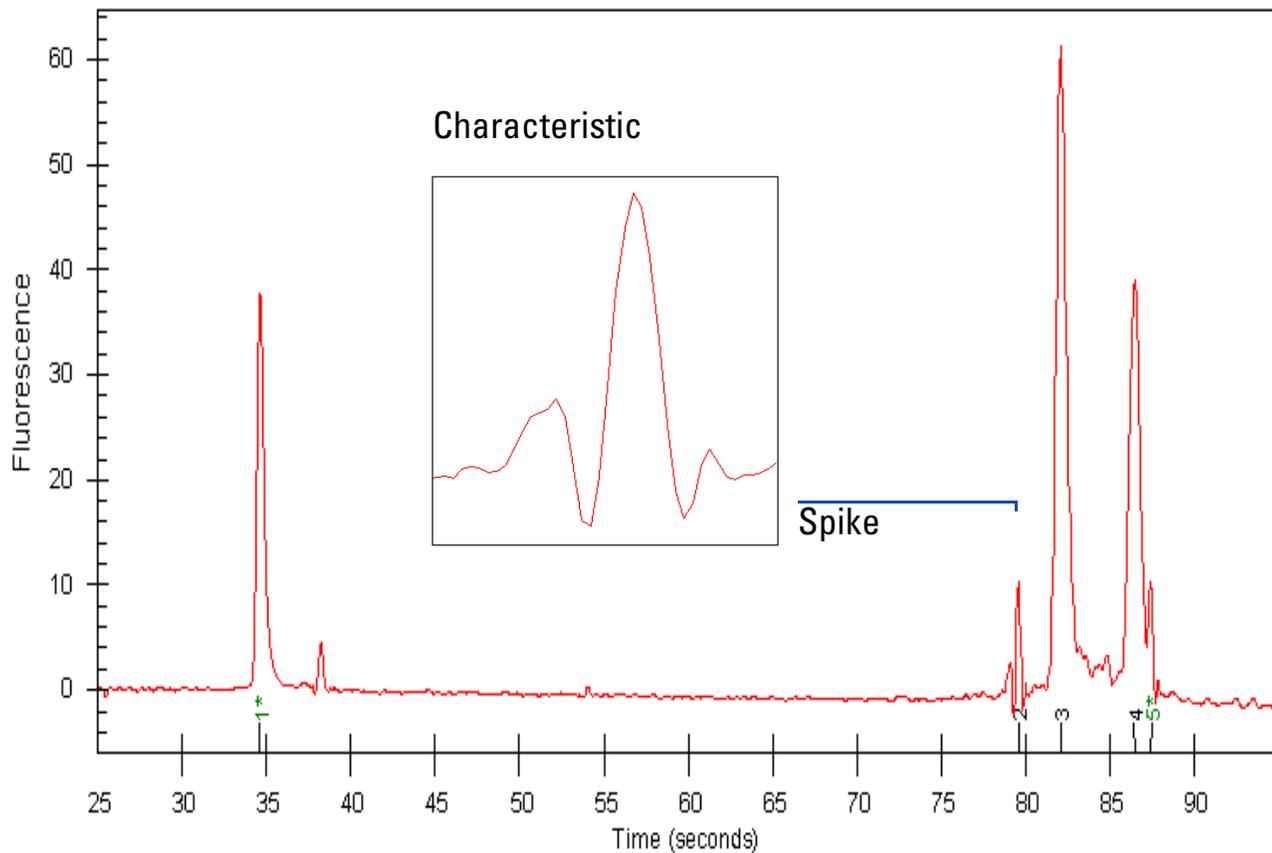
Back to **Symptoms**

Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Probable Causes	Solution
RNA ladder/sample not denatured properly..	Heat ladder/samples at 70°C for 2 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Least Probable Causes	Solution
RNA ladder degraded.	Check expiration date of chemicals
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Spikes/Glitches



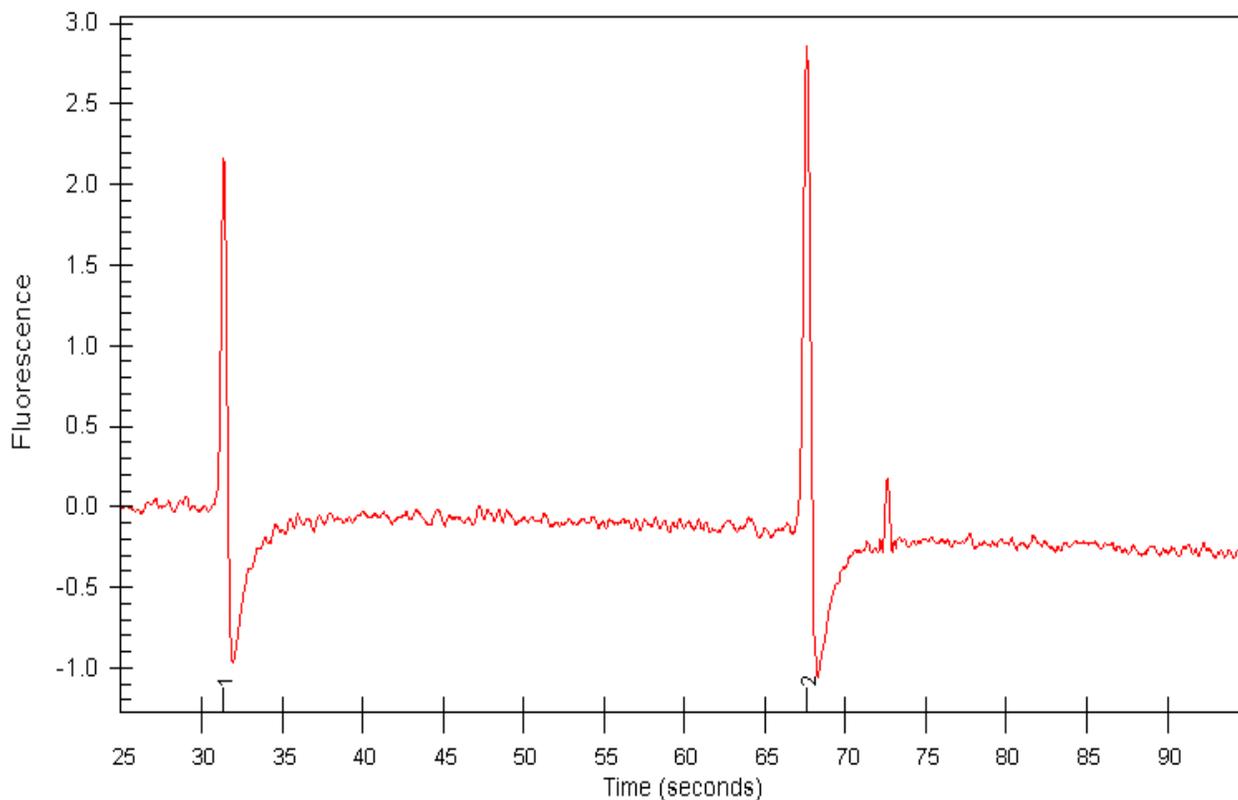
Show me how to solve **Spikes/Glitches**

Back to **Symptoms**

Spikes/Glitches

Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
RNA ladder/sample not denatured properly..	Heat ladder/samples at 70°C for 2 min.
Power outlett	Install power filter.

Poor Sensitivity



Show me how to solve **Poor Sensitivity**

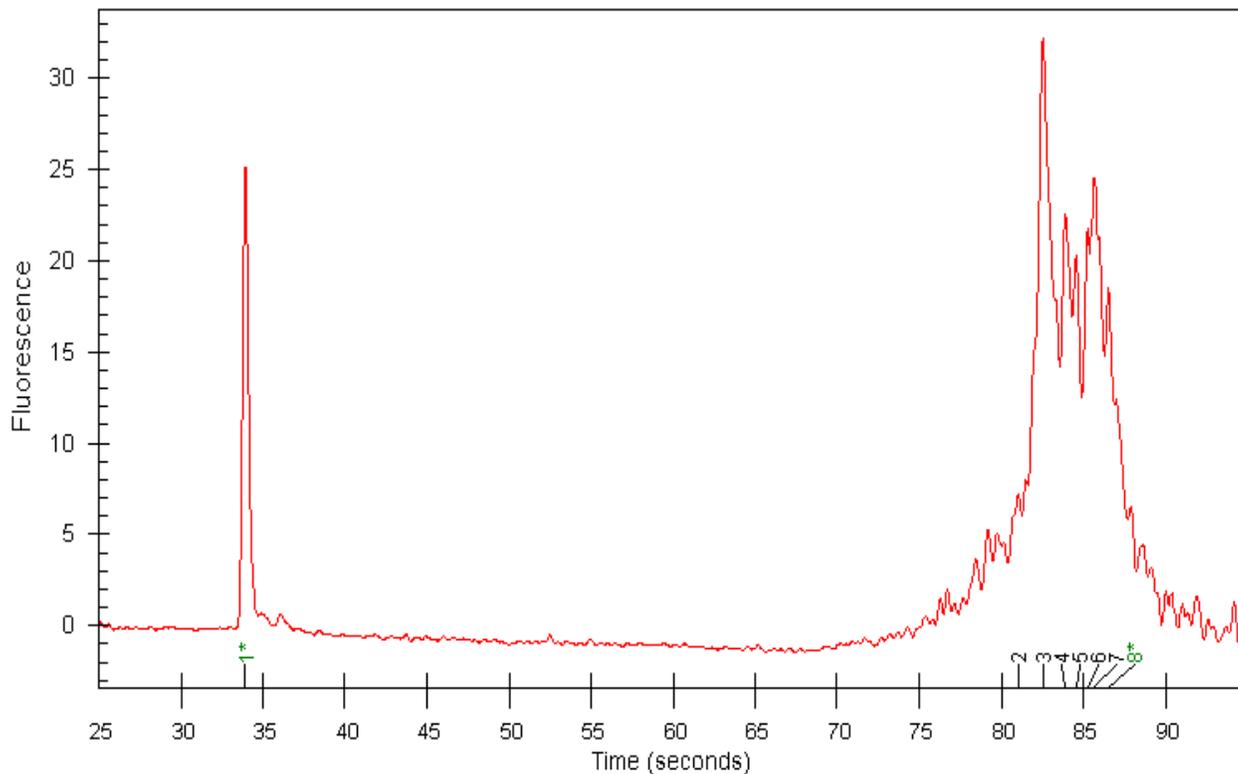
Back to **Symptoms**

Poor Sensitivity

Most Probable Causes	Solution
Insufficient vortexing of chip.	Vortex chip at medium setting for 1 minute.
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Fingerprint on focusing lens.	Clean lens using a dry cloth and isopropanol.
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.

Noisy Electropherogram



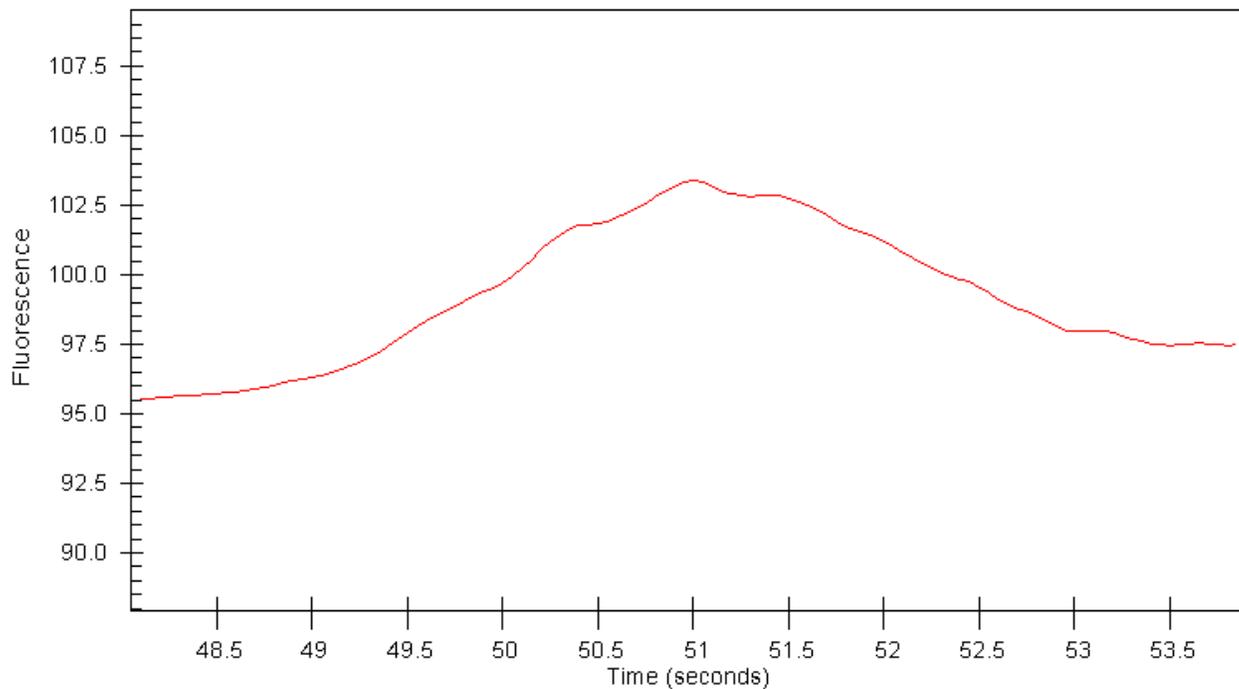
Show me how to solve **Noisy Electropherogram**

Back to **Symptoms**

Noisy Electropherogram

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable Causes	Solution
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Broad Peaks



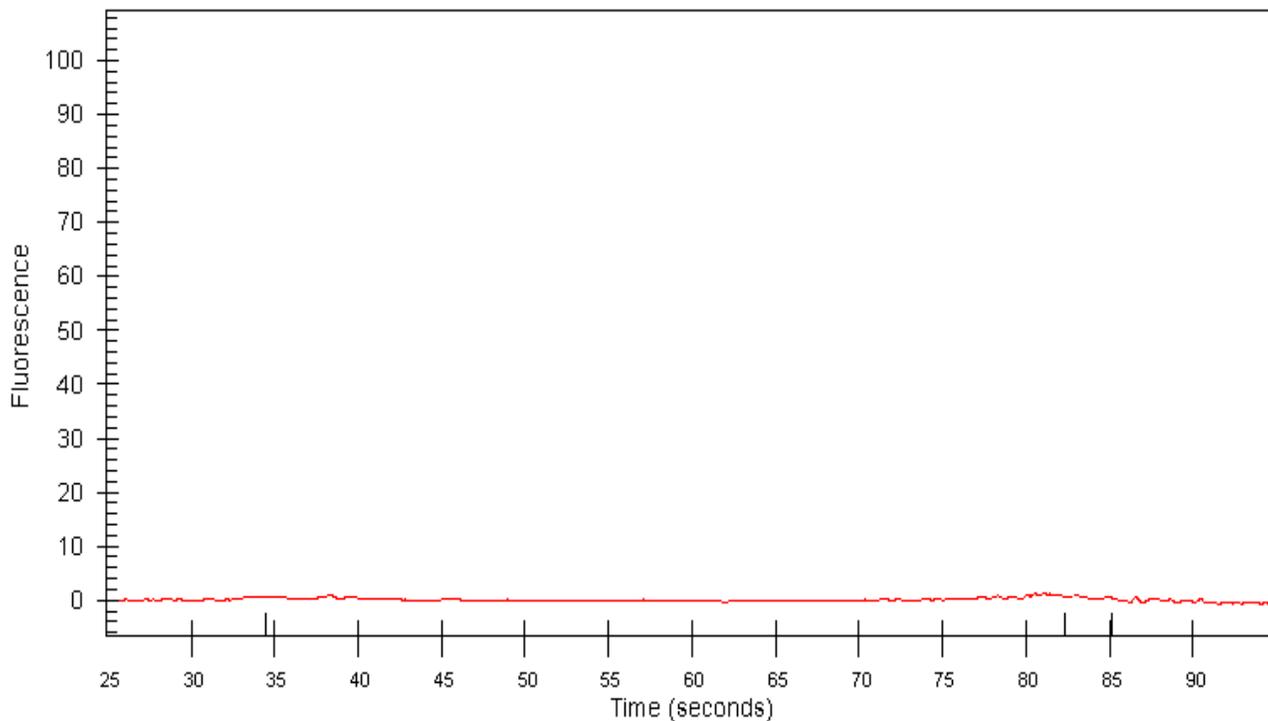
Show me how to solve **Broad Peaks**

Back to **Symptoms**

Broad Peaks

Most Probable Causes	Solution
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance Replace electrode cartridge.
Electrodes contaminated with RNAses.	Clean electrodes with RNaseZAP.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too high.	Use dye concentration according to the RNA Reagent Kit Guide.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Missing Peaks



Show me how to solve **Missing Peaks**

Back to **Symptoms**

Missing Peaks

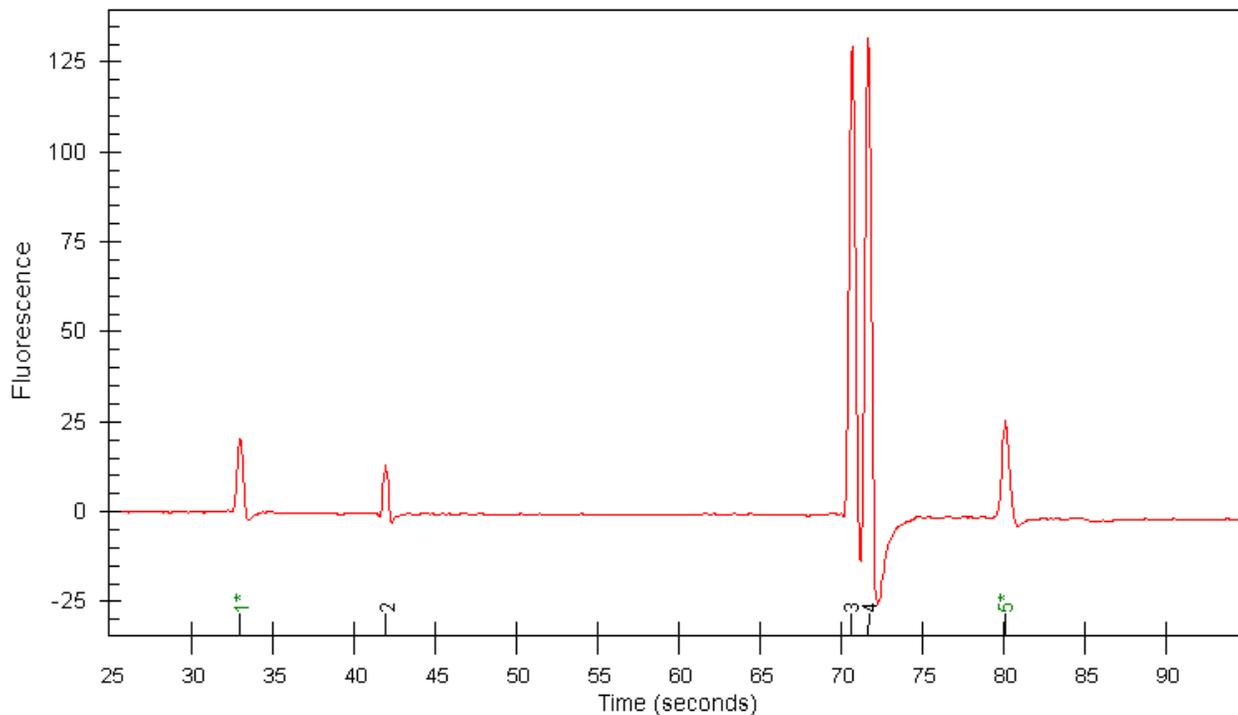
Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.
No ladder in ladder well.	Use a new chip.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
RNA ladder degraded.	Follow decontamination procedure, see the Reagent Kit Guide, and use a new chip.
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
No sample in well.	Pipette sample in all wells.
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance Replace electrode cartridge.
Probable Causes	Solution

Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Dye concentration too low	Use dye concentration according to the RNA Reagent Kit Guide.

