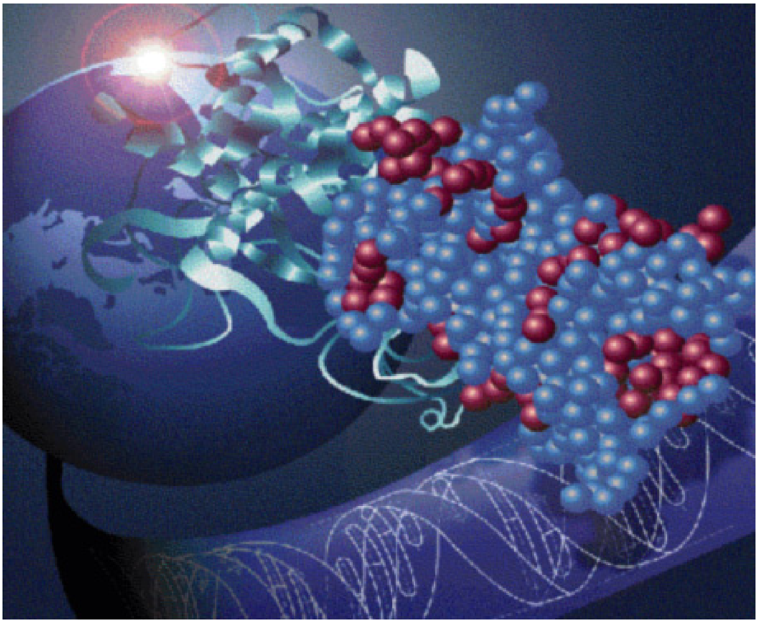

SequencePro®

Data Analysis Application v2.1 Software

User Guide

Applied Biosystems

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Preface

How to Use This Guide

Purpose of This Guide

The Applied Biosystems *SequencePro® Data Analysis Application v2.1 User Guide* provides procedural and reference information related to analyzing data from the PROCISE® Protein Sequencing Systems. It describes how to use the software application to set up preferences, view data and select different analysis parameters.

Audience

This guide is intended for novice and experienced 49X PROCISE Protein Sequencing Systems users who use the system for obtaining N-terminal (PROCISE® or PROCISE® cLC protein sequencer) or C-terminal (PROCISE® C protein sequencer) protein sequencing data.

Assumptions

This guide assumes that your PROCISE Protein Sequencing System has been installed by an Applied Biosystems technical representative and that the latest PROCISE operation software is loaded on the system.

This guide also assumes that you have a working knowledge of the Windows® 2000 operating system.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well microtiter plate.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- ***PROCISE®, PROCISE cLC® and PROCISE® C Protein Sequencing Systems User Guide*** (P/N 4340645) – Describes the PROCISE, PROCISE cLC and PROCISE C protein sequencer hardware and software and provides information on preparing samples, maintaining, and troubleshooting the system.
- ***PROCISE Online Help*** – Describes the PROCISE software and provides explanations for common tasks.

Note: For additional documentation, see “How to Obtain Services and Support” on page xi.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Services and Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Services and Support**.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Overview 1

About This Chapter

This chapter describes the computer specifications to install and use the SequencePro® Data Analysis Application v2.1 software, user management features, an overview of the SequencePro software, and a flow diagram of a typical session using the software.

In This Chapter

This chapter contains the following topics:

Computer System Specifications

Specifications

The following table lists the computer subsystem specifications:

Subsystem	PC
Platform	Pentium or higher-based personal computer
Operating System	Windows® 2000, Windows® XP
RAM	128 MB minimum

Installing SequencePro Software on Windows-Based Platforms

Procedure

To install the SequencePro software:

1. Insert the CD-ROM in the drive.
2. Double-click the Installer icon. A Setup wizard guides you through the installation.
3. Quit the Installer when procedure is complete. Remove and store the CD-ROM.

Registering SequencePro Software

License and Warranty

Before you begin, please read Appendix B, "Software Warranty Information." It explains your rights and responsibilities regarding this software.

About PROCISE and SequencePro User Management Features

Modes

The operation of SequencePro software is determined by the installation of PROCISE® PC 2.1 software. PROCISE software can be installed in two different modes:

- Regulatory
- Non-regulatory mode

The regulatory mode offers a set of user management features that allow an administrator to control access to the features of PROCISE and SequencePro software. The PROCISE software administrator can:

- Create new users
- Create and edit user types
- Manage individual user access to specific screens or capabilities
- Deactivate users

The SequencePro software administrator can:

- Create
- Edit
- Deactivate users

When you install the software in regulatory mode, you are required to log in to each software application with an ID and password. In the regulatory mode, the PROCISE Event Log records your name along with the time and date of the event. In addition, creating or editing cycles, procedures and methods requires verification (re-entry) of user ID and password in order for changes to be saved. The PROCISE Event Log information and the Chemical Log information is transferred to the SequencePro software data file.

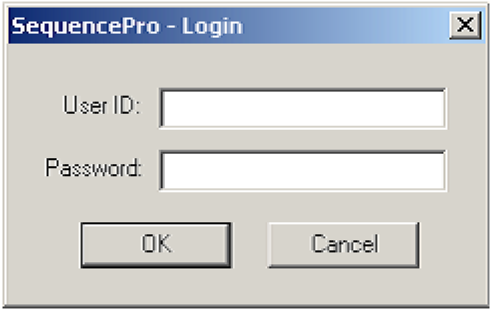
When you open the PROCISE software in the regulatory mode, you can view the Monitor Run and the Event Log screens without a user log-in. Access to any other screens or any of the functionality of the PROCISE software requires the user to log in. You must log in to access SequencePro software features.

Regulatory Mode Log In

In the regulatory mode, all users must log in for access to SequencePro software functions.

To log in:

1. Click on the SequencePro software shortcut to open the SequencePro Login dialog box.



2. Enter your user ID and password and press **OK**. All SequencePro software features will now be accessible.

The initial user ID and password combination for all users is created by your SequencePro software administrator. If a password is lost or forgotten, your administrator will create a new initial password for the user.

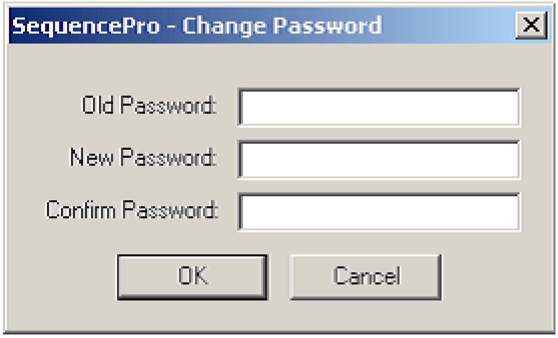
Note: After three failed attempts to log in to the software, the user ID/ password combination will be inactivated and your administrator will need to create a new initial password.

Changing Initial Passwords

The first time you log in to the SequencePro software, you must create a new password. This password should be known only to the user and not shared with your administrator or any other user.