Laser defective.

Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Poor Baseline: Dips



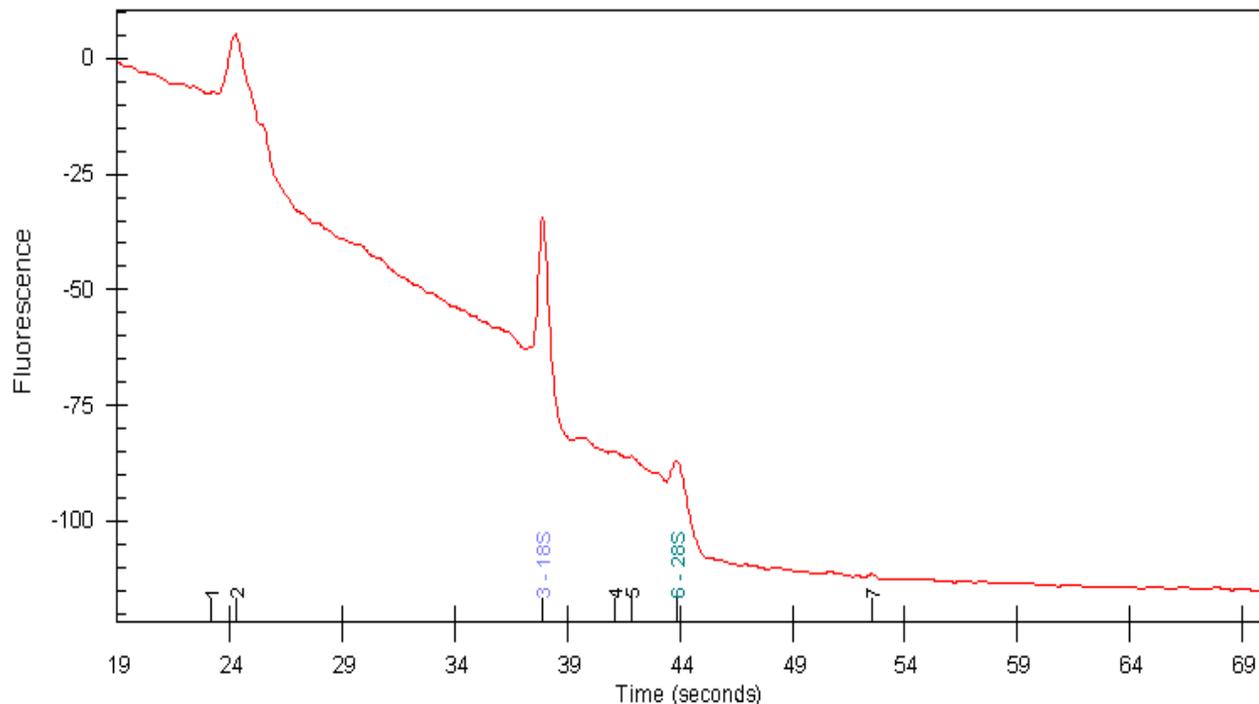
Show me how to solve **Poor Baseline: Dips**

Back to **Symptoms**

Poor Baseline: Dips

Most Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Too high sample concentration.	Use sample concentration according to the RNA Reagent Kit Guide.

Poor Baseline: Drift



RNA Area = 97.05

RNA Conc. = 21.00 ng/ul

28S/18S = 0.31

Show me how to solve **Poor Baseline: Drift**

Back to **Symptoms**

Poor Baseline: Drift

Most Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
Non denaturated RNA sample (for total RNA samples only).	Heat sample to 70°C for 2 min before loading on chip.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Insufficient vortexing of chip.	Change vortex speed to higher (medium) speed.
Leak current due to dirty electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance Replace electrode cartridge.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.

Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
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Least Probable Causes	Solution
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Chip contaminated.	
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	Wear powder-free gloves only.
--	-------------------------------

	Don't touch the underside of the chip.
--	--

	Don't touch the wells of the chip.
--	------------------------------------

	Clean the electrodes.
--	-----------------------

	Load the chip immediately after taking it out of its sealed bag.
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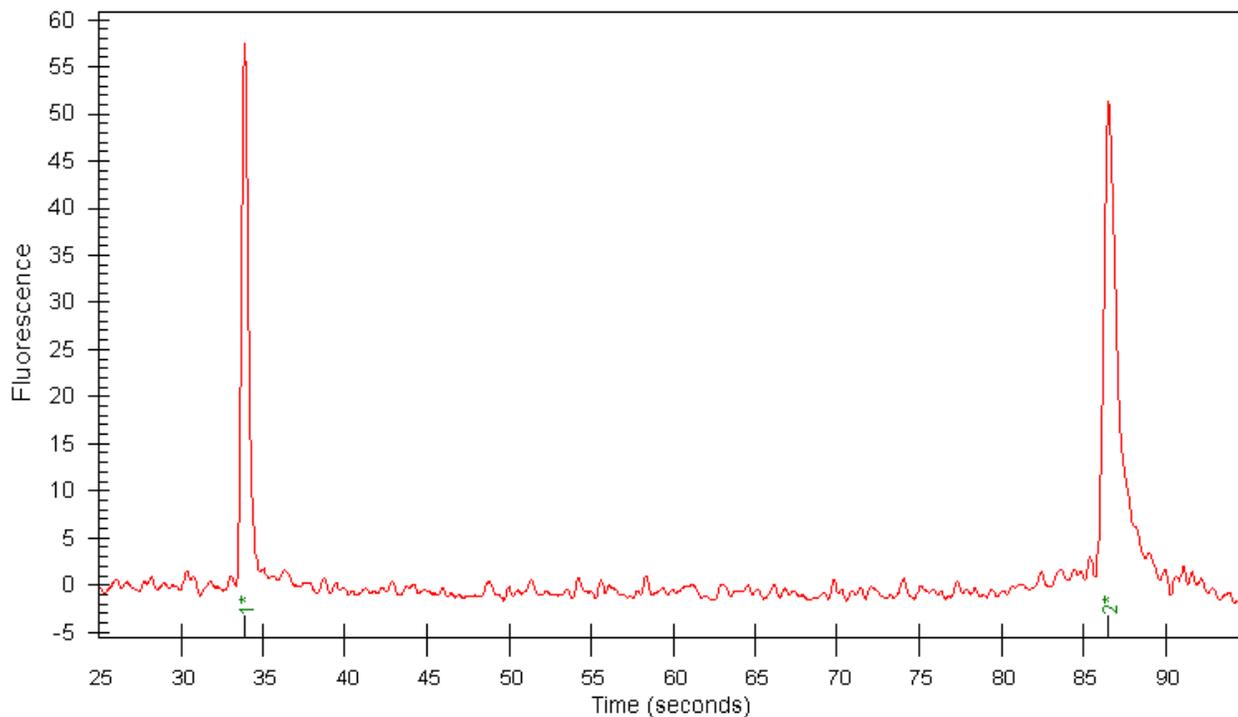
Changes of ambient temperature of more than 5 °C during the run.	
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	Place Agilent 2100 Bioanalyzer in thermally stable environment.
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Laser defective.	
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	Check Laser by using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.
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Poor Baseline: Noise



Show me how to solve **Poor Baseline: Noise**

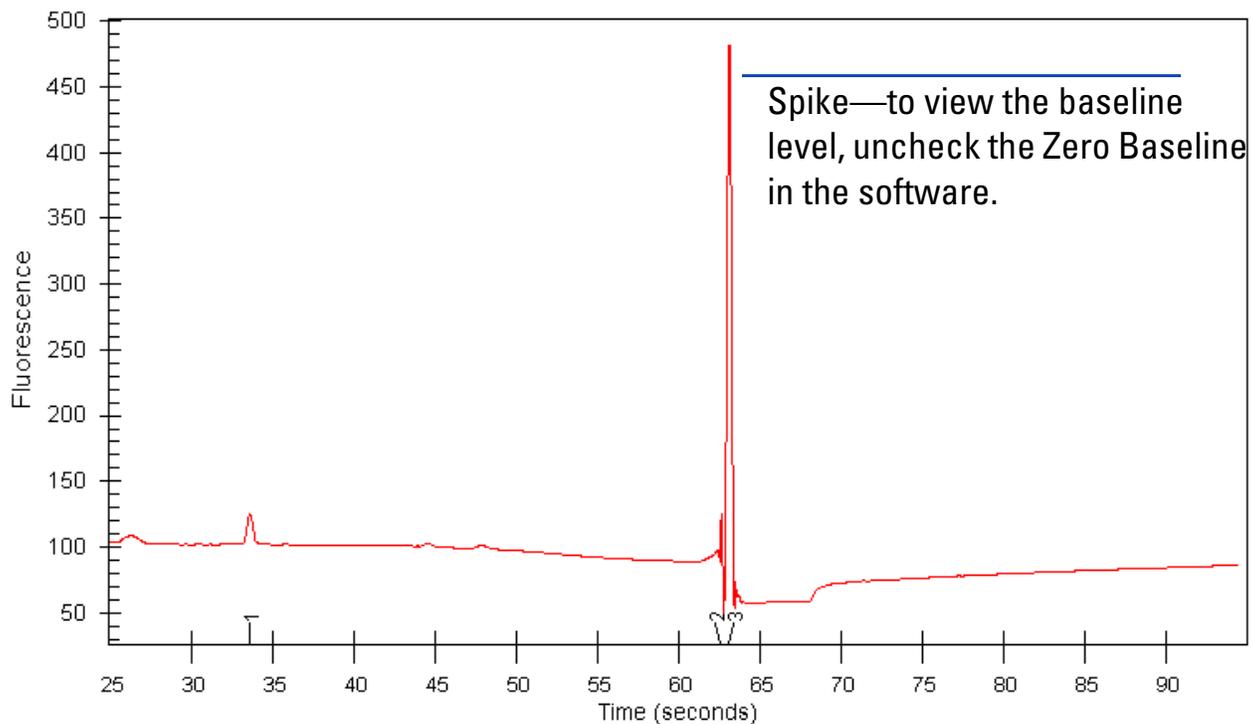
Back to **Symptoms**

Poor Baseline: Noise

Most Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.

Least Probable Causes	Solution
Laser defective.	Check Laser by using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.

No Peaks and High Background



Show me how to solve **No Peaks and High Background**

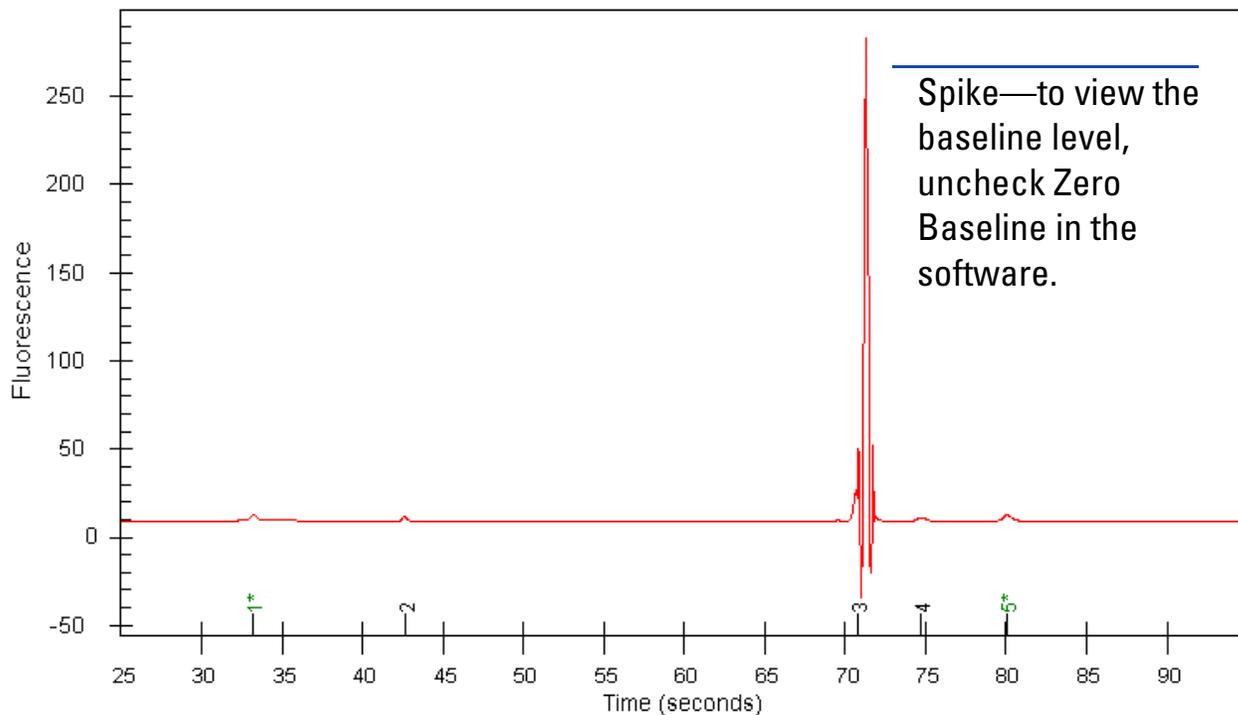
Back to **Symptoms**

No Peaks and High Background

Most Probable Causes	Solution
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance Replace electrode cartridge.
Probable Causes	Solution
Overpriming of chip (gel-dye mix)..	Prime chip for exactly 1 min. Apply proper priming pressure (adjustable clip in medium position).
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
No sample in well.	Pipette sample in all wells.

Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Fingerprint on focusing lens.	Clean lens using a dry cloth and isopropanol.

No Peaks and Low Background



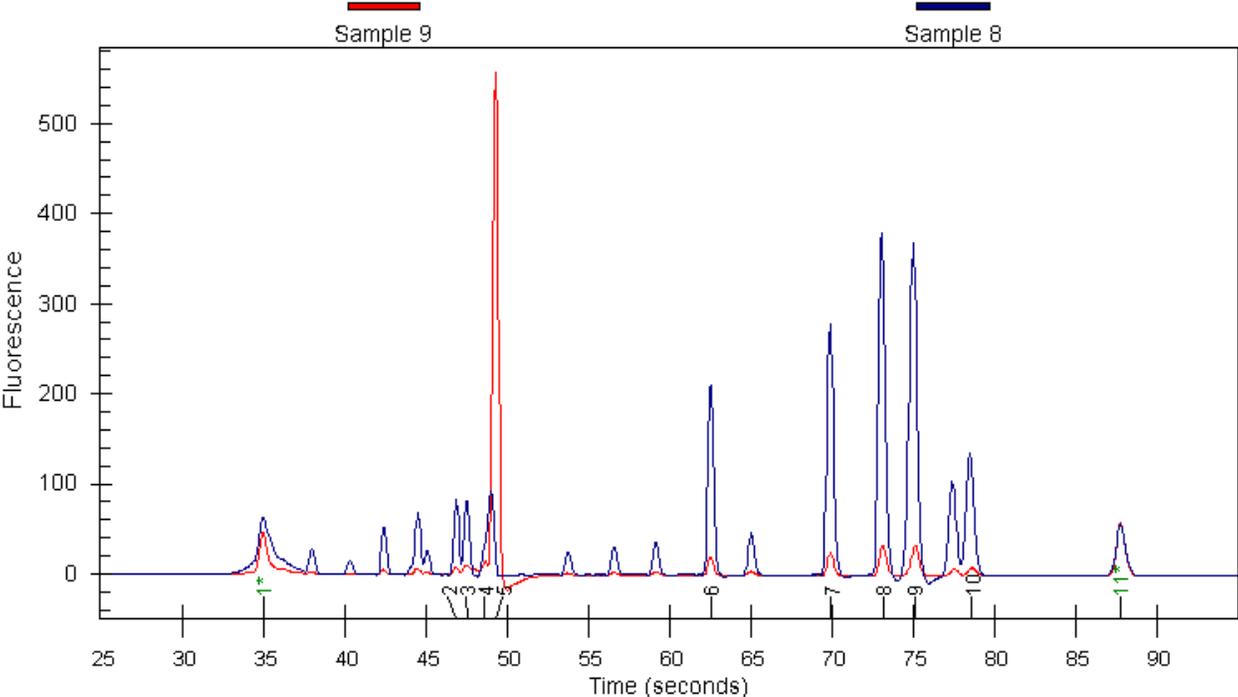
Show me how to solve **No Peaks and Low Background**

Back to **Symptoms**

No Peaks and Low Background

Most Probable Causes	Solution
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide.
Probable Causes	Solution
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).

Cross Contamination



Show me how to solve **Cross Contamination**

Back to **Symptoms**

Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance . Replace electrode cartridge.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Troubleshooting the Protein Application

Good Measurement Practices

For hints on how to handle chips and chemicals, see **Good Measurement Practices—22**.

Troubleshooting the Protein Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms—243**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause
- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Too High Quantitation Results—244

TToo Low Quantitation Results—246

Wrong Sizing Result—247

Error Message: “Poor Chip Performance Detected”—249



Additional Sample or Ladder Peaks—252



Low or Missing Upper Marker—255



Low or Missing Lower Marker—257



No Peaks—259



Broad Peaks—262



Missing Sample Peaks—265



Poor Baseline: High Noise Level—268



Cross Contamination—271

Too High Quantitation Results

Most Probable Causes	Solution
Diluted samples are too old.	Use diluted samples within 30 minutes.
Upper marker not handled according to the instructions.	See Low or Missing Upper Marker—255 .
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Samples not completely denatured.	Heat sample/denaturing solution for 5 min at 100°C.
Sample/denaturing solution are dried out.	Sample/denaturing solution were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturing
Dye concentration too low (marker disappears).	Use dye concentration according to the Protein Reagent Kit Guide.

Least Probable Causes**Solution**

Loaded chip kept for too long before run.

Prepared chips must be used within 10 minutes.

Too Low Quantitation Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Loaded chip kept too long before run.	Prepared chips must be used within 10 min.
Too much upper marker added.	Refer to the Protein Reagent Kit Guide for correct marker concentration.
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide.
Diluted sample are too old.	Use diluted samples within 30 minutes.

Wrong Sizing Result

Most Probable Causes	Solution
Upper and/or lower marker wrongly assigned.	Check assignment of lower/upper marker. See Additional Sample or Ladder Peaks—251
Ladder peaks wrongly assigned.	Check assignment of ladder peaks.
No ladder in ladder well.	Use a new chip.
Protein ladder not properly denatutated.	Heat ladder for 5 min at 100°C.
Upper and/or lower marker missing.	See Low or Missing Lower Marker—256 See Low or Missing Upper Marker—254
Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.

Least Probable Causes	Solution
Protein ladder degraded.	Use fresh ladder aliquot.
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Error Message: “Poor Chip Performance Detected”

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Fill unused wells with lower marker solution or sample replicate.
Loaded chip kept too long before run.	Prepared chips must be used within 10 minutes.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Probable Causes	Solution
Lid sensor broken.	Check lid sensor using the hardware diagnostic tools.
Chip left in instrument after run.	Remove and discard chip after run. Clean electrodes after each run (see Maintenance section of User’s Guide.)
Liquid spill, dirty electrodes.	Clean electrodes after each run (see Maintenance section of User’s Guide.)
No communication between Agilent 2100 Bioanalyzer and PC.	Check whether serial cable is connected. Check status control image of Agilent 2100 Bioanalyzer (open and close the lid.)

High voltage power supply defective.

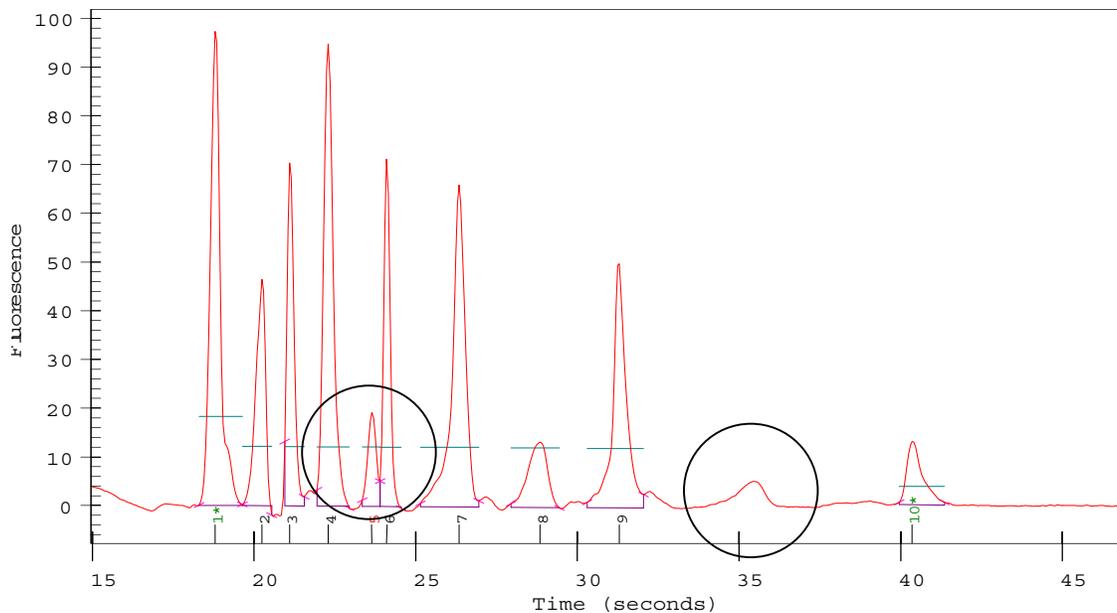
Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.

Least Probable Causes**Solution**

Broken chip.

Don't use a chip that has dropped on the floor, always use a new one.

Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks—252**

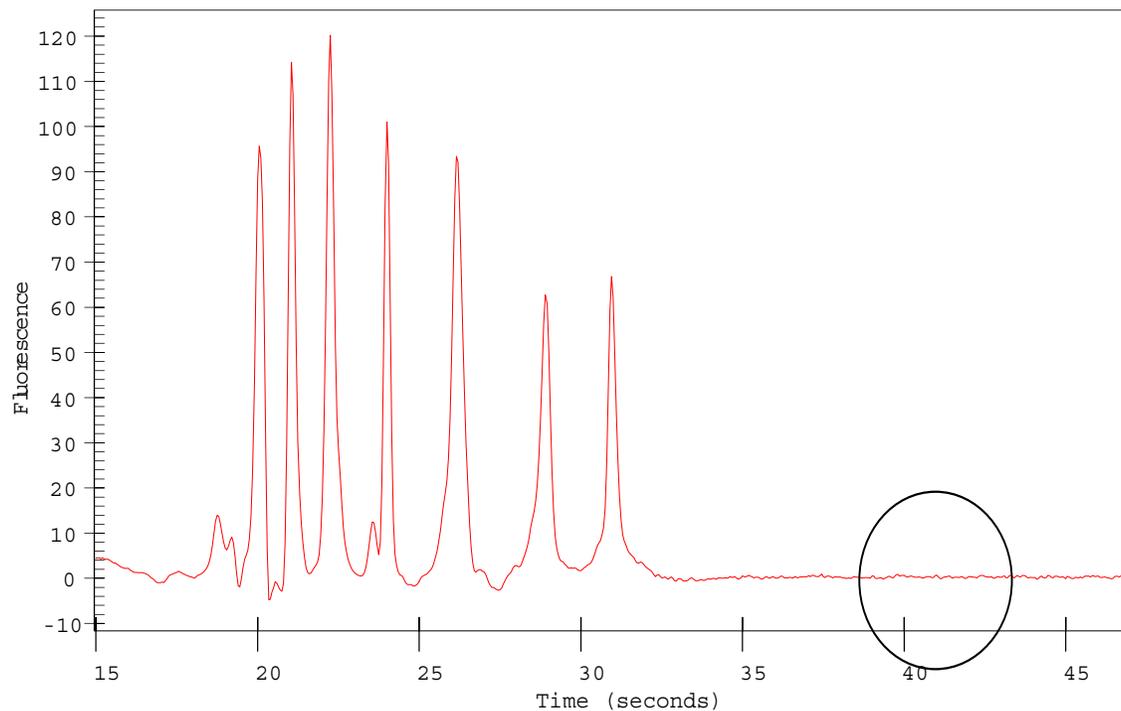
Back to **Symptoms—243**

Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Sample or ladder not denatured properly	Heat sample/denaturing solution and ladder for 5 min at 100°C
Sample/denaturing solution and/or ladder are dried out during denaturation.	Sample/denaturing solution and/or ladder were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturing
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
SDS not completely dissolved in sample buffer and/or dye concentrate.	Let sample buffer and dye concentrate equilibrate to room temperature for 30 min. Vortex sample buffer and dye concentrate well before use. Check for undissolved SDS crystals in the tubes.

Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vacuum pumps, from bench.
Loaded chip kept too long before run.	Prepared chips must be used within 10 min.
Least Probable Causes	Solution
Ladder degraded.	Use fresh ladder aliquot.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Low or Missing Upper Marker



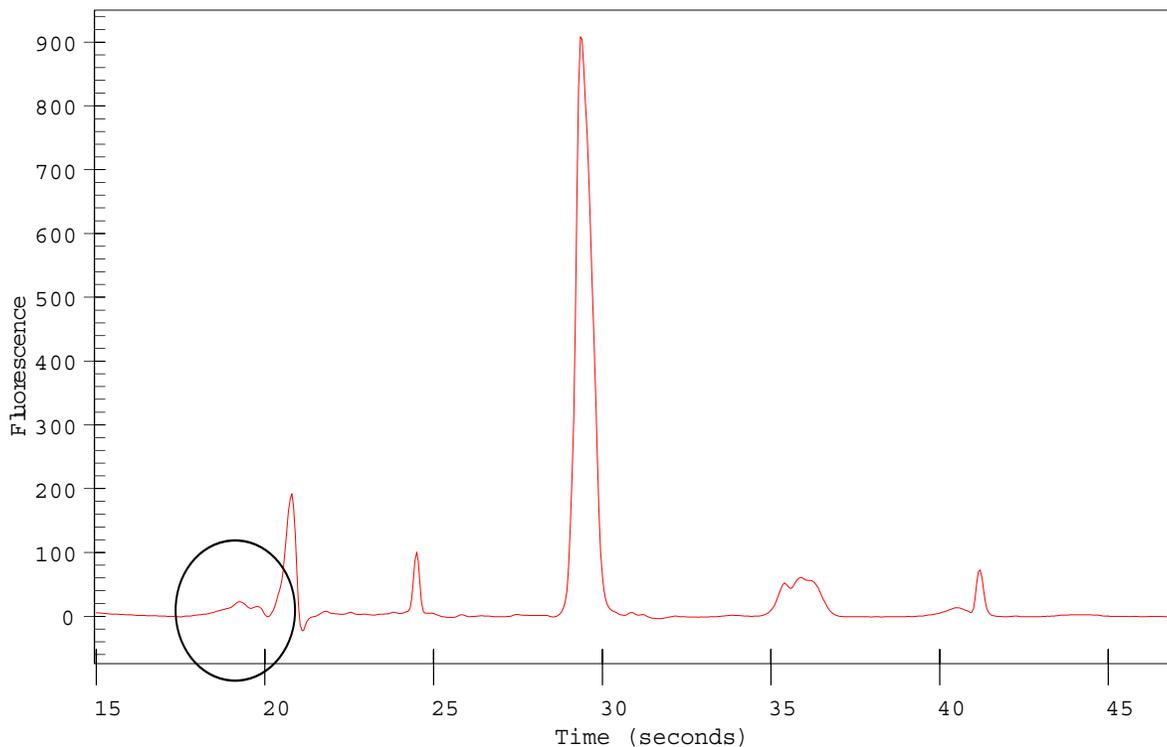
Show me how to solve **Low or Missing Upper Marker—255**

Back to **Symptoms—243**

Low or Missing Upper Marker

Most Probable Causes	Solution
Upper marker not handled according to the instructions.	Aliquote upper marker when kit is used for the first time and keep aliquots frozen until use. Refer to the instructions provided with the upper marker for reconstitution, handling and storage.
Diluted samples too old.	Use diluted samples within 30 minutes.
Sample or ladder not denaturated properly.	Heat sample/denaturing solution and ladder for 5 min at 100°C
Sample/denaturing solution and/or ladder are dried out during denaturation.	Sample/denaturing solution and/or ladder were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturing
Probable Causes	Solution
SDS not completely dissolved in sample buffer and/or dye concentrate.	Let sample buffer and dye concentrate equilibrate to room temperature for 30 min. Vortex sample buffer and dye concentrate well before use. Check for undissolved SDS crystals in the tubes.
Loaded chip kept for too long before run.	Place chip in Agilent 2100 Bioanalyzer within 10 min.

Low or Missing Lower Marker



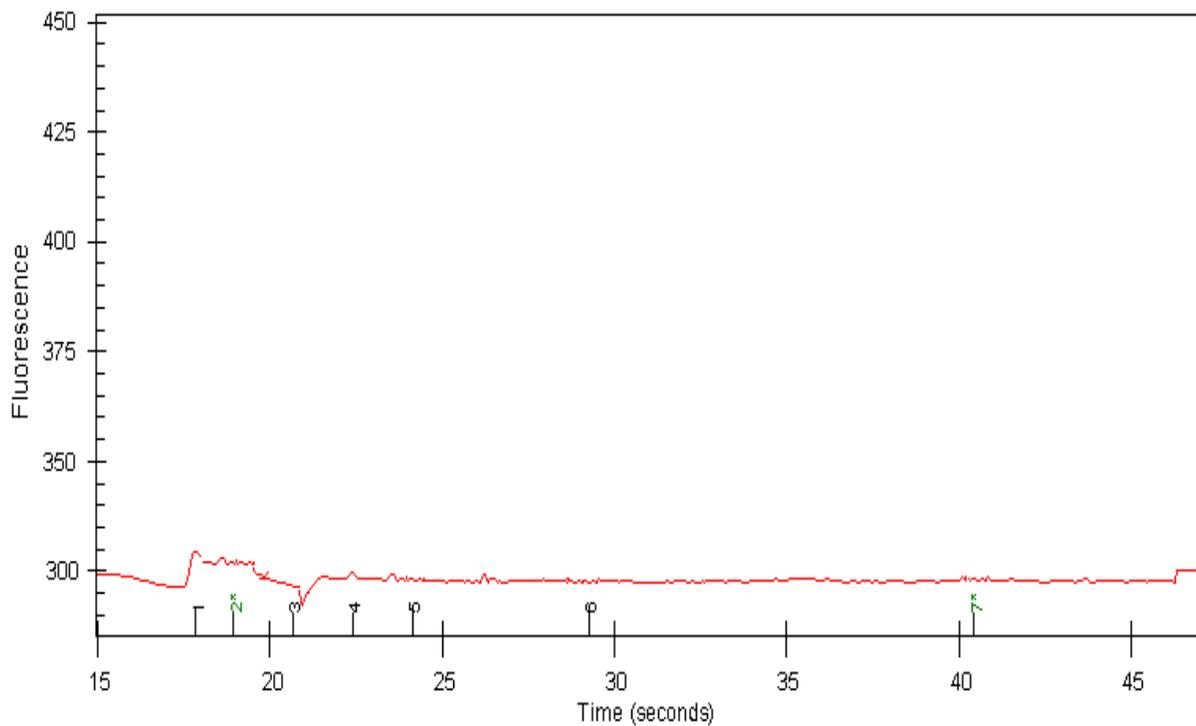
Show me how to solve **Low or Missing Lower Marker—257**

Back to **Symptoms—243**

Low or Missing Lower Marker

Most Probable Causes	Solution
Dye not completely dissolved in DMSO.	Let dye concentrate equilibrate to room temperature for 30 min. Vortex dye concentrate well before use. (check whether dye sticks to the vial bottom by turning it upside down; bottom must be white).
Decomposed lower marker mix.	Protect lower marker and lower marker solution from light.
Lower marker solution too old.	Use marker within 1 hour of preparation.

No Peaks



Show me how to **No Peaks—259**

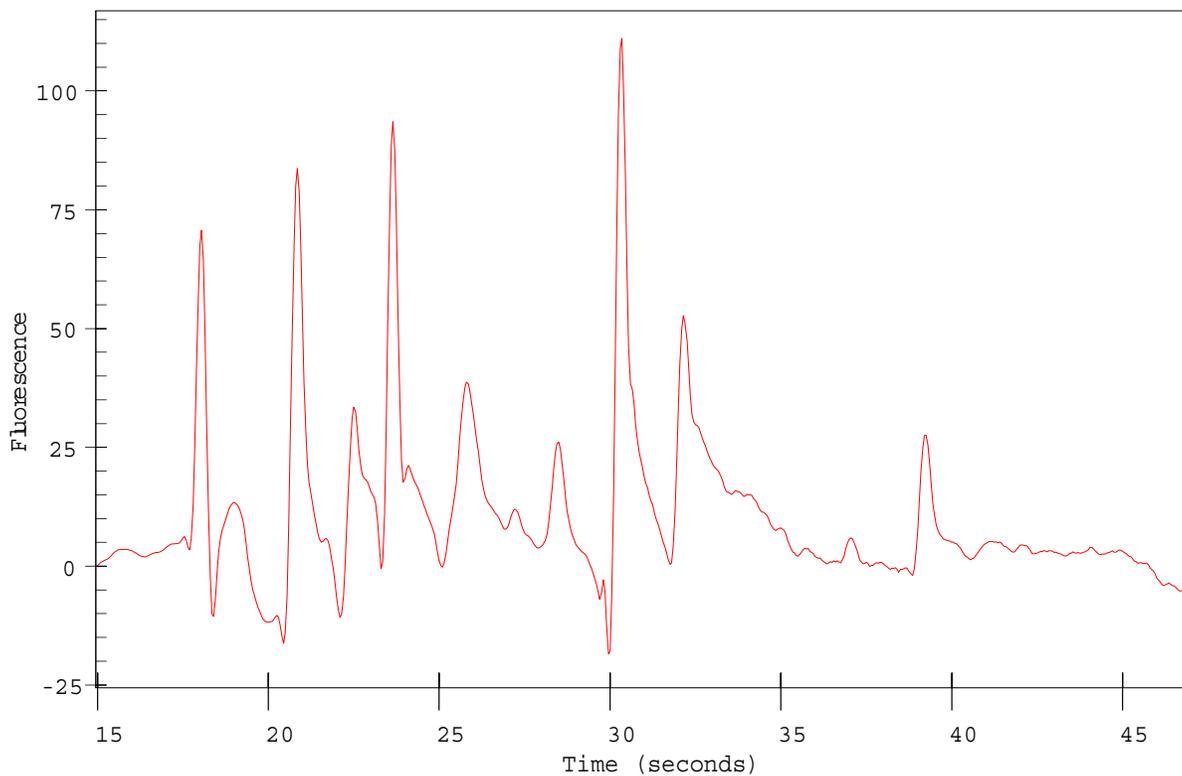
Back to **Symptoms—243**

No Peaks

Most Probable Causes	Solution
Gel dye mix was loaded in the destain well instead of destaining solution.	Discard chip and prepare new chip according to protocol.
Autofocus failure.	Check autofocus using the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Replace electrode cartridge.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too low (marker disappears).	Use dye concentration according to the Protein Reagent Kit Guide.

Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Fingerprint on focusing lens.	Clean lens using a dry cloth and isopropanol.

Broad Peaks



Show me how to solve **Broad Peaks—262**

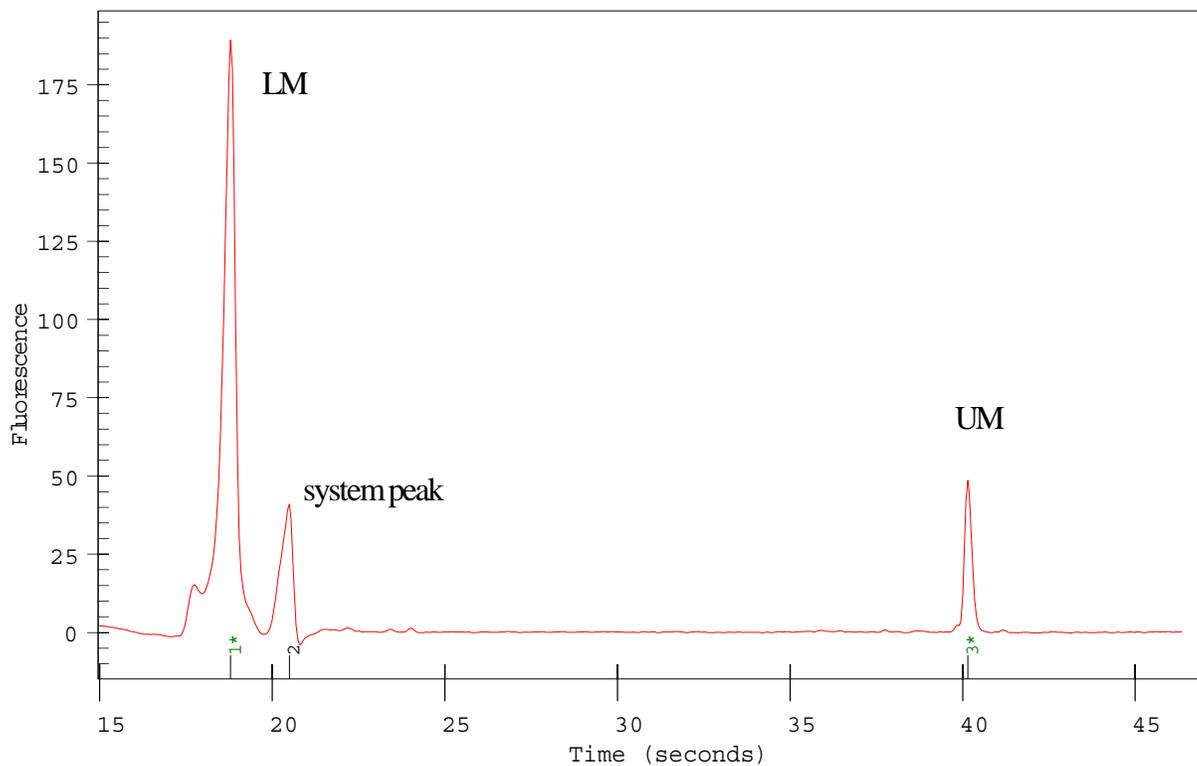
Back to **Symptoms—243**

Broad Peaks

Most Probable Causes	Solution
Wrong peak alignment.	Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip).
Leak Current due to contaminated electrodes. Chip was left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Don't leave chip in instrument after run. Clean electrodes after each run. Replace electrode cartridge.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Dye concentration too high.	Use dye concentration according to the Protein Reagent Kit Guide.
SDS was not completely dissolved in sample buffer.	Allow sample buffer to equilibrate to room temperature before use. Vortex sample buffer vial for 10s to make sure that SDS is dissolved before preparing the denaturing solution.