To change the password:

1. Log in to the SequencePro software to access the Change Password dialog box.



2. In the Change Password pop up menu, enter the:
   a. Administrator-created initial password.
   b. Enter the new password twice, once in the New Password field and once in the Confirm Password field and
3. Click **OK**.

About Passwords

- Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.
- SequencePro software passwords will expire in nine months or less if a shorter expiration is set by the SequencePro software administrator. The SequencePro software will begin prompting the user to create a new password two weeks prior to expiration of the current password.
- If the password expires before a new password is created, the SequencePro software administrator will need to create a new initial password for the user.

Changing Subsequent Passwords

After initial login, you have access to the change password menu. Go to the User Management pull-down menu and select **Change Password**. The dialog box shown in the preceding procedure appears; the password may be changed in the same manner as the initial password.

Note: For users without administrative rights, the other user management selections will be unavailable.

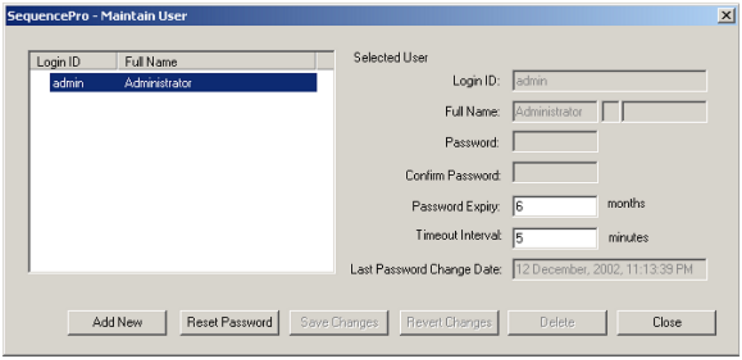
About Timeout Intervals

All users have a timeout setting. This feature automatically logs you out after a specified period of inactivity. The length of the timeout interval is the amount of idle time allowed before the software will log out a user and is set by the SequencePro software administrator. If you are away from the system for longer than the timeout interval, the software will log you out and the you will need to log on again to resume the session.

Managing Users

The SequencePro software administrator has access to the user management feature of the SequencePro software. The administrator can create new users, set password expiration and screen timeout duration, and deactivate users.

The Create/ Maintain User dialog box allows your administrator to manage user access to the SequencePro software. From the User Management pull down, select **Create/Maintain User** and the Maintain User dialog box appears.



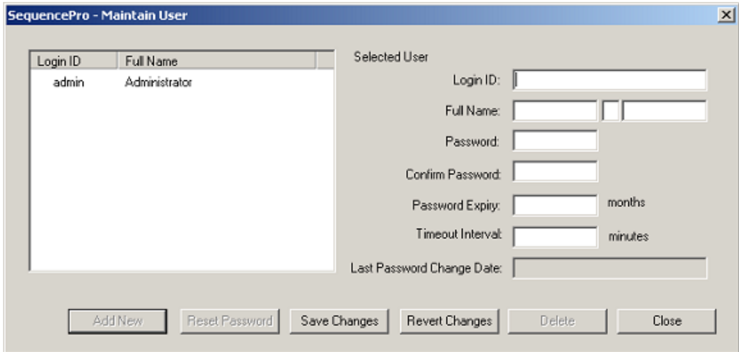
All fields will be grayed out except the Password Expiry and the Timeout Interval.

Note: The default values are six months for password expiration and five minutes for the timeout interval. Custom default values can be set at this point or these values can be set individually as each user is set up.

Creating a New User

To create a new user:

1. Click the **Add New** button on the Maintain User dialog box to activate all the entry fields.



2. Enter a Login ID for the new user. The Login ID can be any combination of 3 to 15 alphanumeric characters.
3. Enter the full legal name of the user associated with the Login ID. The user's full name will be recorded with all actions reported to the Event Log during the session.
4. Set and confirm an initial password for the user. Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.
5. Set password expiration and timeout interval if values other than the default values are required.
6. Click **Save Changes** to store the new user. Click **Revert Changes** to clear all entries.

Changing User's Password

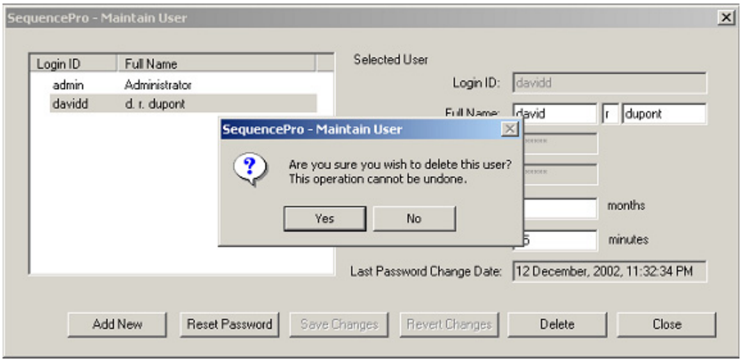
To change the password for a user:

1. Highlight the user from the list at the left of the Maintain User dialog box (shown preceding).
2. Click **Reset Password** and enter and confirm the new initial password. Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.

Inactivating a User

To inactivate a user:

1. Highlight the user from the list at the left of the screen.
2. Click **Delete**. A pop up appears requiring confirmation.



3. Click **Yes**. The user is marked as inactivated in the list but not removed from the list.

   Note: The default administrator cannot be inactivated.

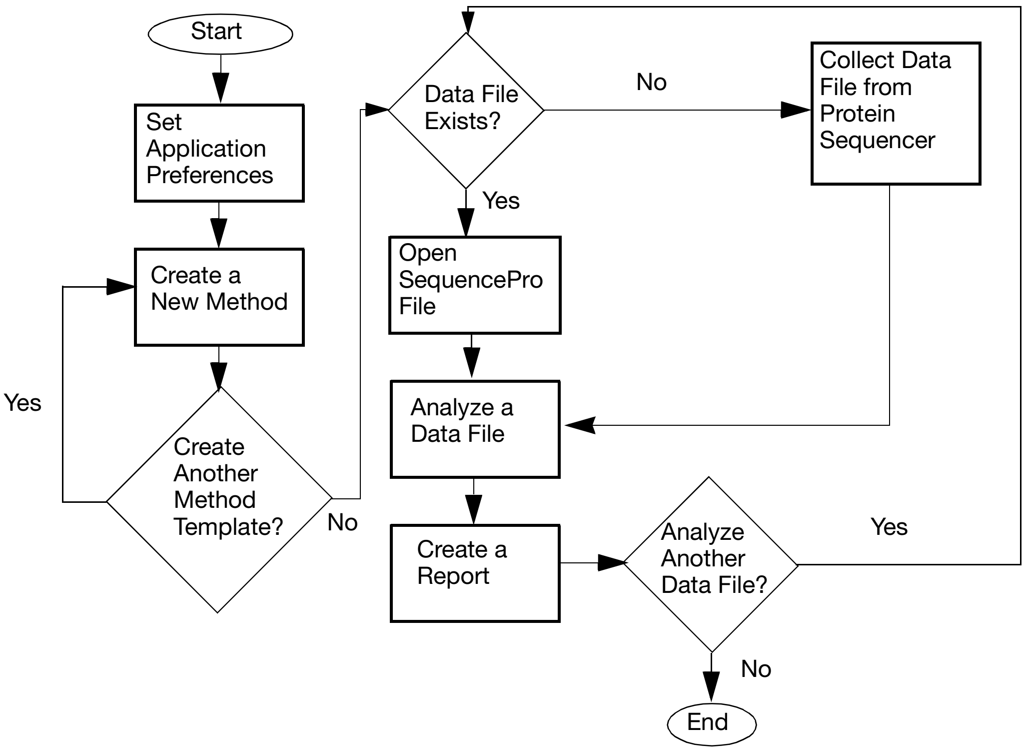
About the SequencePro Software

The SequencePro software analyzes protein data obtained from PROCISE®, PROCISE® cLC, and PROCISE® C Protein Sequencing Systems. The software runs on IBM-compatible computers running the Microsoft Windows® 2000 operating system.

SequencePro Software Flow Diagram

Flow Diagram

The following flow diagram shows a typical SequencePro software session.



This manual takes you through each of the steps in this flow diagram.

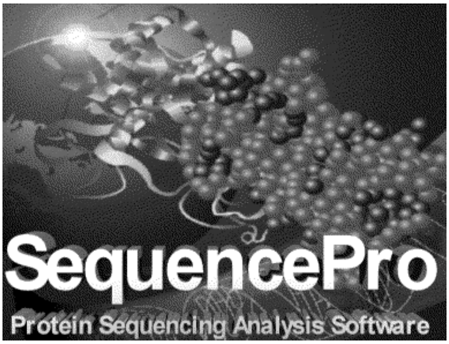
Starting the SequencePro Software

Opening the SequencePro Software

To open the SequencePro software:

1. Double-click the SequencePro software icon, or select **Program Files** and then select **SequencePro**.

   The splash screen appears for a few seconds.



   The splash screen is replaced by the Select a task dialog box.

   Note: The Select a task dialog box does not appear if an acquisition is in progress.

2. Click a button in the Select a task dialog box to:
   - Open an existing file
   - View or modify the Data Dictionary

Setting Preferences and Using Toolbars

2

About This Chapter

This chapter discusses how to set the preferences for SequencePro® Data Analysis Application v2.1 and information about the various pages and toolbars in the application.

In This Chapter

This chapter contains the following topics:

Setting SequencePro Software Preferences

Description

Specify software preferences the first time you use the SequencePro software. This entails:

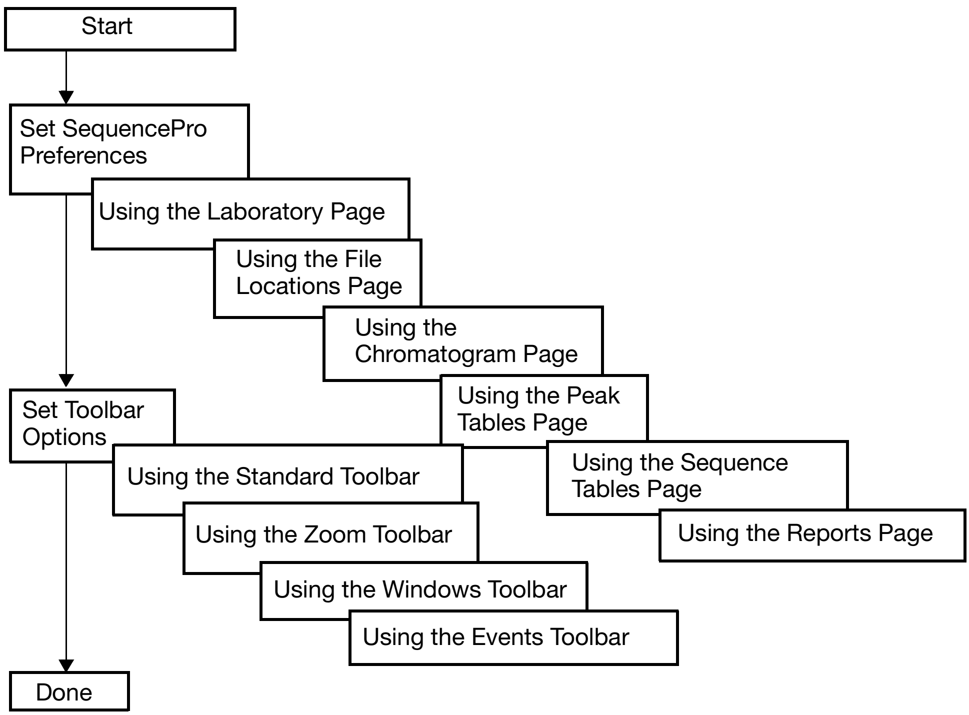
- Naming the laboratory and all users
- Defining acquisition and chromatogram settings
- Setting options for formatting chromatograms, peak lists, reports, and sequence tables

In this process, you also set the on-screen Toolbar options.

Note: Preferences are editable at any time.

Flow Diagram

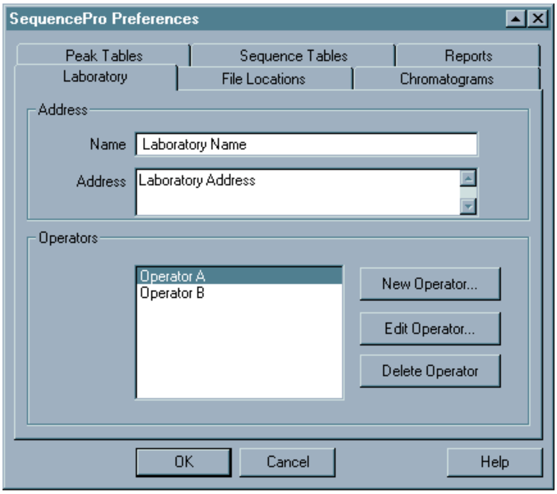
The following flow diagram illustrates setting application preferences.



Setting Preferences

To set preferences:

1. From the View menu, select **Preferences** to open the **Preferences** dialog box.



2. Click each of the following tabs in the SequencePro Preferences dialog box and type in the required information on each page.
3. Click a new tab after you complete each page.

Page	See Page
Laboratory page	2-4
File Locations page	2-6
Chromatogram page	2-8
Peak Tables page	2-10
Sequence Tables page	2-12
Reports page	2-14

Note: These pages may be completed in any order.

4. Click **OK** in the SequencePro Preferences dialog box when you have completed entering information in all of the pages.