Probable Causes	Solution
Sample was not denaturated properly.	Heat sample/denaturing solution for 5 min at 100°C.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Add 3 μ l of BME (β -mercapto ethanol) to denaturing solution to work under reducing conditions.
One or more wells was not filled correctly.	Fill all wells with volumes as given in the Protein Reagent Kit Guide. Fill unused wells with lower marker solution or sample replicate.
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.

Missing Sample Peaks



Show me how to solve **Missing Sample Peaks—265**

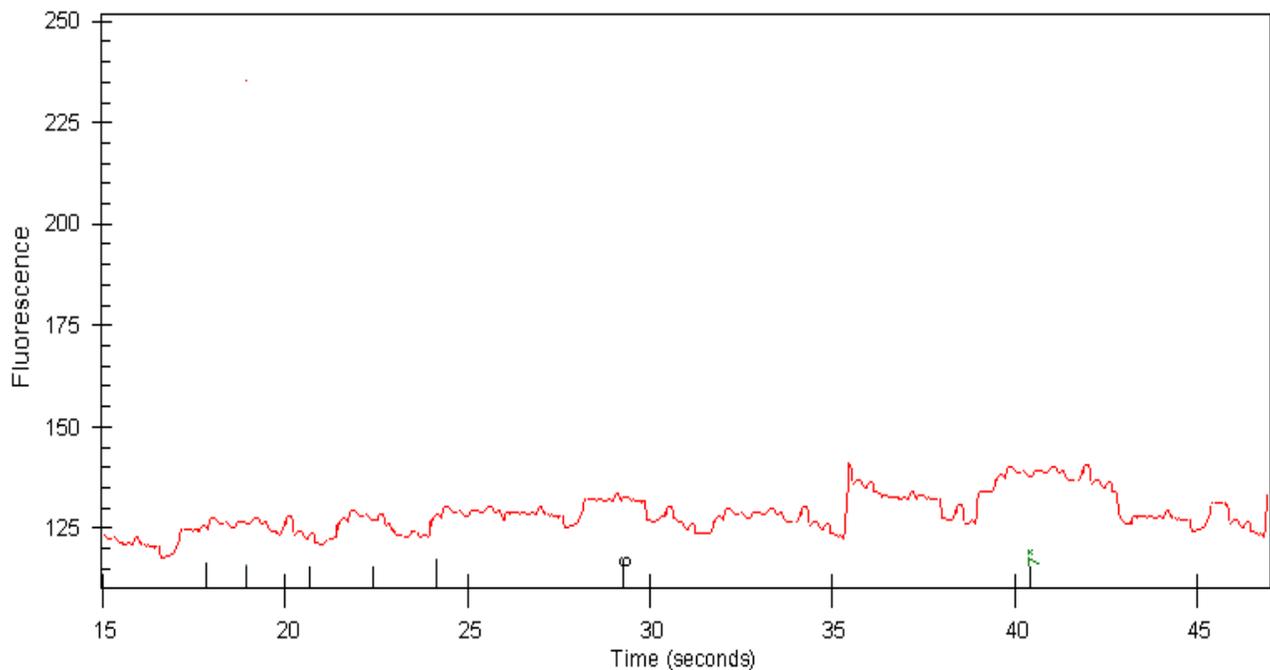
Back to **Symptoms—243**

Missing Sample Peaks

Most Probable Causes	Solution
Sample concentration too low.	Use sample concentration according to the Protein Reagent Kit Guide.
Salt concentration too high.	Use salt concentration according to the Protein Reagent Kit Guide.
Loaded chip kept for too long before the run.	Prepared chips must be used within 10 minutes.
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Probable Causes	Solution
Leak current due to contaminated electrodes. Chip left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Don't leave chip in instrument after run. Clean electrodes after each run. Replace electrode cartridge.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).

Pipetting error during preparation of mixtures.	<p>Check dilution procedure.</p> <p>Check calibration of pipette.</p>
Chip pipetting error.	<p>Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.</p> <p>Use appropriate pipette and tips.</p>
Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.
Laser defective.	Check laser using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Poor Baseline: High Noise Level



Show me how to solve **Poor Baseline: High Noise Level—268**

Back to **Symptoms—243**

Poor Baseline: High Noise Level

Most Probable Causes	Solution
Dirty electrodes. Chip left in instrument after run.	Clean electrodes after each run (see Maintenance section of User's guide). Don't leave chip in instrument after run.
Loaded chip kept for too long before run.	Prepared chips must be used within 10 minutes.
Dye concentration too low (marker disappears).	Use dye concentration according to the Protein Reagent Kit Guide.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal. Check if clip and base plate of priming station are in the right position (see chip priming station manual).
Autofocus failure.	Check autofocus by means of the hardware diagnostic tools. If autofocus fails, call Agilent Technologies.
Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Least Probable Causes	Solution

Chip contaminated.

Wear powder-free gloves only.

Don't touch the underside of the chip.

Don't touch the wells of the chip.

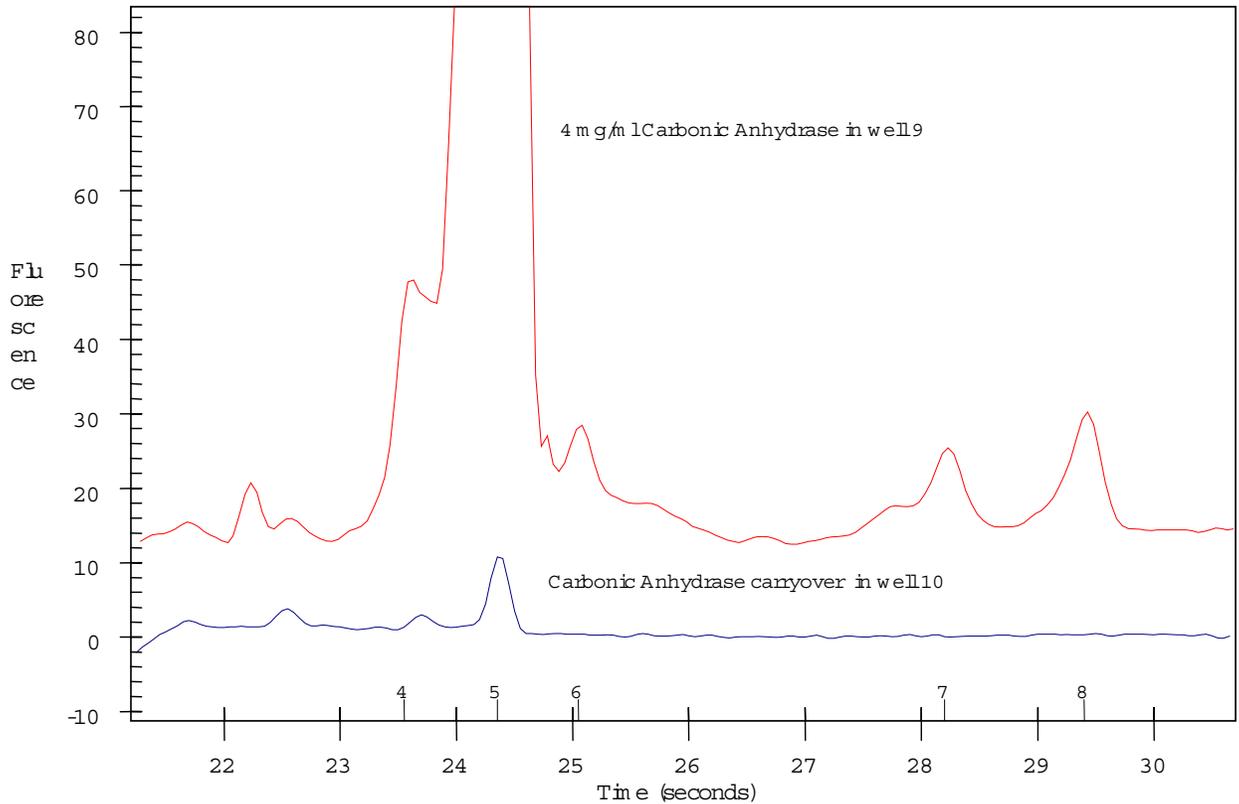
Clean the electrodes.

Load the chip immediately after taking it out of its sealed bag.

Laser defective.

Check Laser by using the hardware diagnostic tools. If the laser is defective, call Agilent Technologies.

Cross Contamination



Show me how to solve **Cross Contamination—271**

Back to **Symptoms—243**

Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Contaminated electrodes. Chip left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 . Don't leave chip in instrument after run. Clean electrodes after each run.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see Electrode Cartridge Maintenance—273 Replace electrode cartridge.

Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the hardware diagnostic tools. If the power supply is defective, call Agilent Technologies.
Broken chip.	Don't use a chip that has dropped on the floor, always use a new one.

Maintenance

Electrode Cartridge Maintenance

Daily Basis—Electrode Cleaner

To avoid cross-contamination due to contaminated electrode tips, clean electrodes after each run.

- 1 Slowly fill one of the wells of the the electrode cleaner with 350 μ L deionized analysis-grade water.

NOTE Never fill too much water in the electrode cleaner. This could cause liquid spill or contamination of the electrodes.

- 2 Open the lid and place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 3 Close the lid and leave it closed for about 5 seconds.
- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 10 seconds for the water on the electrodes to evaporate.

Depending on the sensitivity of your measurements and the adhesive forces of your sample, you have to change the water in the electrode cleaner after each use.

WARNING Never use a cloth to clean the electrodes. Electrostatic discharge might damage the high voltage power supplies.

Daily Basis—For RNA Assay Only

To avoid decomposition of your RNA sample, follow this electrode decontamination procedure on a daily basis.

- 1 Slowly fill one of the wells of an electrode cleaner with 350 μ L RNaseZAP.
- 2 Open the lid and place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 3 Close the lid and leave it closed for about 1 minute.
- 4 Open the lid and remove the electrode cleaner—label the electrode cleaner and keep for future use. You can reuse the electrode cleaner for all the chips in the kit. If the electrode cleaner dries out, simply refill.
- 5 Slowly fill one of the wells of *another* electrode cleaner with 350 μ L RNase-free water.
- 6 Place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 7 Close the lid and leave it closed for about 10 seconds.
- 8 Open the lid and remove the electrode cleaner.
- 9 Wait another 10 seconds for the water on the electrodes to evaporate.

Every Six Months

Evaporation of liquid from the chip could cause salt to coat the electrodes and the area between the electrodes. Leak currents, which distort the measurement results, could result.

- 1 Remove electrode cartridge from the Agilent 2100 Bioanalyzer.
- 2 Dip a toothbrush in distilled water and gently brush the electrodes and the area between the electrodes.
- 3 Wait at least 24 hours for the water to evaporate and check visually for residues of salt contamination.
- 4 Clean all electrodes, and the areas between the electrodes, until the electrode cartridge is free of any trace of salt contamination.
- 5 Put the electrode cartridge back into the Agilent 2100 Bioanalyzer.

Lens Maintenance

Liquid spill may lower the light throughput of the focusing lens underneath the chip. To avoid reduced signal-to-noise ratios, or absorbing coatings on the lens, apply the following procedure on a quarterly basis or after liquid has been spilled on the lens.

- 1 Remove chip from the Agilent 2100 Bioanalyzer.
- 2 Dampen a lens tissue with reagent-grade isopropanol and gently swab the surface of the lens. Repeat several times with clean tissues and alcohol each time.

Specifications

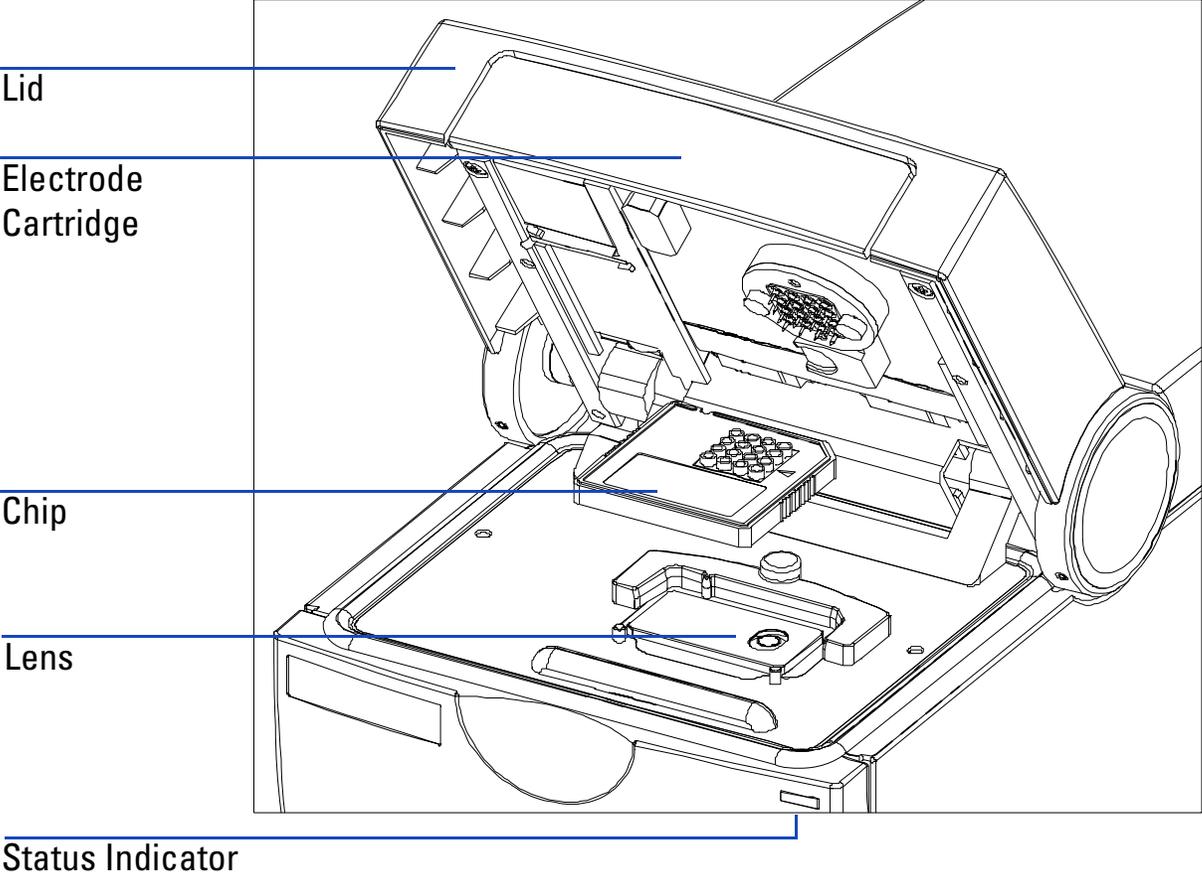
For physical specifications of the Agilent 2100 Bioanalyzer (for example, power consumption, accepted humidity range, and so on), see the *Site Preparation and Safety Manual* that comes with the Agilent 2100 Bioanalyzer.

NOTE The specifications are subject to change without notice. For the most recent specifications, see the appropriate Reagent Kit Guide, or visit the Lab-on-a-Chip web site at:

<http://www.agilent.com/chem/labonachip>

Hardware Reference

The Agilent 2100 Bioanalyzer



The major components of the Agilent 2100 Bioanalyzer are:

- Housing—made of plastic.
- Lid and electrode cartridge—the lid contains a slot which accommodates an electrode cartridge. The cartridge contains 16 electrodes that fit into the wells of the chip. Each electrode in the cartridge is powered by its own power supply. Different electrode cartridges can be used for different types of assays and correspond to different types of chips.
- chip receptacle—the recessed space that is designed to hold the chip in place. The receptacle is keyed to the chip so that you cannot insert the chip improperly.

For details of the power switch and status indicator, see *Site Preparation and Safety Manual*.

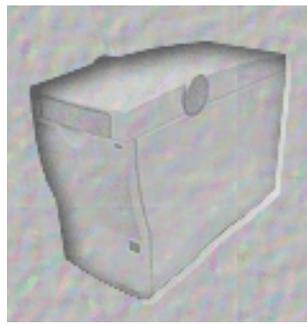
Communication Between Agilent 2100 Bioanalyzer and PC



Lid open



Lid closed

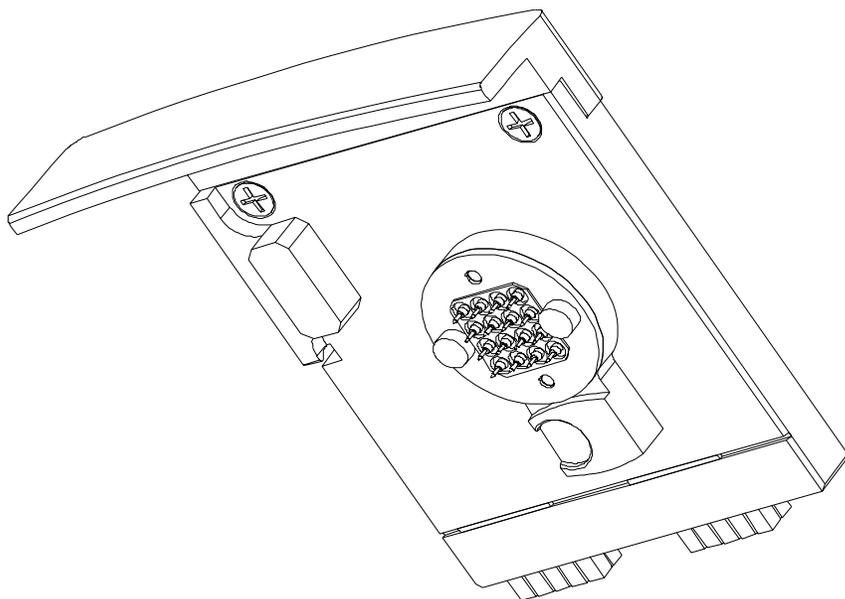


Not communicating properly

A dimmed icon such as the one shown above means that the Agilent 2100 Bioanalyzer and the PC are not communicating properly. The problem could be with a cable or connection between the Agilent 2100 Bioanalyzer and PC, a communications setting that is not correct, or another problem. For details, see **Communication—109**.

Changing the 16-Pin Cartridge

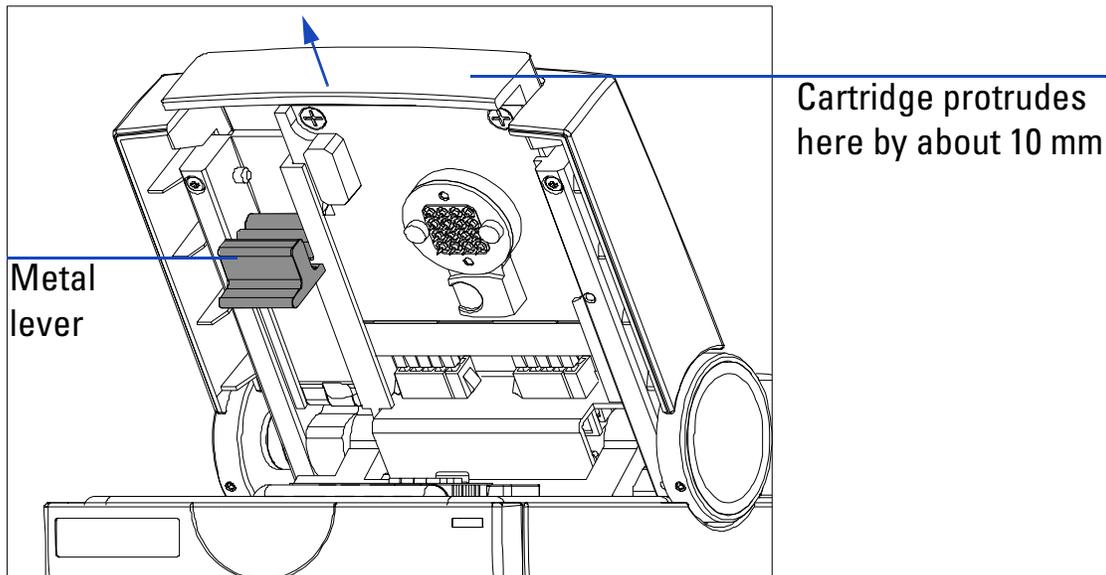
The 16-pin cartridge (reorder number 5064-8244) contains 16 electrodes configured to fit in the wells of a LabChip. The electrodes make contact with the liquid in the wells when the lid of the Agilent 2100 Bioanalyzer is closed. The cartridge can be removed if the electrodes become contaminated or damaged.



Removing the 16-Pin Cartridge

CAUTION Do not touch the electrodes while the Agilent 2100 Bioanalyzer is turned on—the electrodes and high voltage power supplies can be easily damaged.

- 1 Turn off line power to the Agilent 2100 Bioanalyzer. The power switch is located at the rear of the Agilent 2100 Bioanalyzer.
- 2 Open the lid.
- 3 Move the metal lever on the left of the inside of the lid to the vertical position. When the lever is in the vertical position, the cartridge protrudes by about 10 mm.
- 4 Gently pull the cartridge out of the lid.

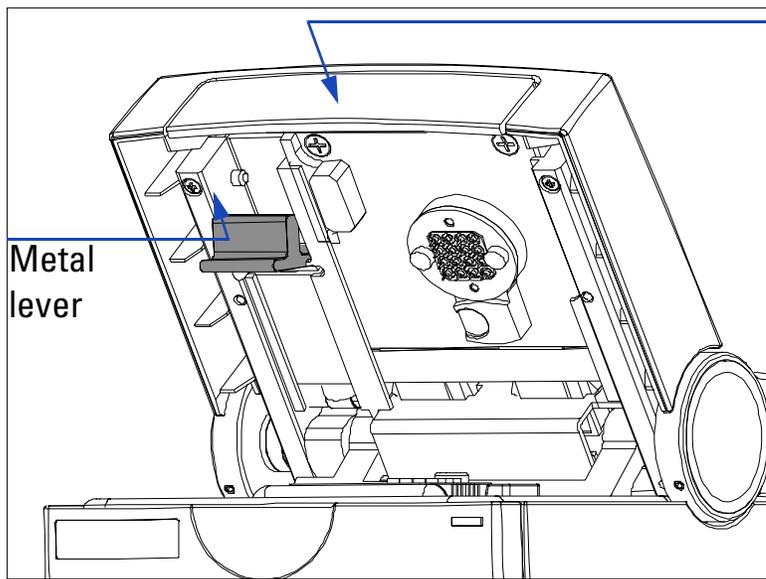


Inserting the 16-Pin Cartridge

- 1 Slide the 16-pin cartridge into the lid.
- 2 Move the metal lever to the flat (closed) position.
- 3 Push the metal front of the 16-pin cartridge to ensure a tight connection to the Agilent 2100 Bioanalyzer.

NOTE

Make sure the 16-pin cartridge is connected tightly to the Agilent 2100 Bioanalyzer.

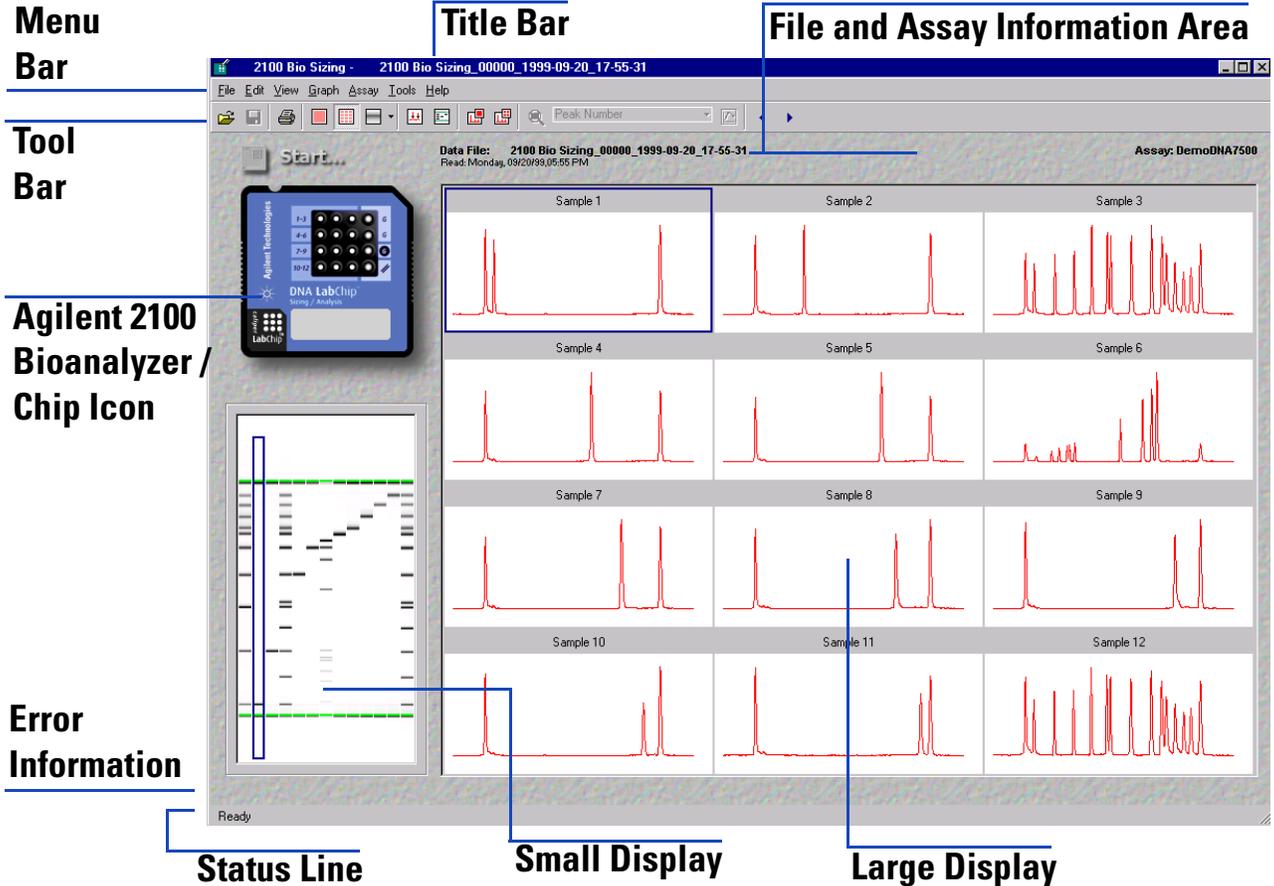


Metal
lever

Push here to
ensure tight
connection

Software Reference

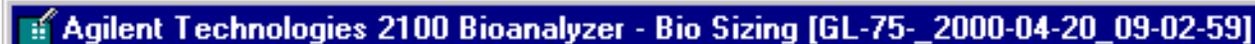
The Agilent 2100 Bioanalyzer Software Screen



The main screen of the Agilent 2100 Bioanalyzer software includes a title bar, menu bar, tool bar, Agilent 2100 Bioanalyzer icon, file information, large display area, small display area, error area, and status line. These elements are described here.

When you first load the Agilent 2100 Bioanalyzer software, the default view is in a window that fills the screen. You can size this window smaller: the Agilent 2100 Bioanalyzer software will remember the window size and position and will restore the settings the next time you start the software.

Title Bar

A screenshot of a window title bar from the Agilent 2100 Bioanalyzer software. The title bar is dark blue with white text. On the left side, there is a small icon of a document with a pencil. The text reads: "Agilent Technologies 2100 Bioanalyzer - Bio Sizing [GL-75-_2000-04-20_09-02-59]".

Agilent Technologies 2100 Bioanalyzer - Bio Sizing [GL-75-_2000-04-20_09-02-59]

The title bar extends across the top of the window, inside the window borders. It displays the name of the file and indicates whether it is the active window or not. The title bar of an active window is highlighted and any other window title bars on your desktop are dimmed.

Dragging the title bar repositions the window on the screen (in window view only; if the window has been maximized, dragging will not work). The buttons that appear at the right end of the title bar can be used to minimize the window so that it appears only on the task bar, maximize the window to full screen size, or to close the window.

Menu Bar



File Edit View Graph Assay Tools Help

The menu bar is the area across the top of the window directly below the title bar. It contains the names of the menus that group together related commands. Clicking a menu name displays a list of commands that can be used to access software functions.

The menus contained in the Agilent 2100 Bioanalyzer software menu bar are:

- **File Menu—302**
- **Edit Menu—303**
- **View Menu—304**
- **Graph Menu—306**
- **Assay Menu—307**
- **Tools Menu—310**
- **Help Menu—312**

Tool Bar



Each button on the tool bar represents a menu command (and is a shortcut to activating that command). The buttons on the tool bar are:



(Open) Brings up a dialog box allowing you to open a previously saved data file.



(Save) Saves the data file currently showing on the screen. (Note: raw data can not be changed; only the items that affect how that data is displayed are resaved).



(Print) Opens a dialog box asking you to choose the elements you would like to print.



(Single Well view) Switches to a view of the selected well in the large display.



(multiwell View) Switches to a view of all of the wells in the large display.



(Gel View) Switches to a view of the gel in the large display. Clicking the small arrow to the right of this button opens a drop-down menu allowing you to choose the coloring of the gel image.



(Peak Find Setting Dialog Box) Shows the current settings used to determine peaks. You can change the settings shown in this box.



(Analysis On/Off) Turns analysis (alignment of markers to the ladder) on and off (toggle). Note that the state of this setting is saved, even when the Agilent 2100 Bioanalyzer is powered down, until you change it again.



(Scale to Selected Well) Scales all peaks to the scale (with all peaks visible) of the selected well.



(Scale All Wells) Causes all of the data to be visible within the individual well windows by scaling each well to itself. If you hold down the Shift key while clicking this button or choosing this command, each well is scaled to the well data having the highest peak.



(Unzoom Tool) If you have zoomed in for a closer view of a single well, this tool will reset the standard view.



Drop-down menu showing labeling options for the single-well view graph.



(Show/Hide Data Points) Turns the display of data points on and off for the single well view.



(Backward/Forward) On the single-well display, allows you to move backward and forward through the wells.

Agilent 2100 Bioanalyzer / Chip Icon

The Agilent 2100 Bioanalyzer or chip is represented on the left side of the screen. What is shown in this display depends upon whether or not the lid to the Agilent 2100 Bioanalyzer is open or closed or a chip has been inserted.

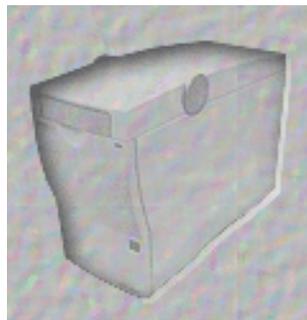
When the lid of the Agilent 2100 Bioanalyzer is open, the picture on the screen will show an open door, letting you know that a chip has not been inserted and the lid has not been closed. The picture will change to a closed door when you have closed the lid but no chip has been inserted. A communication problem with the Agilent 2100 Bioanalyzer will cause the icon to be dimmed.



Lid open

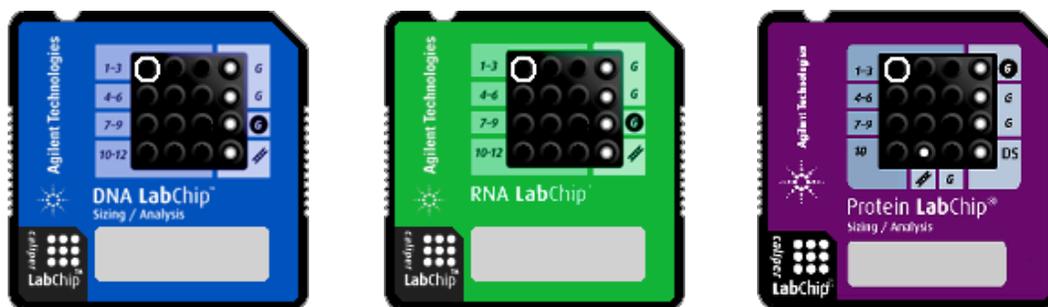


Lid closed but no
chip inserted



Dimmed icon:
not communicating properly

When a chip is inserted in the Agilent 2100 Bioanalyzer and you close the lid, the icon will change to show the chip, depending on the assay selected.



Chip inserted and lid closed

The chip icon is more than just a picture—the currently selected well has a circle around it; click a different well on the chip icon and the rest of the displays will update to reflect the new well choice.

During a run, the white spot in the center of the well that is currently being assayed will blink.

File and Assay Information Area

The file and assay information areas are located just above the large display and show the following information:

```
Data File: BioSizing_00000_2000-11-23_18-00-38
Read: 11/23/00,6:00:38 PM
```

Left side of the display: file information

- The name of the data file (if saved; otherwise the area on the left is blank)
- The date and time the data file was created

Assay: 2100 DNA 7500

Right side of the display: assay information

- The assay that was used to generate the data (on the right)

Should an error occur with data collection during an assay, a red circle with an X in it will appear to the left of the file information and a third line of data file information will appear, listing the type of error that occurred. Clicking on the red circle with an X in it will open the help for that particular error message, allowing you to view possible causes for the problem as well as any potential solutions.

 **Data File: 2100 Bio Sizing_00000_1999-09-20_18-45-58**
Read: Monday, 09/20/99,06:45 PM
Status: Aborted by the user

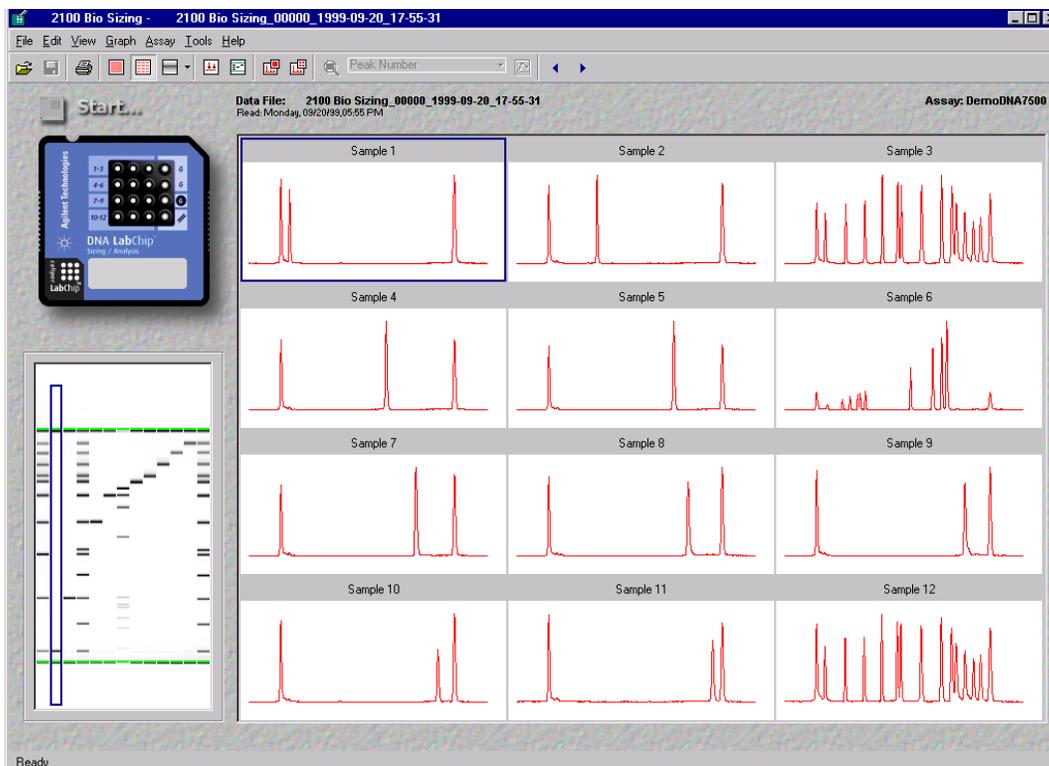
You can add or amend notes for the run by accessing the notes section of the File Properties dialog box: double-click the filename shown above the large display. The File Properties dialog box that appears also contains a button allowing you to view the Run Log for that data file.

Double-clicking the assay information shown on the right side above the large display opens the Assay Properties dialog box. The tab labeled Global Peak Find within this dialog box allows you to change the peak find settings.

Large Display

Large Multiwell Graph View

This area of the workspace shows a multiwell view (default) of the data received from the chip, a single-well display of a selected well, or a gel view. When the multiwell or single-well views are selected, the small display area shows the gel view and vice versa.

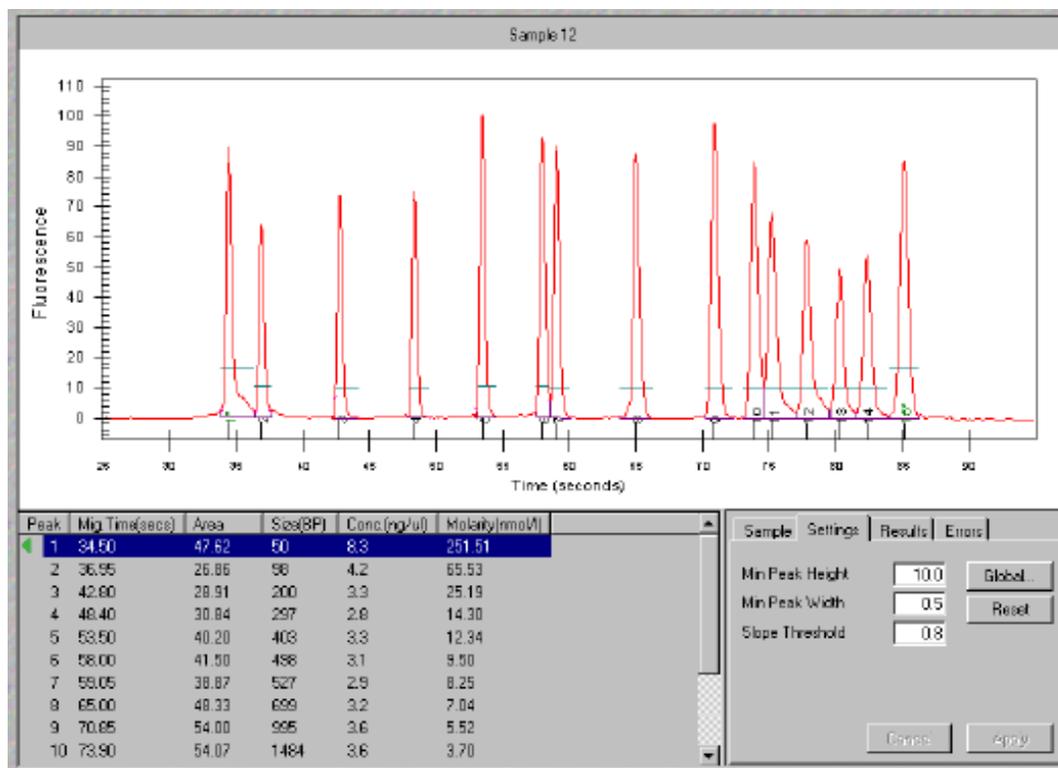


This is the default view when you start the Agilent 2100 Bioanalyzer software. As data is acquired, the selected well (Sample 1, in the example above) will increment to the well that is currently being run, and the data will appear in the display in real time. The screen above shows the large display after all data for the run has been collected.