Using the Laboratory Page

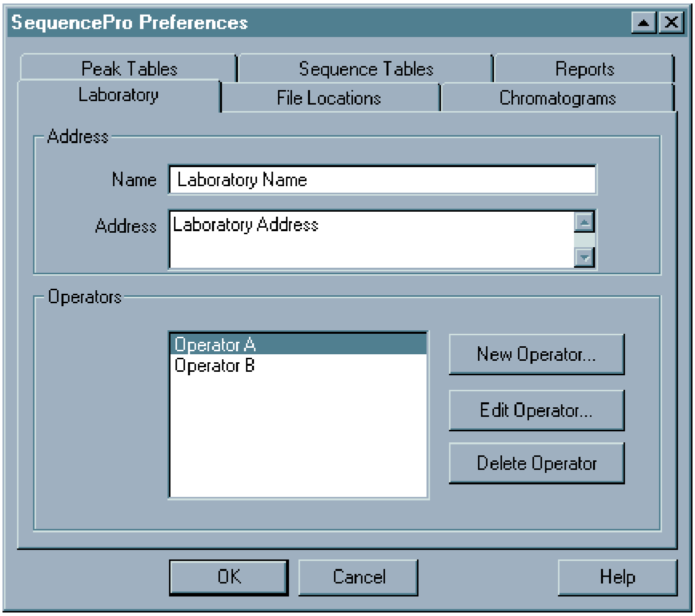
Description

The Laboratory page allows you to enter information about your laboratory and the names of all of the sequencer operators. The information entered here is automatically included in all new data files.

Setting Laboratory Page Options

To set the Laboratory options:

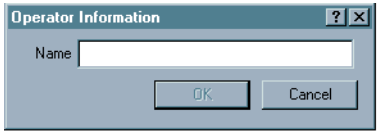
1. With the SequencePro Preferences dialog box displayed, click the **Laboratory** tab to open the Laboratory page.



2. Type the name and address of the laboratory in their respective fields.

<table>
<tr><th>If you want to...</th><th>Then...</th></tr>
<tr><td>add an operator's name</td><td>

1. Click the **New Operator** button to open the Operator Information dialog box.



2. Enter the name of the new operator in the Name field, and then click **OK**.

The OK button will not be activated unless data is entered in the Name box.

</td></tr>
<tr><td>edit an operator's name</td><td>

1. Select the name you want to edit.
2. Click the **Edit Operator** button.
3. Type your edits in the Operator Information dialog box, then click **OK**.

</td></tr>
<tr><td>delete an operator's name</td><td>

1. Select the name of the operator you want to delete.
2. Click the **Delete Operator** button.

The operator's name is deleted from the list.

</td></tr>
</table>

3. Click another tab or, if you are done, click **OK** in the SequencePro Preferences dialog box.

Using the File Locations Page

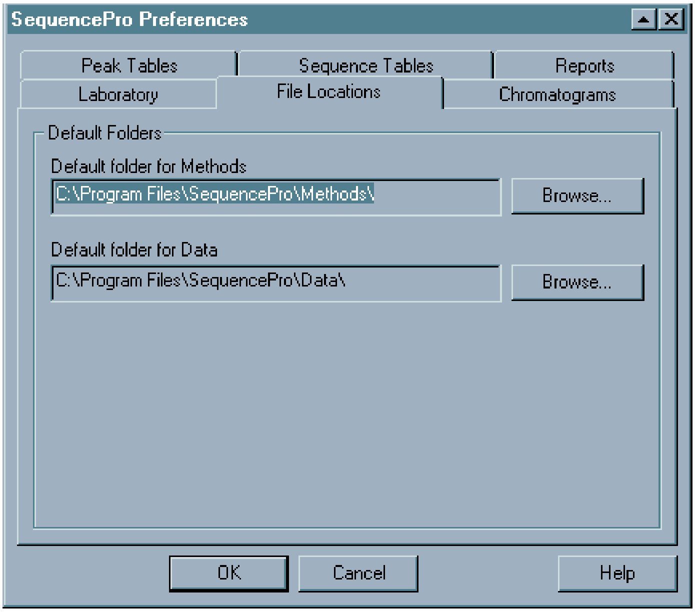
Description

The File Locations page allows you to enter information about the default folders for opening and saving method and sample files.

Setting File Locations

To set the File Locations:

1. With the SequencePro Preferences dialog box displayed, click the **File Locations** tab to open the File Locations page.



2. To select the default folder for Method templates:

   a. Click the **Browse** icon to the right of the field labeled Default folder for Methods. A dialog box opens, allowing you to choose a directory.

   b. Use the dialog box to select the folder you want to use as the default folder for the Method template, then click **OK**.

   The path to this folder now appears in the default folder for Methods field on the File Locations page.

   Note: You cannot directly edit this field. To change the default folder, repeat steps a and b.

3. To select the default folder for data files:
    a. Click the **Browse** icon to the right of the field labeled Default folder for Data.

       A dialog box opens, allowing you to choose a directory.
    b. Use the dialog box to select the folder you want to use as the default folder for the data file, then click **OK**.

   The path to this folder now appears in the default folder for Data Field in the File Locations page.

   Note: You cannot directly edit this field. To change the default folder, repeat steps a and b.
4. Click another tab or, if you are done, click **OK** in the SequencePro Preferences dialog box.

Using the Chromatogram Page

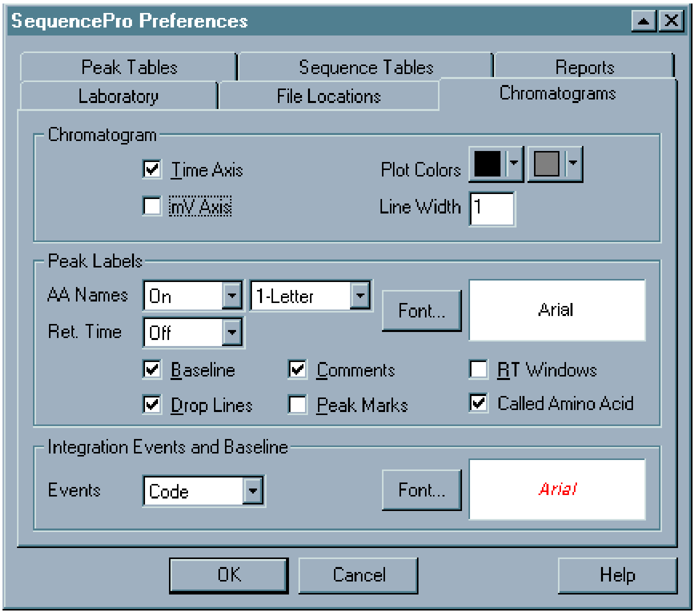
Description

The Chromatogram page allows you to specify the default display settings for chromatograms.

Defining Chromatogram Options

To define the Chromatograms Options:

1. In the SequencePro Preferences dialog box, click the **Chromatograms** tab to open the Chromatogram page.



2. In the Chromatogram section:

Use the...	To...
Time Axis and mV Axis check boxes	select the parameters that you want to appear.
Plot Colors button	select the plot colors for chromatograms.
Line Width text box	enter the line width you want for the chromatogram.

3. In the Peak Labels section:

Use the...	**To...**
Name pull-down menu	select where you want the amino acid name to appear (On or Off).
Letter pull-down menu	select how you want the amino acid name to appear (1- Letter, 3-Letter, or Full Name).
Font button	select the font for peak labels.
Ret. Time pull-down menu	display the retention time above the label (On or Off).
Check boxes	select the parameters you want to appear in the chromatogram. Parameters include: • Baseline • Drop Lines • Comments (indicated by yellow box above peak in Chromatogram view and by footnote in Report view.) • Peak Marks • RT Windows • Called Amino Acid

4. In the Integration Events and Baseline section:

Use the...	**To...**
Events pull-down menu	select how you want to indicate the events information (Code, Full Text, or Off).
Font button	select a font, font style, and color, as in step 3.

5. Click another tab or, if you are done, click **OK** in the SequencePro Preferences dialog box.

Using the Peak Tables Page

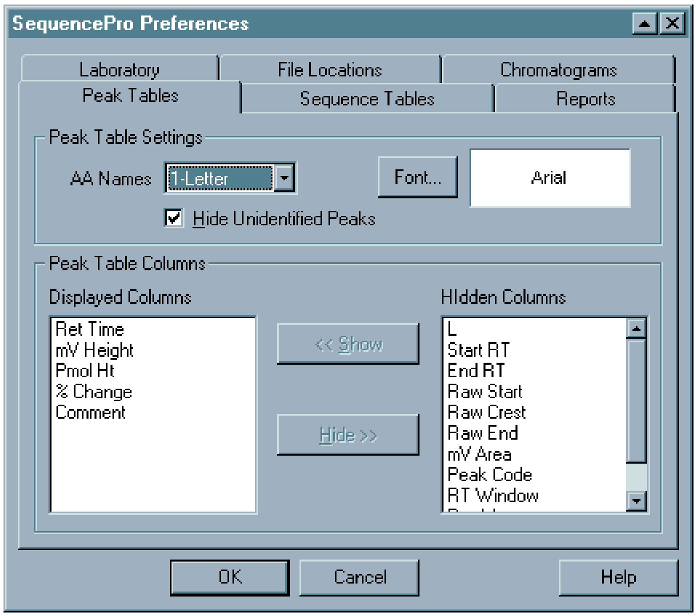
Description

The Peak Tables page allows you to enter the default display settings for peak tables.

Setting Peak Table Options

To set the Peak Tables options:

1. With the SequencePro Preferences dialog box displayed, click the **Peak Tables** tab to open the Peak Tables page.



2. In the Peak Table Columns section, you can:

Use the...	To...
AA Names pull-down menu	select the Full Name, 1-Letter, or 3-Letter amino acid designation.
Font button	select the font for the peak tables.

Use the...	To...
Hide Unidentified Peaks check box	cancel display of unidentified peaks in the peak table.

If you want to...	Then...
show a column	1. From the Hidden Columns list, select the column(s) you want to appear in the peak tables. 2. Click the **<<Show** button. The selected columns appear in the Displayed Columns list.
hide a column	1. From the Displayed Columns list, select a column(s). 2. Click the **Hide>>** button. The selected columns are deleted from the Displayed Columns list.
move a column	1. Click the column you want to move. 2. Hold the left mouse button down and drag the column name to the desired position in the list.

3. Click another tab or, if you are done, click **OK** in the SequencePro Preferences dialog box.

Using the Sequence Tables Page

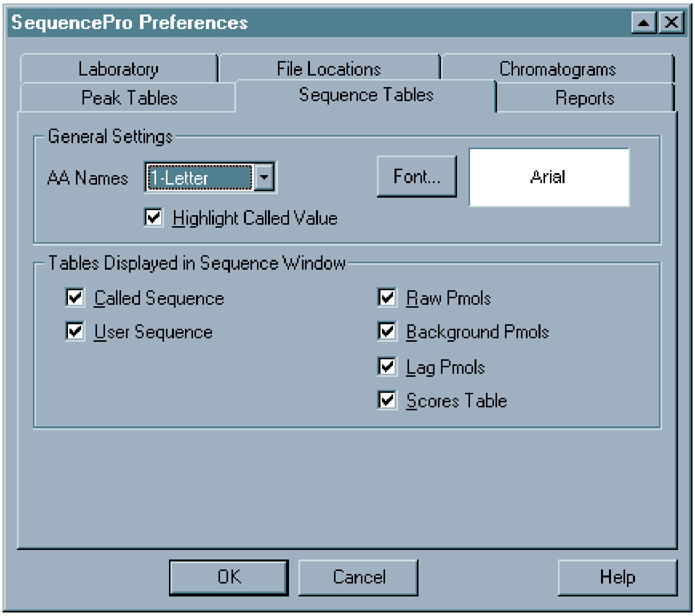
Description

The Sequence Tables page allows you to enter the default display settings for sequence windows.

Setting Sequence Tables Page Options

To set the Sequence Tables options:

1. With the SequencePro Preferences dialog box displayed, click the **Sequence Tables** tab to open the Sequence Tables page.



2. Set the Sequence Tables options:

In this section...	You can...
General Settings	select the Full Name, 1-Letter, or 3-Letter amino acid designation from the AA Names pull-down menu.
	click the check box to highlight the called value.
	set the font by taking the following action: 1. Click the **Font** button to select the font for sequence tables, using the Font dialog box. 2. When you are finished, click **OK**.
Tables Displayed in Sequence Window	click the check boxes for the tables you want to display in the Sequence window.

3. Click another tab or, if you are done, click **OK** in the SequencePro Preferences dialog box.

Using the Reports Page

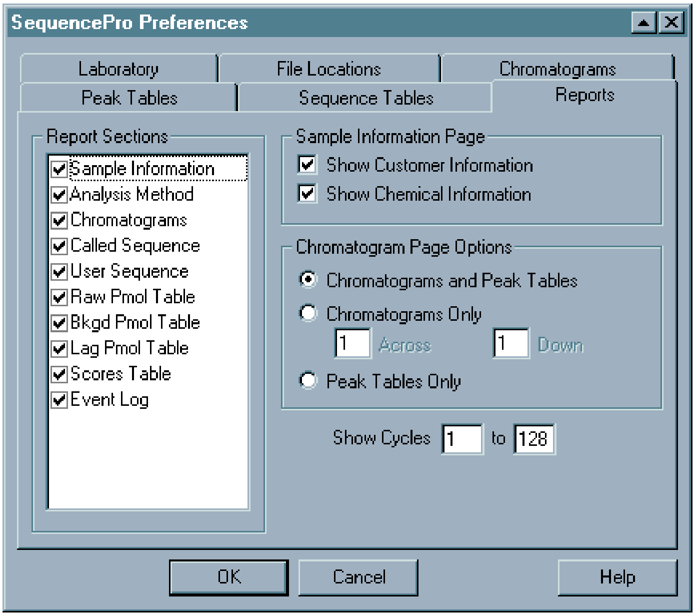
Description

The Reports page allows you to set report preferences, including what information is included in your report, and how information appears on the page.