To see a single well view, either select a well and click the single-well display button in the tool bar, or double-click the desired well in the multiwell view.

You can change the names of the sample wells to any names you wish. To change a name, highlight the existing name and type over it. When the file is saved, the new well names will be saved with the file.

Large Single-Well Graph View

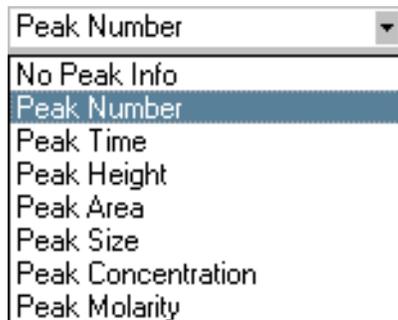


When the large display shows a single well, you can zoom in to see the data in the graph more closely. Drag the mouse in a rectangle that bounds the area you wish to view in more detail. This area will enlarge to fill the large display area.

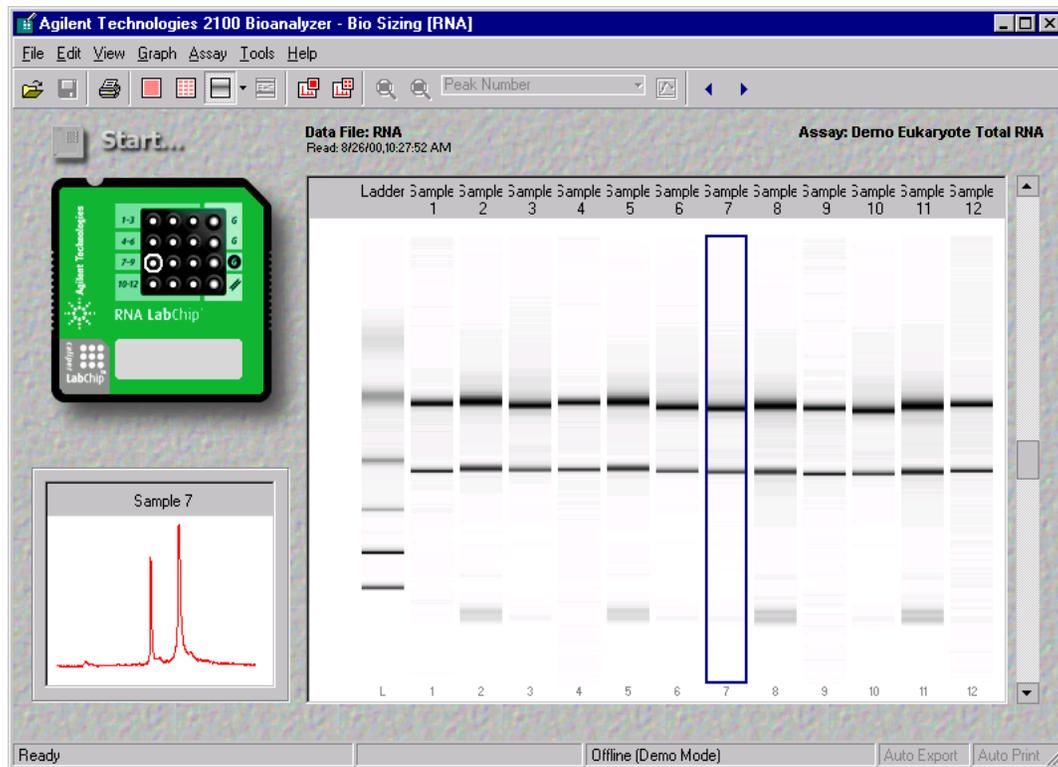
The dividing line between the graph and the Results Table and the electropherogram display can be moved in the vertical direction, giving more or less space to the Result Table. This allows you to view all of the results at once, for example, in wells having several peaks.

You can also overlay the graphs from more than one well in a single-well display. To learn more about this feature, see **Graph Menu—306**.

Peak Number is the default for the single well display but other options are available:



Large Gel View



In this view, the gel which is typically shown in small format on the left side of the screen is displayed in the large display and a small single well graph is shown on the left side of the screen.

One lane of the large gel view will be surrounded by a box. This is the selected lane (one lane is always selected) which corresponds to a selected well in the chip icon; the small display will show the well graph corresponding to the selected lane. Clicking a different lane will select that lane and the small display will update to show the new well graph.

When a new run is made, the gel display will first be blank and the first lane which corresponds to the ladder well will be selected. As data is acquired in the first and subsequent lanes/wells containing samples, the selection box will increment to show the well and lane that is currently acquiring data. If you select a lane/well that is earlier in sequence than the current well, however, the display will no longer increment as new data is acquired but will remain on the selected lane/well.

Moving the mouse pointer over a gel in the large display will cause numbers to appear next to a crosshair pointer. What is displayed depends on the type of assay selected:

- With a DNA assay, you will see the base pair measurements for the area of the lane beneath the crosshair of the pointer (shown by a "+"). If the cursor is positioned over a recognized band, the cursor will change to show a target and the concentration and molarity will also be shown.
- With RNA assays, nothing is shown except over recognized bands where the area and percent of total area will be shown.
- With a Protein assay, positioning the cursor anywhere in the gel image will show the size of the protein (in kDa) for the area of the lane beneath the crosshair of the pointer (shown by a "+"). If the cursor is positioned over a recognized band, the cursor will change to show a target and the concentration will also be shown.

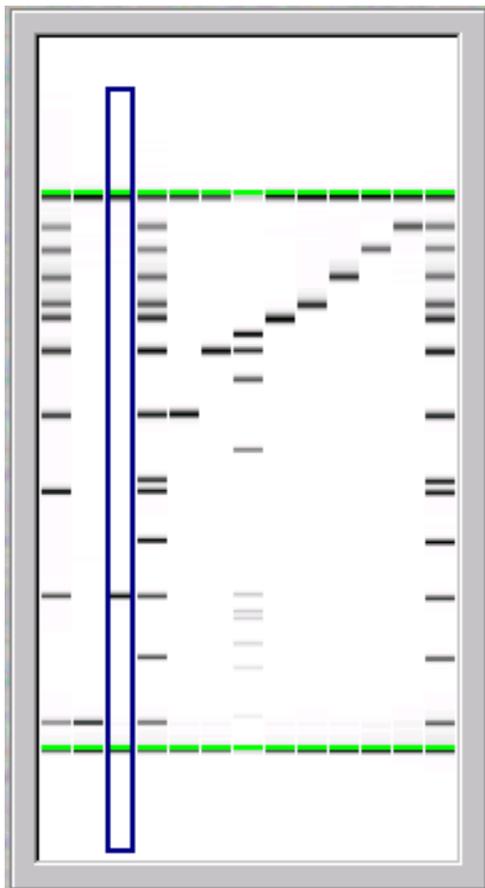
NOTE

The display of the gel can be changed to a number of different color combinations. These are selected using the arrow to the right of the gel button in the toolbar or via the View menu. For more information, see **View Menu—304**.

Small Display

Small Gel View

This area of the workspace shows either a gel (default) view of the data received or a single-well display (if the large display is showing a gel view).

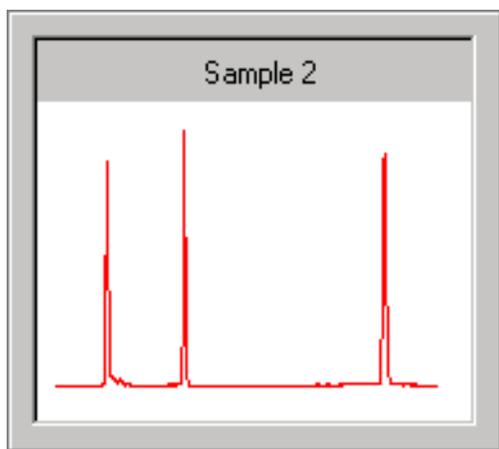


One lane of the small gel view will be bounded with a box. This is the selected lane, which corresponds to a selected well in the chip icon (or in the multiwell display). Clicking on a different lane will cause that lane to be selected and, if a single well is shown in the large display, the large display will update to show the currently selected well data.

When a new run is begun, the display will be blank and the first lane/ladder well will be selected. As data is acquired in lanes/wells, the selection rectangle around the lane will increment to show the lane that is currently acquiring data. If you select a lane/well that is earlier in sequence than the current well, however, the display will no longer increment as new data is acquired but will remain on the selected lane/well.

Small Graph View

When a gel view is selected in the large display, the small display will show a single well view of the data in the selected well.



Error Information

 Marker peaks in Sample C3 cannot be aligned to the ladder

The area of the screen just above the status line is where most error messages will appear.

NOTE

Click  for context-sensitive help.

Error messages can result from hardware or software problems. Most are the result of peaks not being located by the analysis algorithms of the software. This can be due to a sample peak or ladder peak not appearing as expected; the settings in the software (via the Peak Find Settings dialog box) can also cause peaks to go undetected which can cause errors. Additionally, manually excluding a peak from analysis (done in the result table) can cause errors with the Peak Find algorithm.

Status Line

Reading 'Ladder' (59 of 80 seconds)

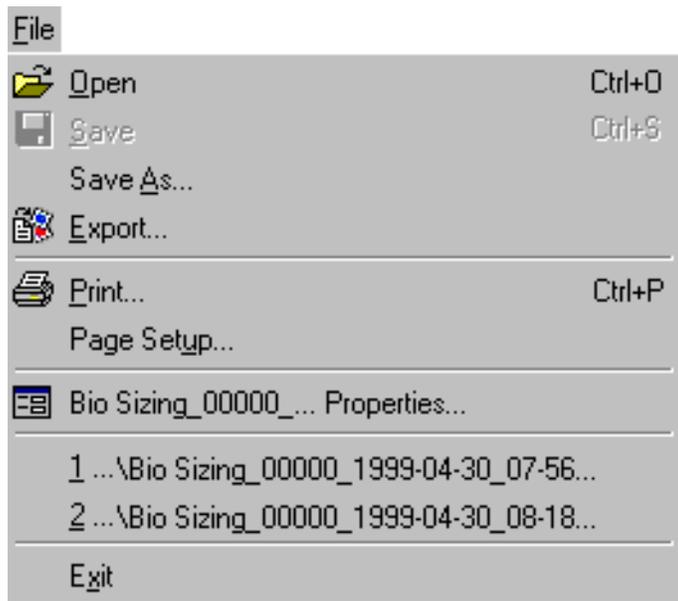


The status line is found at the bottom of the Agilent 2100 Bioanalyzer software screen and displays information relevant to whatever is currently taking place in the Agilent 2100 Bioanalyzer.

When the software is ready to run an assay, the Status Line will display Ready. When you begin an assay, it will show each step as it starts, including the total amount of time that step will require and how much time remains for that step to complete. A progress bar on the right side of the Status Line provides a graphical representation of the same information.

Menu Items

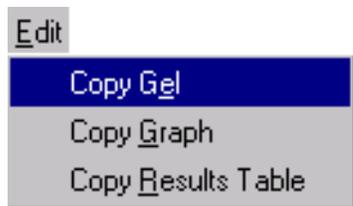
File Menu



Open	Opens a previously saved data file.
Save	Saves an unsaved or changed data file.
Save As...	Saves a data file under a new name.
Export	Causes a dialog box to appear allowing you to choose the type of data that will be exported.

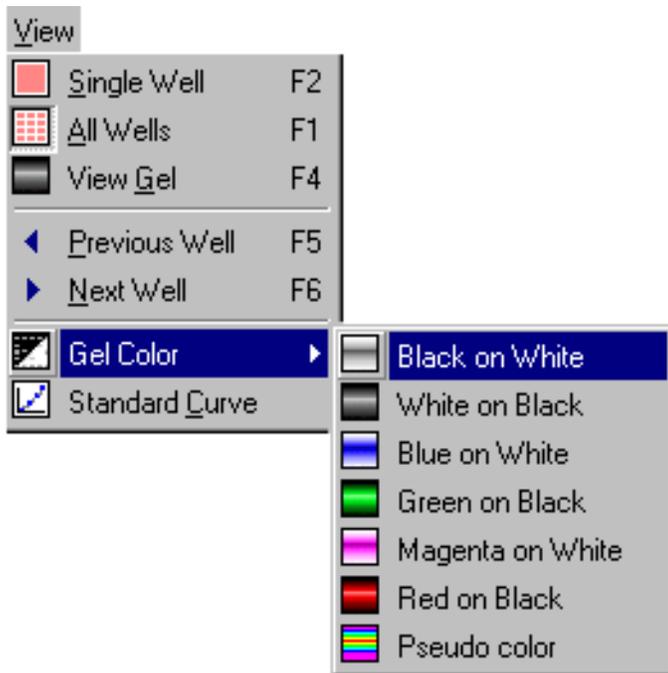
Print	Opens the Print dialog box allowing you to choose the items that will be sent to the printer.
Page Setup	Opens the Page Setup dialog box, allowing you to change page orientation and margins.
1-10 Recent Files	The ten data files that were opened most recently (the most recent file is labeled 1).
Exit	Allows you to close the software. If you have unsaved data, you will first be asked if you want to save it.

Edit Menu



The copy functions in this menu allow you to copy a gel, graph, or a result table for use with another software.

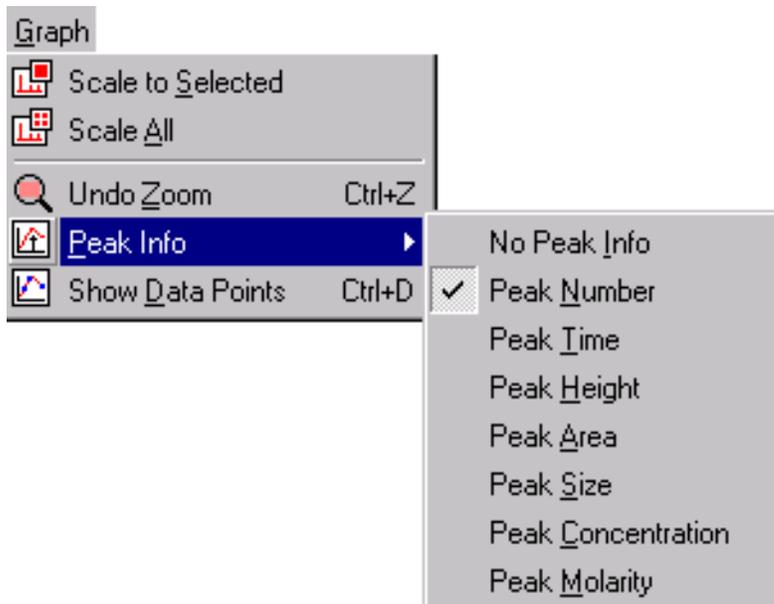
View Menu



Single Well	Shows the single well view (of the selected well) in the large display.
All Wells	Shows all of the wells in the large display.
View Gel	Shows the gel in the large display.
Previous Well	In any display, decrements the view to the previous well or lane. If you are viewing Sample 1, clicking this button (or pressing the left arrow button on the keyboard) takes you to the ladder well/lane. If you are viewing the ladder well, clicking this button (or pressing the left arrow button on the keyboard) takes you to the last well/lane.

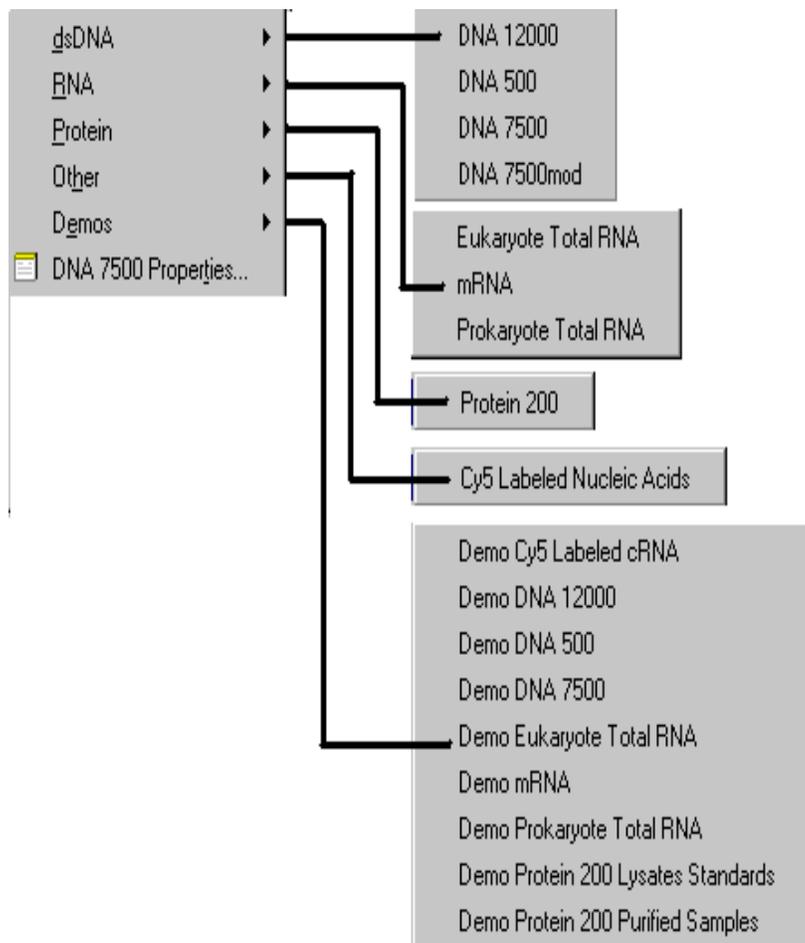
Next Well	In any display, increments the view to the next well or lane. If you are viewing the ladder, clicking this button (or pressing the right arrow button on the keyboard) takes you to Sample 1. If you are viewing the last sample, clicking this button (or pressing the right arrow button on the keyboard) takes you to the ladder well/lane.
Gel Color	This pulldown menu presents different color choices for viewing a gel in the large display. The colors are designed to approximate various actual gel staining and imaging techniques. The Pseudo color choice provides more detail (1280 colors) since it maps the signal into a larger color space than is available with the other monochrome options (256 levels of brightness).
Standard Curve	Opens the Standard Curve dialog box, allowing you to view the DNA ladder as a curve with a point-to-point fit.

Graph Menu



Scale to Selected	Scales the data in all wells to the data in the selected well.
Scale All	Scales display of each well to itself allowing all of the peaks to be visible. Holding down the Shift key and choosing Scale All causes all of the wells to be scaled relative to each other.
Undo Zoom	Returns to the standard (unzoomed) view of the single well. Double-clicking in the single well display performs the same function.
Peak Info	Allows you to choose the type of information that is shown in the Result Table of the single-well display. Default is Peak Number.
Show Data Points	Enables/disables the display of the data points used to generate the graph. Data points are visible only in the single-well display.