Viewing the Reports Page

To set the Reports options:

1. With the SequencePro Preferences dialog box, click the **Reports** tab to open the Reports page.



2. Set the reports options:

In this section...	You can use...
Report Sections	the check boxes to select the information you want to appear in your report. Only those sections that are checked will appear.
Sample Information Page	the check boxes to choose customer and chemical information in the report.

In this section...	You can use...
Chromatogram Page Options	to specify chromatogram, peak tables, or both in the report.
Show Cycles	to specify a range of chromatogram cycles to be included in the report.

3. Click another tab, if you are done, or click **OK** in the SequencePro Preferences dialog box.

Setting Toolbar Options

Description

Setting the Toolbar Options allows you direct access in the Chromatogram window to the following toolbars:

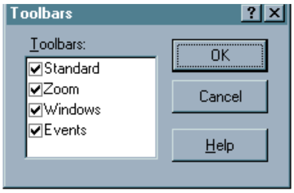
- Standard toolbar
- Zoom toolbar
- Windows toolbar
- Events toolbar

Setting Toolbar Options

To set toolbar options:

1. Choose **Toolbars** from the View menu.

   The **Toolbars** dialog box opens.

2. Click the **Standard** check box to make the Standard toolbar open whenever the Chromatogram window is open.

   For more information, see “Using the Standard Toolbar” on page 2-18.

3. Click the **Zoom** check box to make the Zoom toolbar open whenever the Chromatogram window is open.

   For more information, see “Using the Zoom Toolbar” on page 2-19.

4. Click the **Windows** check box to make the Windows toolbar open whenever the Chromatogram window is open.

   For more information, see “Using the Windows Toolbar” on page 2-20.

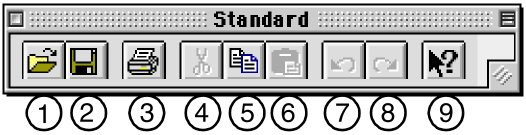
5. Click the **Events** check box and the Events toolbar will appear whenever the Chromatogram window is open.

   For more information, see "Using the Events Toolbar" on page 2-21.

Using the Standard Toolbar

Standard Toolbar Diagram

The figure below shows the Standard toolbar.



Using the Standard Toolbar Buttons

Click a button in the Standard toolbar to create new or to open existing Sample files, or perform standard computer functions.

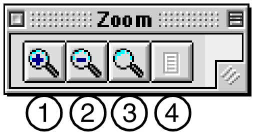
The table below summarizes the Standard toolbar buttons.

Button	Description	Function
1	Open Sample	Opens an existing Sample file.
2	Save	Saves the file.
3	Print	Prints the active window.
4	Cut	Cuts the selection.
5	Copy	Copies the selection.
6	Paste	Pastes the selection.
7	Undo	Undoes the last action.
8	Redo	Redoes the last action.
9	Help	Opens the Help file.

Using the Zoom Toolbar

Zoom Toolbar Diagram

The figure below shows the Zoom toolbar.



Using the Zoom Toolbar Buttons

Click a button in the Zoom toolbar to enlarge or to reduce the selected area in the active window.

The table below summarizes the functions of the Zoom toolbar buttons.

Button	Description	Function
1	Zoom In	Magnifies the display.
2	Zoom Out	Reduces the display.
3	View 100%	Shows the display in normal size.
4	Page View	Always displays the entire page.

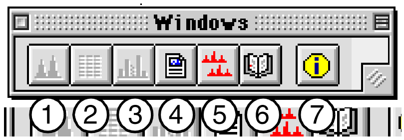
Using the Windows Toolbar

Description

Click a button in the Windows toolbar to view various windows.

Windows Toolbar Diagram

The figure below shows the Windows toolbar.



Using the Windows Toolbar Buttons

The table below summarizes the Windows toolbar buttons.

Button	Description	Function
1	Chromatogram Window	Displays the chromatogram window.
2	Sequence Window	Displays the Sequence window.
3	Graph Window	Displays the Graph window.
4	Report Window	Displays the Report window.
5	Compare Window	Displays one or more chromatograms.
6	Event Log	Displays the Event Log.
7	Sample Information	Displays the Sample Information table.

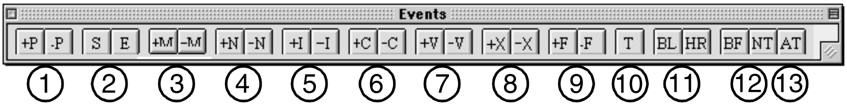
Using the Events Toolbar

Description

Use the buttons on the Events toolbar to modify the integration parameters. All modifications made with the Events toolbar are local (*i.e.*, they apply only to the currently active chromatogram).

Events Toolbar Diagram

The figure below shows the Events toolbar.



Using the Events Toolbar Buttons

See the table below for descriptions and functions of the Events toolbar buttons.

Note: For a detailed description of the integration parameters see “Using Events” on page A-17.

Events Toolbar Buttons

Button Set	Description	Function
1	+P, Peak detection on	Enables peak detection (default).
	–P, Peak detection off	Disables peak detection.
2	S, Start or split peak	Specifies the starting point of a split peak.
	E, End of peak	Specifies the end point of a split peak.
3	+M, Start manual integration	Adjusts a baseline manually and prevents normal baseline adjustment.
	–M, Stop manual integration	Turns off manual baseline adjustment and allows normal baseline adjustment.

Events Toolbar Buttons *(continued)*

Button Set	Description	Function
4	+N, Negative peak detection on	Enables negative peak detection.
	–N, Negative peak detection off	Disables negative peak detection (default).
5	+I, Inhibit end of peak	Prevents finding the end of a peak.
	–I, Allow end of peak	Lets the software find the end of a peak (default).
6	+C, Force common baseline	Draws a common baseline for a series of peaks.
	–C, Discontinue common baseline	Discontinues drawing a common baseline for a series of peaks.
7	+V, Force valley to valley baseline	Draws separate baselines for each peak in a series of peaks.
	–V, Discontinue valley to valley baseline	Discontinues drawing separate baselines for each peak in a series of peaks.
8	+X, Exponential skim on	Forces an exponential skim to start on a peak.
	–X, Exponential skim off	Prevents an exponential skim from starting on a peak, if the peak meets the criteria for exponential skimming.
9	+F, Force horizontal baseline forward	Forces a horizontal baseline forward from the timed event.
	–F, Discontinue horizontal baseline forward	Discontinues the horizontal baseline.
10	T, Tangential skim on	Forces a tangential skim to start on a peak.

Events Toolbar Buttons *(continued)*

Button Set	Description	Function
11	BL, Force baseline to point	Forces the baseline to the start of a peak.
	HR, Force horizontal baseline backward	Forces the baseline backward from the time of the event.
12	BF, Reset bunching factor	Resets the numerical value of the bunching factor.
	NT, Reset noise threshold	Resets the numerical value of the noise threshold.
13	AT, Reset area threshold	Resets the numerical value of the area threshold.