Assay Menu

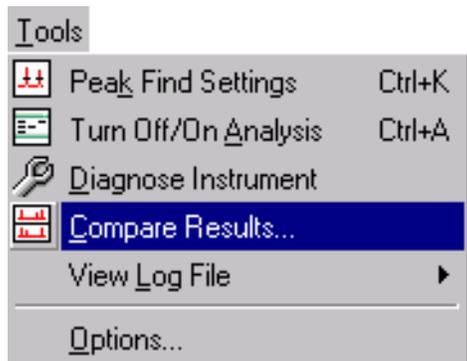


The Assay menu includes the default assays that are shipped with the Agilent 2100 Bioanalyzer software, as well as any assays that have been created and saved subsequently into the Assays > Assay type folder.

dsDNA DNA 12000 DNA 7500	The dsDNA assays included with the Agilent 2100 Bioanalyzer software are a Demo assay, which can be used as a learning tool, and two assays designed for separation of DNA. To choose an assay that is different from the one run previously (the default, for which Properties are shown in this menu), choose Assay > DNA > one of the assays on the pull-out menu. It is possible to alter the parameters for one of the assay is shipped with the Agilent 2100 Bioanalyzer software and save it as a new assay for future use.
RNA mRNA TotalRNA	The RNA assays included with the Agilent 2100 Bioanalyzer software are designed to determine the concentration of total RNA and mRNA preparations. The RNA assay is designed to separate RNA up to 6000 base pairs.
CY5	This assay can be used to analyze Cy5-labeled cRNAs and cDNAs before hybridizing these samples to microarrays. A rough size estimate can be obtained and failed cDNA labeling reactions can be identified.
Protein	The Protein assay included with the Bio Sizing software is designed to analyze multiple types of proteins, such as column fractions, cell lysates, and purified proteins.

Assay Properties	This menu shows the name of the current assay. Choosing this command opens the Assay Properties dialog box which displays the settings used to determine the ladder peaks and other settings required for analysis. The settings on the first three tabs of this dialog box are not changeable by the user. Settings on the last tab, Peak Find, are changeable (see Peak Find Settings for more information).
Open Assay	Brings up the Open dialog box, allowing you to open an assay that is not currently shown in the Assays menu (residing in another location).
Save Assay	Choosing this command will save an assay file with updated properties. This command is dimmed if the current assay has not been altered in any way. Choosing this command while using one of the default program assays will cause an error message to appear, since those assay files are "read-only" and will open the Save As... dialog box instead.
Save Assay As...	Opens the Save As... dialog box, allowing you to save the assay as it is currently configured under a different assay name.
Start	Opens the Start dialog box, allowing you to enter information regarding the run. Clicking Start in this dialog box will initiate the run. Note that the Start dialog box also allows you to change the file prefix that will be used in automatically naming the data file.

Tools Menu



Peak Find Settings	Opens the Peak Find Settings dialog box which lists the settings used to determine whether or not a peak will be kept for analysis.
Turn Off/On Analysis	The default is analysis on, which causes the marker peaks run with the samples to be aligned to marker peaks in the ladder. Choosing this command turns analysis off which removes the marker peak alignment.
Diagnose Instrument	Opens the Diagnose Interface for checking the hardware components of the Agilent 2100 Bioanalyzer.
Compare Results	Opens the Data Comparison program as a stand-alone program. You can compare results from wells within a single run, or between runs or assays that have been saved previously.

View Log File

Four types of log files are maintained by the Agilent 2100 Bioanalyzer software:

1) a System Log, which maintains a running record of all events that occurred with the Agilent 2100 Bioanalyzer while it is online with the Agilent 2100 Bioanalyzer software (including the dates and times that the system went on and offline, the version history, etc.).

2) a Run Log, which includes information about the particular run including the date and time of the run, any problems that occurred, the assay that was used to generate the data, and the name for the saved data file. An example of information stored in a Run Log might look like this:

04-30-1999 07:57:37 Start Data:

C:\PROGRAM FILES\HP\BIO SIZING\Assays\DNA\DNA7500.asy

C:\PROGRAM FILES\HP\BIO SIZING\Data\Bio

Sizing_00329_1999-04-30_09-57-37.cld

04-30-1999 10:02:12 End Data:

13 wells read (0 1 2 3 4 5 6 7 8 9 10 11 12)

3) a Diagnose log.

4) an IQ log.

Options

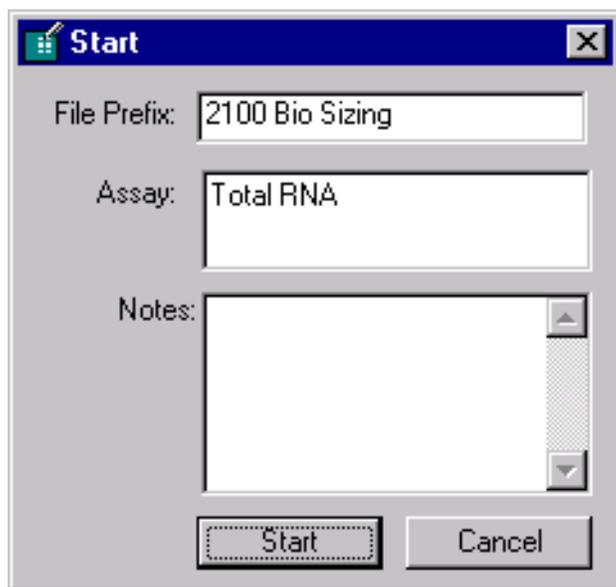
Opens the Options dialog box which contains four tabs: Data Files, Reader, Chip Alert, and Advanced.

Help Menu



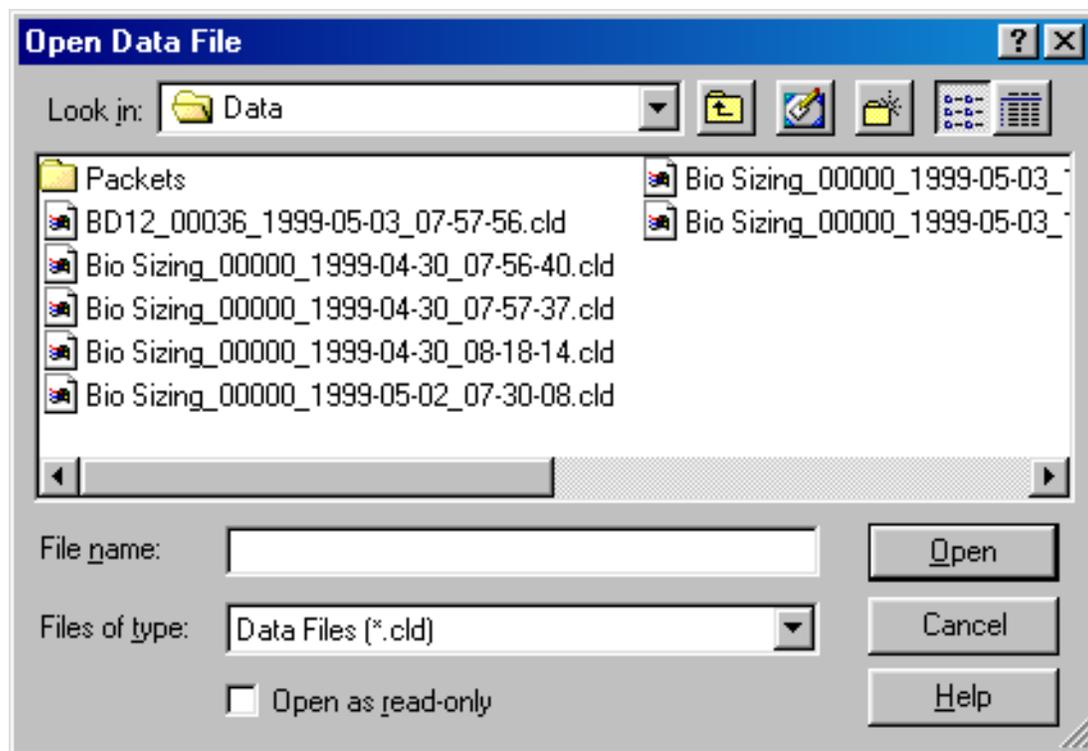
Contents and Index	Opens the Contents/Index page for this Help function.
About 2100 Bio Sizing	Opens the About box, showing the Agilent 2100 Bioanalyzer software version number, the Agilent 2100 Bioanalyzer software authors, and so on.

Start Dialog Box



This dialog box appears when you click the Start button above the chip icon. You can enter a file prefix other than the one shown in the dialog box and enter any notes about the run in the box at the bottom. When you are ready to begin the run, click Start.

Open Data File Dialog Box



When you choose Open from the File menu, this dialog box appears. Choose a data filename from the list in the box and click the Open button or double-click a filename to open that data file.

This dialog box contains a checkbox which opens the file as read-only and (Open Read-Only) is displayed after the filename in the title bar. A read-only file may be edited but may not be saved under the same filename. If you attempt to save an edited, read-only file, an error message will be displayed explaining that the file is a read-only file. Clicking Okay in the error box will open the Save As... Dialog box. Entering the same name as the read-only file causes another error message to be displayed telling you to save the file with a different name or to a different location.

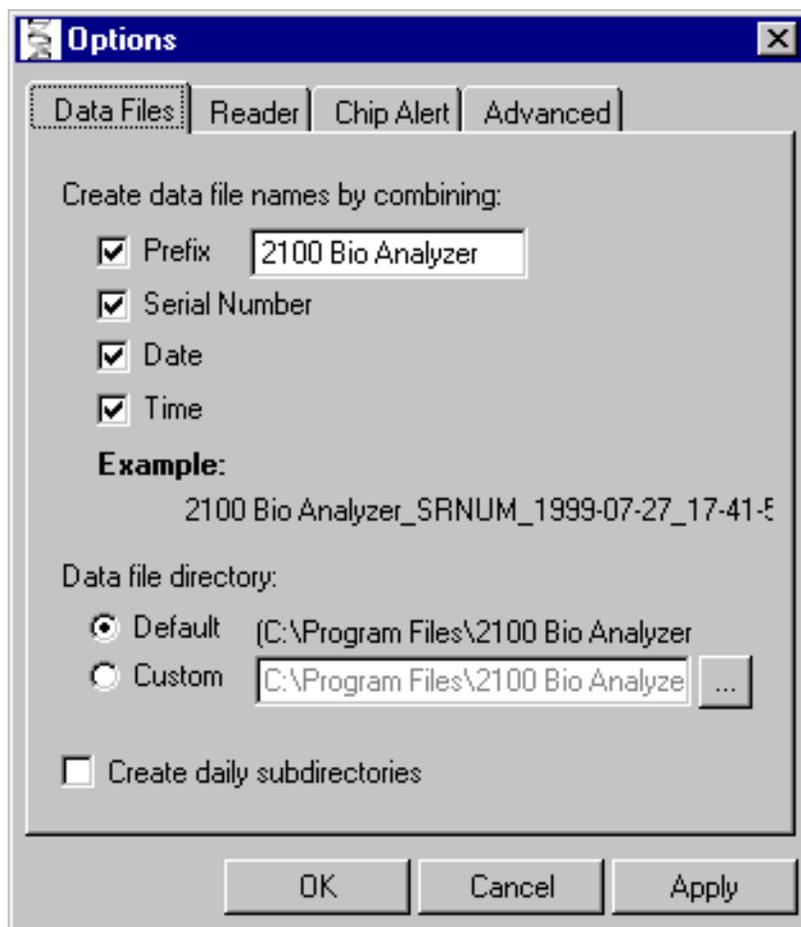
The benefit of opening a file as read-only is to prohibit you or other users from making changes that would alter the file in any way. Since the Agilent 2100 Bioanalyzer software allows you to open data files, reanalyze them using different assays, alter peak find parameters, etc., and saves these new parameters with the file when it is saved, you may prefer to ensure that the original parameters which were used to create the file are not altered.

Options Dialog Box

The Options dialog box contains four tabs: Data Files, Reader, Chip Alert, and Advanced.



Data Files Tab



Data File settings allow you to determine the way in which data files are automatically named. You can include a prefix of your choice, the serial number of the Agilent 2100 Bioanalyzer, the date and/or the time of the run. For example: including a prefix such as Bio Sizing, as well as the date and time of the run, files would bear names such as Bio

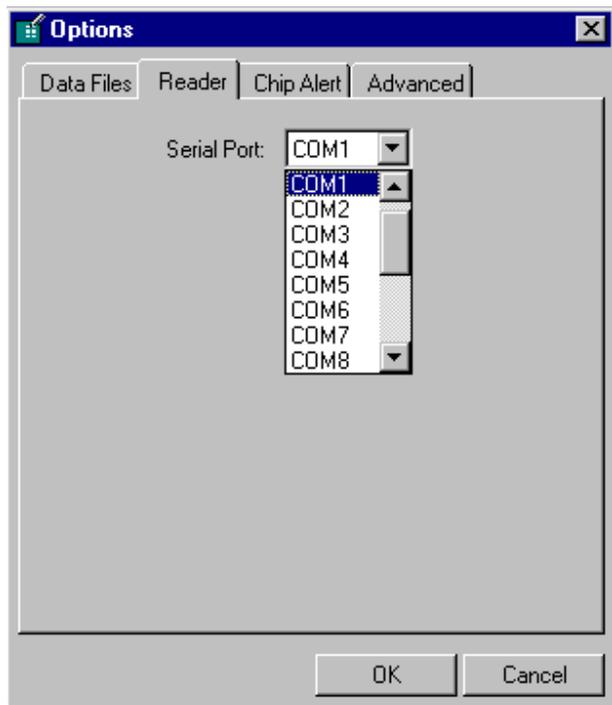
NOTE

If you choose not to use the time of the run as part of the data filename, the Agilent 2100 Bioanalyzer software will automatically append a -1, -2, and so on, for each subsequent run made that day.

This tab also allows you to choose the directory in which data files will be stored. The default stores them in a directory with the software files but you can create and use a custom directory, if desired. You can also choose to have daily subdirectories created for file storage each day.

Saved files can be altered and resaved or saved to a different name, if desired.

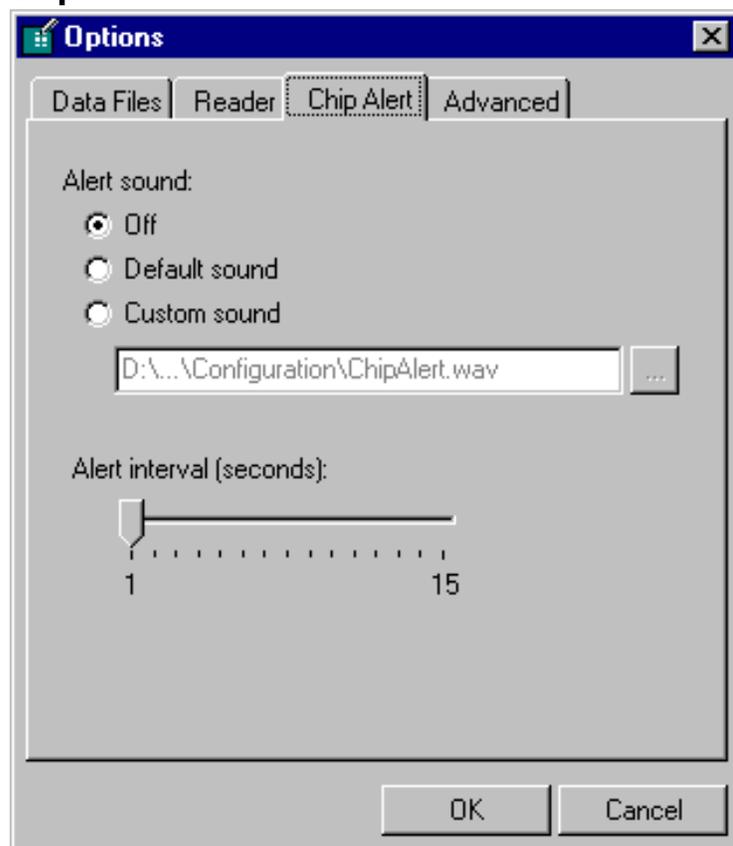
Reader Tab



Serial Port

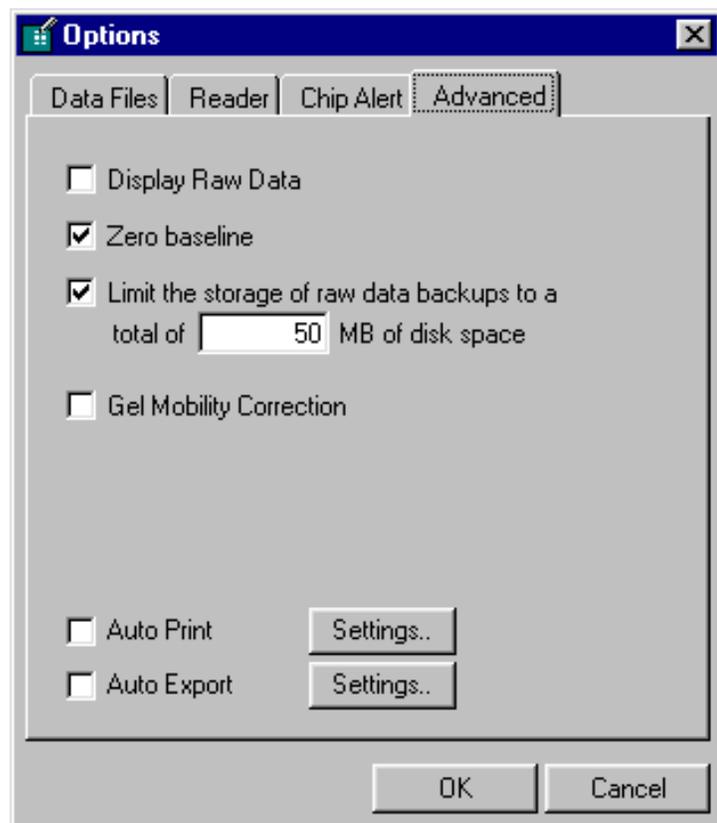
This setting allows you to choose the serial (Com) port to which the Agilent 2100 Bioanalyzer is connected (Com1 through Com16). A setting of None is also available (if you're running the Agilent 2100 Bioanalyzer software alone without a connection to an Agilent 2100 Bioanalyzer).

Chip Alert Tab



The settings under this tab have to do with the alert sound that is made when a chip needs to be removed from the Agilent 2100 Bioanalyzer. Choices are to turn the sound off, leave it at the default sound setting, or use a custom sound which can be any .wav file of your choice. You can also change the interval in seconds between the alert sounds from the default at 1 second to a maximum of 15 seconds.

Advanced Tab



Display Raw Data

Choosing Display Raw Data shows the electropherograms as they were acquired. The data is still filtered, however, using the polynomial filter (the settings for this filter can be changed in the Peak Find Settings dialog box).

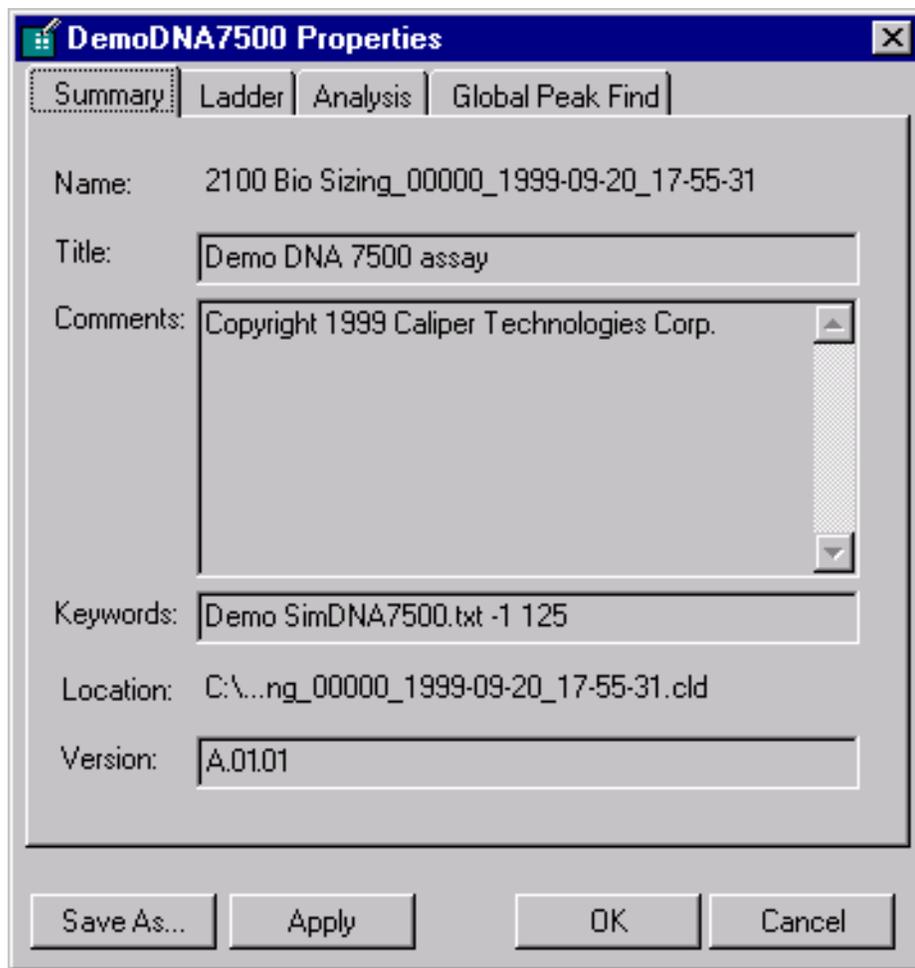
Zero Baseline

The Zero Baseline setting is used to offset the graphs shown for the individual wells but does not affect analysis.

Limit the storage...	You can also choose to limit the storage of raw data backups to a certain amount of disk space. The default is 50 MB, which corresponds to 50 assays. When the limit is reached, the data of the first assay is overwritten.
Gel Mobility Correction	Gel Mobility Correction is applied to the gel display. Each point is plotted against mobility, instead of time, where mobility equals distance/time. By plotting against the reciprocal of time (1/time), the separation of the peaks is proportional to the mobility and is more comparable to a photo of a real gel. Applying mobility correction expands the fast peaks and compresses the slower ones.

Assay Properties Dialog Box

The Assay Properties dialog box displays the settings used to determine the ladder peaks and other values required for analysis. The settings in this box (with the exception of the settings on the Peak Find tab) are not changeable by the user.



The image shows a Windows-style dialog box titled "DemoDNA7500 Properties". It has four tabs: "Summary" (selected), "Ladder", "Analysis", and "Global Peak Find". The "Summary" tab contains the following fields:

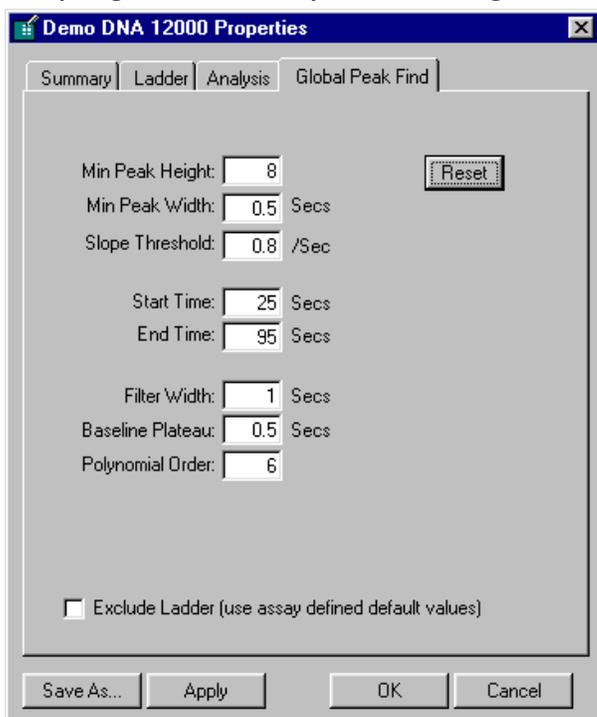
- Name: 2100 Bio Sizing_00000_1999-09-20_17-55-31
- Title: Demo DNA 7500 assay
- Comments: Copyright 1999 Caliper Technologies Corp. (text is visible in a scrollable area)
- Keywords: Demo SimDNA7500.txt -1 125
- Location: C:\...ng_00000_1999-09-20_17-55-31.cld
- Version: A.01.01

At the bottom of the dialog box are four buttons: "Save As...", "Apply", "OK", and "Cancel".

This dialog box allows you to make changes to the peak find settings, apply them, and, if you are satisfied with the result, save the changes you have made to a new assay file. This assay file can be used with subsequent readings to generate new data files and will be called up if any such file is reopened.

Global Peak Find Settings

Choosing Assay > Assay Properties... opens the Assay Properties dialog box. The settings found on the Global Peak Find tab can be changed to alter the way in which the program locates peaks or fragments (the other tab settings are not changeable).



The choices on the Global Peak Find tab determine how peaks are detected and shown in the display. Settings on this tab are user-changeable and have the following functions:

Minimum Peak Height	Determines the limit below which a peak will not be detected. When the Settings tab is displayed in the Sample Information area, the single-well display depicts the minimum peak height setting by means of horizontal green lines on the peaks.
Minimum Peak Width	Determines the limit (in seconds) under which a peak will not be detected.
Slope Threshold	This setting represents the amount of change in absorbance units over time required to indicate that a peak has occurred. Changing this setting may cause certain peaks that were previously detected to be ignored or to interpret noise as peaks.
Start Time	Shown on the single-well display as a vertical green line, this setting determines the time after the start of a run when the first peak can appear (any peaks appearing before this time are ignored). The vertical green line is shown as a solid line when markers are not aligned (analysis off); it is shown as a dotted line when markers are aligned.
End Time	This setting determines the time after which peak detection stops. It is shown in the single-well display as the end of the graph window scale.

Filter Width	This setting determines the width, in seconds, of the polynomial to be convolved with the data. If you change the setting, ensure that the value is less than twice the width of the peaks of interest or the peaks will be distorted.
Baseline Plateau	This is a baseline parameter for peak finding. The signal is its baseline whenever the slope of the data is less than the slope threshold setting (positive or negative) for longer than the time specified for Baseline Plateau. This setting is used to reject brief, low slope areas such as at peaks in between non-baseline-resolved peaks.
Polynomial Order	This setting is used to define the power series applied to fit the raw data. The higher the number you set, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.
Exclude Ladder	Excludes the ladder from any changes you may make to the peak find settings (the default ladder settings from the assay are used instead).

If you save the changes you make, the new peak find settings are saved along with the file and will be used the next time that file is opened.

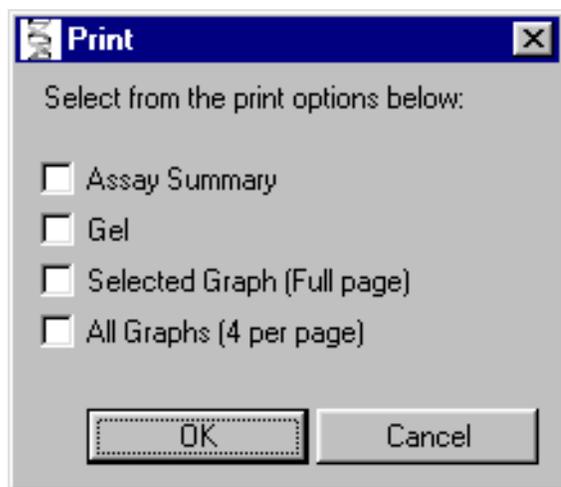
Clicking the **Reset** button causes the Peak Find settings to revert to the values saved previously.

The Peak Find tab has an additional **Save As...** button. Clicking this button allows you to save the current values entered for the Peak Find settings as the defaults for a new assay or to save the current values with an existing assay. The default assay folder will be shown but you can save the assay to any folder of your choice.

NOTE

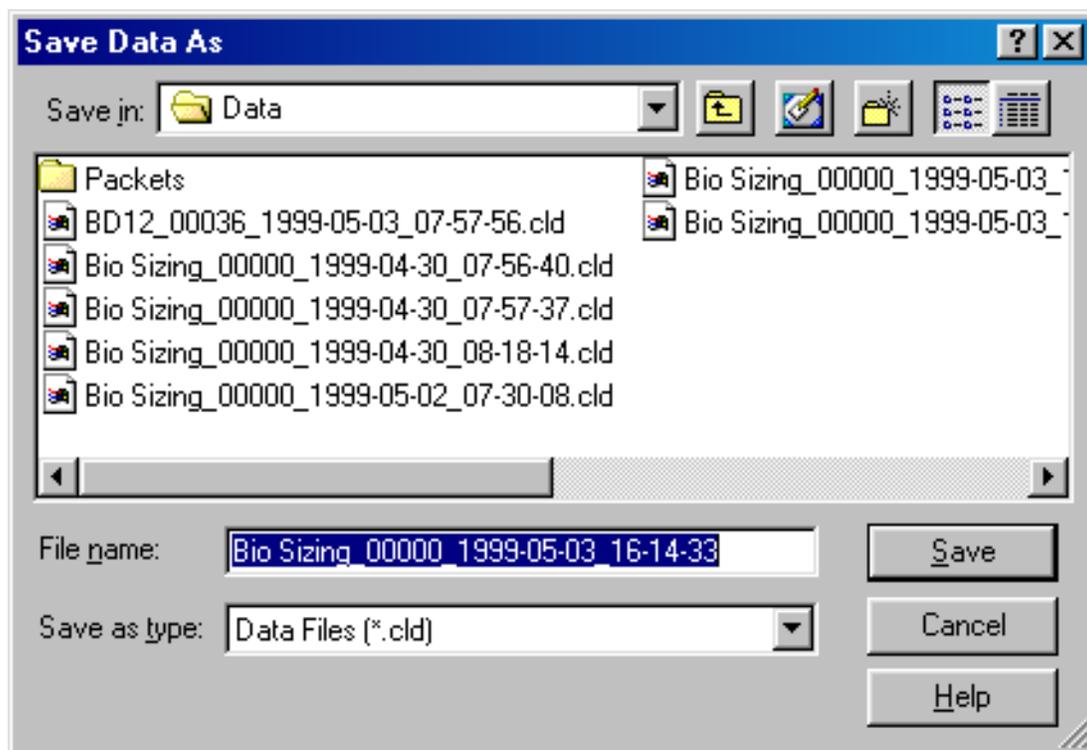
Settings that are saved with an assay file are the peak find settings, gel color default, and well names.

Print Dialog Box



When you choose Print from the File menu, the dialog box shown above appears, providing four options for printing. You can choose from one to all four options. Clicking OK sends the print request. See **Printing a Report—113** for more information.

Save Data As... Dialog Box



This dialog box appears when you choose Save As from the File menu. It allows you to save a data file under a new or different filename. You can also save as the same filename—a dialog box will ask if you want to overwrite the old file. The file can also be saved (under the same or a different name) to a different location on your computer.

Saving Data and Assay Files

File data is saved automatically at the end of a run. Files are given a name that corresponds to the choices you have made regarding the prefix, the serial number, the date and time of the run (see **Data Files Tab—315** for more information).

You can also save files manually by choosing File > Save or File > Save As...

NOTE After data has been acquired and you make changes to the file display, the raw data acquired from the Agilent 2100 Bioanalyzer is *not* changed—only the display of the data is changed and saved.

If you alter the data shown in any way after it has been saved and try to exit the software or acquire new data, a dialog box will appear asking whether or not you wish to save the changed file.

The Save As... dialog box contains a checkbox for saving the file as read-only. A read-only file may be opened (the title bar will show Read-Only at the end of the filename) and edited but may not be saved under the same filename. If you attempt to save an edited read-only file, an error message will be displayed explaining that the file is a read-only file. Clicking OK in the error box will open the Save As... Dialog box. Entering the same name as the read-only file causes another error message to be displayed telling you to save the file with a different name or to a different location.

The benefit of saving a file as read-only is to prohibit you or other users from making changes that would alter the file in any way. Since the Agilent 2100 Bioanalyzer software allows you to open data files, reanalyze them using different assays, alter

peak find parameters, etc., and saves these new parameters with the file when it is saved, you may prefer to ensure that the original parameters which were used to create the file are not altered.

Changes made to an assay file (altered peak find settings, for example) can be saved to the same assay file or to a new one, if desired. Clicking the Save as... button on the Assay Properties dialog box opens the Save As... dialog box allowing you to save the assay.

Tips and Shortcuts

Keyboard Shortcuts

Commands, windows, or dialog boxes can be accessed by selecting them from menus, but the same items can be activated by keystroke combinations or by clicking buttons on a tool bar.

Description (Menu Name)	Keyboard Shortcut	ALT Key Shortcut
File Menu		ALT+F
Open	CTRL+O	ALT+F, O
Save	CTRL+S	ALT+F, S
Save As...		ALT+F, A
Export		ALT+F, E
Print	CTRL+P	ALT+F, P
Page setup		ALT+F, U
File Properties		ALT+F, T
Exit		ALT+F, X
Edit Menu		ALT+E
Copy Gel		
Copy Graph		
Copy Result Table		

Description (Menu Name)	Keyboard Shortcut	ALT Key Shortcut
View Menu		ALT+V
Single Well	F2 or CTRL+W	ALT+V, S
All Wells	F1	ALT+V, A
View Gel	F4	ALT+V, G
Previous Well	F5 or Left Arrow	ALT+V, P
Next Well	F6 or Right Arrow	ALT+V, N
Standard Curve		ALT+V, C
Chip Run Summary		ALT+V, U
Graph Menu		ALT+G
Scale to Selected		ALT+G, S
Scale All		ALT+G, A
Undo Zoom	CTRL+Z	ALT+G, Z
Undo Zoom Completely		ALT+G, U
Show Data Points	CTRL+D	ALT+G, D
Assay Menu		ALT+A
dsDNA Menu		ALT+A, D
RNA		ALT+A, R
Protein		ALT+A, P
Other		ALT+A, H

Description (Menu Name)	Keyboard Shortcut	ALT Key Shortcut
Demos		ALT+A, E
Assay Properties		ALT+A, T
Open Assay		ALT+A, O
Save Assay		ALT+A, V
Save Assay As...		ALT+A, A
Start...		ALT+A, S
Tools Menu		ALT+T
Turn On/Off Analysis	CTRL+A	ALT+T, A
Diagnose Instrument		ALT+T, D
Compare Results		ALT+T, C
Temperature Monitor		ALT+T, T
View Log File		ALT+T, V
Run Log		ALT+T, V, R
System Log		ALT+T, V, S
Options...		ALT+T, O
Help Menu		ALT+H
Contents and Index	CTRL+H	ALT+H, C
About Bio Sizing		ALT+H, A
Selecting Wells		

Description (Menu Name)	Keyboard Shortcut	ALT Key Shortcut
Select Well Above	Up Arrow	
Select Well Below	Down Arrow	
Select First Well (Ladder)	Home	
Select Last Well	End	
After Zooming In on a Plot		
Scroll Horizontally	Left/Right Arrow	
Scroll Vertically	Up/Down Arrow	
Page Horizontal Scroll Right	Shift+Page Up	
Page Horizontal Scroll Left	Shift+Page Down	
Undo zoom and show regular plot	Home	

Mouse Shortcuts

Single Left Mouse Button (Left Click)

In Small Gel	Selects a well
In Multiwell Large Display	Selects a well
In Single-Well Large Display	RNA assays: If on a long-dashed line, move start/end times for that well only; move start/end points for individual peaks DNA assays: if on a peak, selects the peak (shows highlighted in the Results Table and pointer appears over the peak)

In Chip	Selects a well
In Tool Bar	Activates function associated with button in tool bar

Double Left Mouse Button (Left Double-Click)

In Small Gel	Goes to Single-Well View for lane double-clicked
In Multiwell Large Display	Goes to Single-Well View for well double-clicked
In Chip	Goes to Single-Well View for well double-clicked
In Single-Well Large Display	Undoes current zoom (shows all of graph in window)

Single Right Mouse Button (Right Click)

In Small Gel	Selects a well
In Multiwell Large Display	Selects a well
In Title Bar	Activates pop-up menu for sizing of window and closing the application
In Large Gel Display	Activates pop-up menu with combination of items from the Tools and Graph menus
In Single-Well Large Display	Activates pop-up menu with combination of items from the Tools and Graph menus
In Multiwell Large Display	Activates pop-up menu with combination of items from the Tools and Graph menus
In Results Table (Single Well)	Activates pop-up menu with combination of items from the Tools and Graph menus

CTRL+Left-Click (Left Mouse Button + CTRL Key)

In Small Gel Display

In a single-well view in the large display, overlays second and subsequent well data over original well data (for each CTRL+click on a lane in the small gel). Each set of peaks is shown in a different color and line style.

Information about Your Computer

A System Info... feature has been shipped and installed with the Agilent 2100 Bioanalyzer software. This feature can be used to examine your computer and show information about the operating system, fonts, printing, screen display, and more. This information may be useful to the technical support representative if you call for assistance.

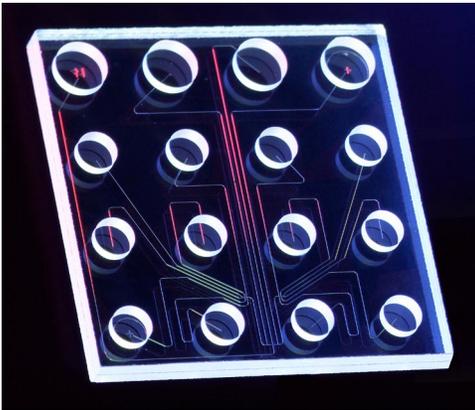
To view information using the System Info feature:

- 1 On the Help menu, click About Agilent 2100 Bioanalyzer.
- 2 Click System Info.
- 3 Click a category for the type of information you want.

Tip: You can save or print information from the System Info dialog box.

Principles of Nucleic Acid Sizing on a Chip

Each chip contains an interconnected set of gel-filled channels that allow for molecular sieving of nucleic acid samples. A series of electrodes control sample movement within the chip. These make contact with the samples when the instrument lid is closed. Each electrode is connected to an independent power supply, providing maximum control and flexibility. The electrodes are also removable, providing the flexibility to implement different configurations depending on the design of a chip.



Microchannels are fabricated in glass to create interconnected networks of fluid reservoirs and pathways.

Glossary

electokinetic forces Electokinetic forces are used to move, switch, mix and separate the nucleic acid samples. Active control over voltage gradients directs the movement of materials using the phenomenon of electrophoretic flow.

electrophoretic flow A macroscopic phenomenon that results from an electrical double layer formed by ions in the fluid and surface electrical charges immobilized on the capillary walls. When an electric field is applied, the bulk solution moves towards one of the electrodes. Electrodes sit in the reservoirs that connect to the ends of the various channels. Electrode potentials are applied to the various reservoirs in a time dependent fashion to move the fluid in the direction you desire it to go.

electrophoresis A standard technique of separating molecules on the basis of their charge-to-mass ratios. An electric potential is applied across a capillary containing a sample in a fluid medium. Positive molecules migrate to the cathode and negative molecules migrate to the anode at different speeds depending on the charge-to-mass ratios.

lab-on-a-chip The generic term for a microfluidic product, signifying a chemical process or material movement taking place on a microchip. In contrast to analysis in a standard laboratory which relies on human intervention at several stages to manipulate or observe samples and record results the self-contained lab-on-a-chip represents an almost *hands-free* technology.

microfluidics The technology term to indicate miniaturization of chemical processes. Microfluidics are generally systems involved in the control of fluid flow. This includes pumps, valves, jets, and microchannels.

molecular separation techniques Processes such as gel electrophoresis, liquid chromatography and capillary electrophoresis which can separate bimolecular organic substances from other compounds

miniaturized laboratories on a microchip Expression used to describe Lab-on-a-Chip technology.

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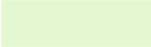
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