Viewing Sample Data 3

About This Chapter

This chapter describes how to use the windows, commands, and other options of the SequencePro® Data Analysis Application v2.1 software.

In This Chapter

This chapter contains the following topics:

Using the Chromatogram Window

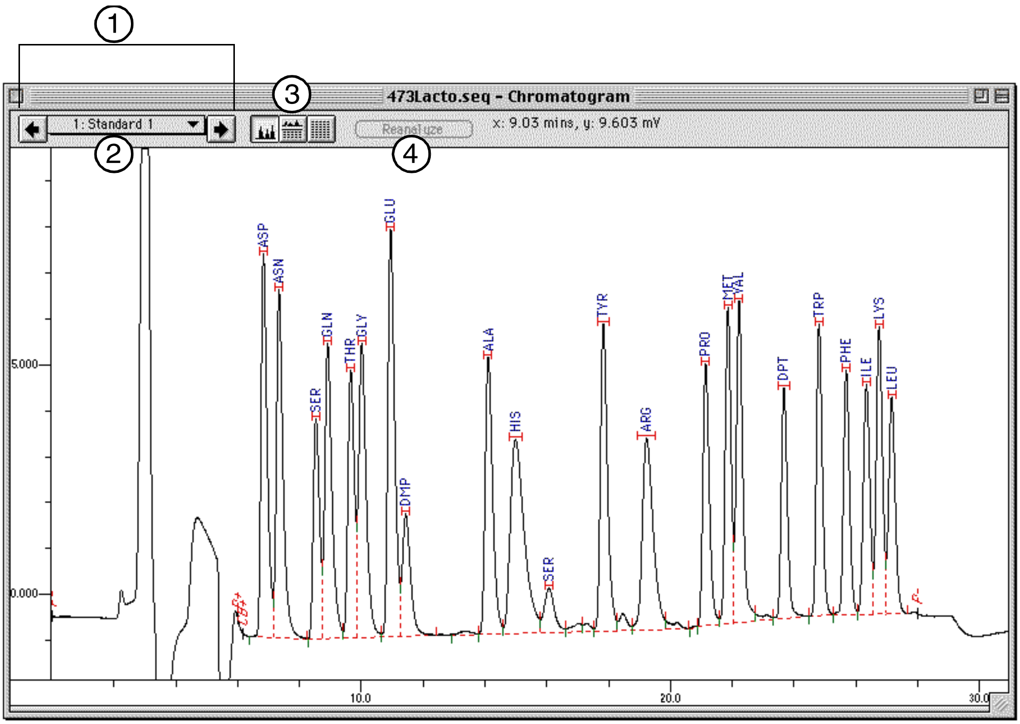
Description

The chromatogram window displays the chromatogram and/or peak table for each chromatographic cycle in a sample file. You can modify and customize the appearance of the chromatogram window to suit your needs.

The chromatogram window is displayed by default whenever you open a sample file.

Chromatogram Window Example

The following is an example of the chromatogram window. A table follows to explain the numbered areas.



Chromatogram Window Description

The following table lists the numbers from the previous diagram:

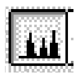
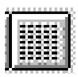
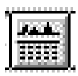
No.	Description	For more information, see...
1	View next or previous cycle	"Cycle Viewing Options" on page 3-4.
2	View any cycle	
3	View chromatogram and or peak table	"Chromatogram and Peak Table Viewing Options" on page 3-3.
4	Reanalyze button	"Reanalyze Button" on page 3-3.
5	View split windows	"Viewing the Chromatogram and the Peak Table" on page 3-5.

Reanalyze Button

The Reanalyze button is active if Automatic Analysis is off. If you make a change when the Automatic Analysis is off, for example, adding an event, then click reanalyze to update the chromatogram.

Chromatogram and Peak Table Viewing Options

The following table lists the chromatogram window viewing options:

If you want to view the...	Then click...
chromatogram only	show chromatogram icon button. 
peak table only	show peak table icon button. 
chromatogram and the peak table together	show chromatogram and the peak table icon button. 

Viewing Multiple Chromatogram Windows for Multiple Sample Files

To view multiple chromatogram windows simultaneously:

1. Open each one using the **File Open** command.
2. Click on the window you wish to view to bring it to the front.

   Other windows will hide behind the active chromatogram.

Viewing Extra Chromatogram Windows for the Same File

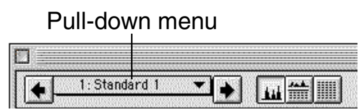
Viewing extra chromatogram windows for the same file can be useful for comparing the cycles in the sample.

To view extra chromatogram windows for the same file:

1. Open the chromatogram window.
2. Select **New Chromatogram Window** from the Window menu.
3. Select **Tile Horizontal** from the Window menu.
4. Select **Synchronize Cycles** from the Chromatogram menu.
5. Click either the right or left arrow to cycle through the sequences.

Cycle Viewing Options

You have the following options:

If you want to view...	Then...
the next or the previous cycle in the sample	click the right- or left-pointing arrows. 
a specific chromatogram or cycle, or any residue chromatogram	select it from the pull-down menu.

Viewing the Chromatogram and the Peak Table

Split-Window Option

The split-window option allows you to view both the chromatogram and the peak table. All chromatogram windows have the split-window option.

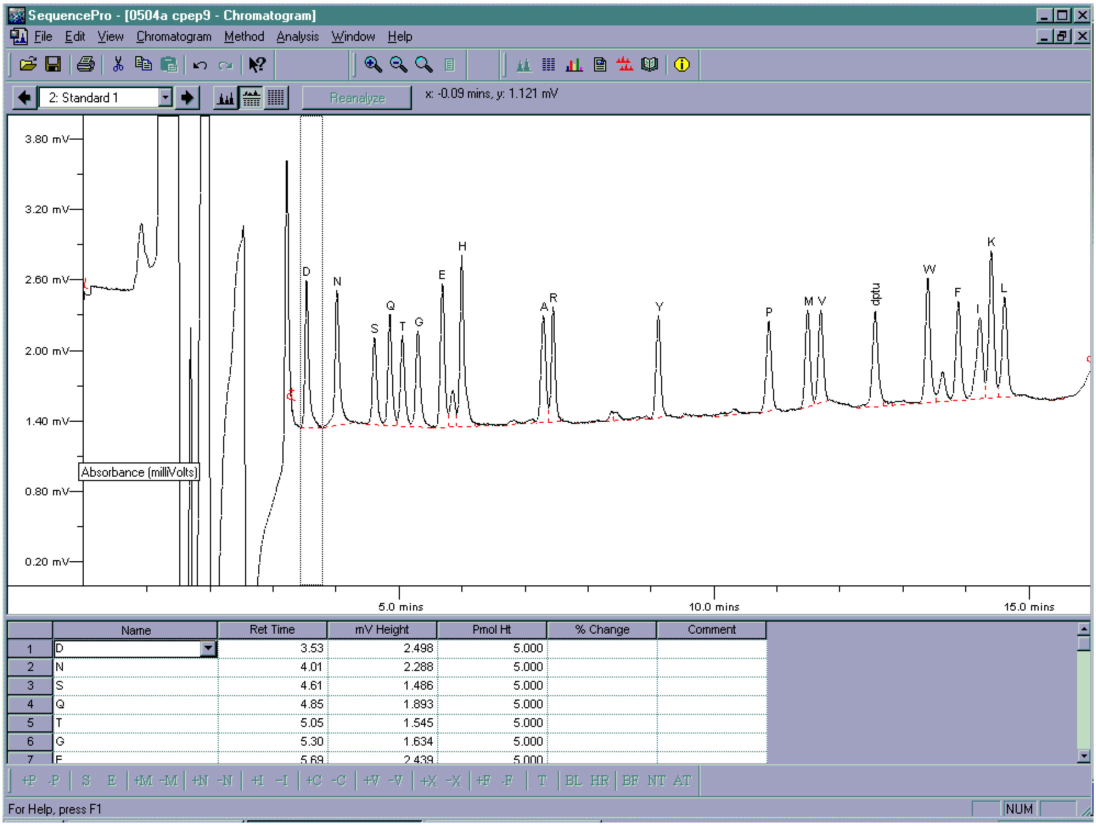
To split the window, move the cursor to the:

- Bottom of the chromatogram, or
- Top of the table, if you are in the Peak table view

You can do the following when the cursor changes from an arrow to the window splitter icon:

<table>
<tr><th>If you are in...</th><th>Then...</th><th>Result</th></tr>
<tr><td>Chromatogram view</td><td>hold the mouse button down and drag the cursor up</td><td rowspan="2">The chromatogram pane is compressed as the table pane enlarges (see “Split-Window Example” below).
The peak table shows information about the peaks in the cycle.</td></tr>
<tr><td>Peak table view</td><td>hold the mouse button down and drag the cursor down</td></tr>
</table>

Split-Window Example



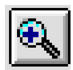
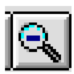
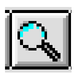
Using the Peak Table

To use the peak table, you can take the following action:

If you want to...	**Then...**
go to a specific peak in the peak table	double-click the arrow icon that appears when you place the cursor on a peak.
go to a specific peak in the chromatogram window	Click the row in the table. The selected peak is highlighted, as a box outlines the peak in the chromatogram window.

Zooming Options

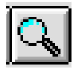
Use the Zoom toolbar as follows:

If you want to...	**Then...**
zoom in of the chromatogram window.	click 
zoom out of the chromatogram window.	click 
restore the full chromatogram window view.	click 
view a specific region of the chromatogram	Hold down the mouse button and drag over the area of interest.

Note: The Axes Settings dialog box also allows you to zoom in or out. For more information see “Setting Scale Properties” on page 3-18.

Home View

To set the view of the chromatogram so that it opens as the same size for every cycle in the sample, take the following action:

If you want to...	Then...
preserve a chromatogram view	select **Save Home View** from the chromatogram menu.
restore the view	you can either: • Click the  or • From the **View** menu, select **100%**

Changing Settings

You can take the following action:

If you want to change settings...	Then select...
in the current file	Settings from the chromatogram menu.
for all new files (Default)	Preferences from the View menu.

Using the Context Menus

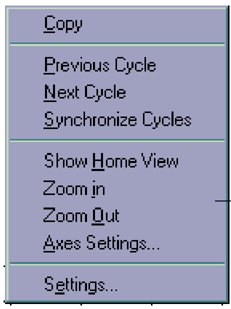
Description

A context menu is available in each window. It displays a list of commands that can be used on the selected item.

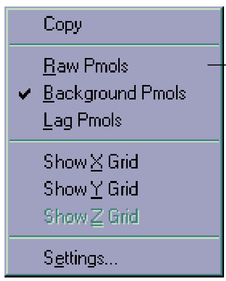
Viewing Context Menus

To view a context menu right-click in the window. The context menu opens at the cursor.

Two examples of context menus are shown below.



Context menu, as it appears in the chromatogram



Context menu, as it appears in the graph window

Using Multiple Windows

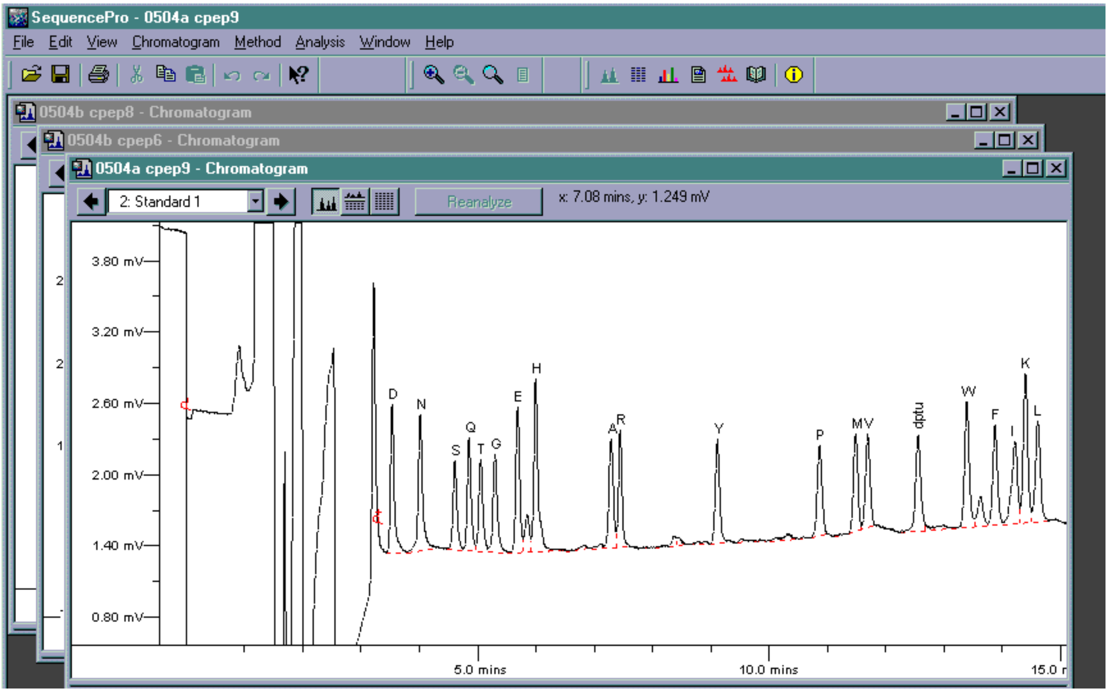
Description

When more than one window is open on the screen, you can use the Window pull-down menu to organize them in one of the following arrangements:

- Cascade
- Tile Horizontal
- Tile Vertical

Cascade

This option displays open windows in a cascade pattern, as follows:

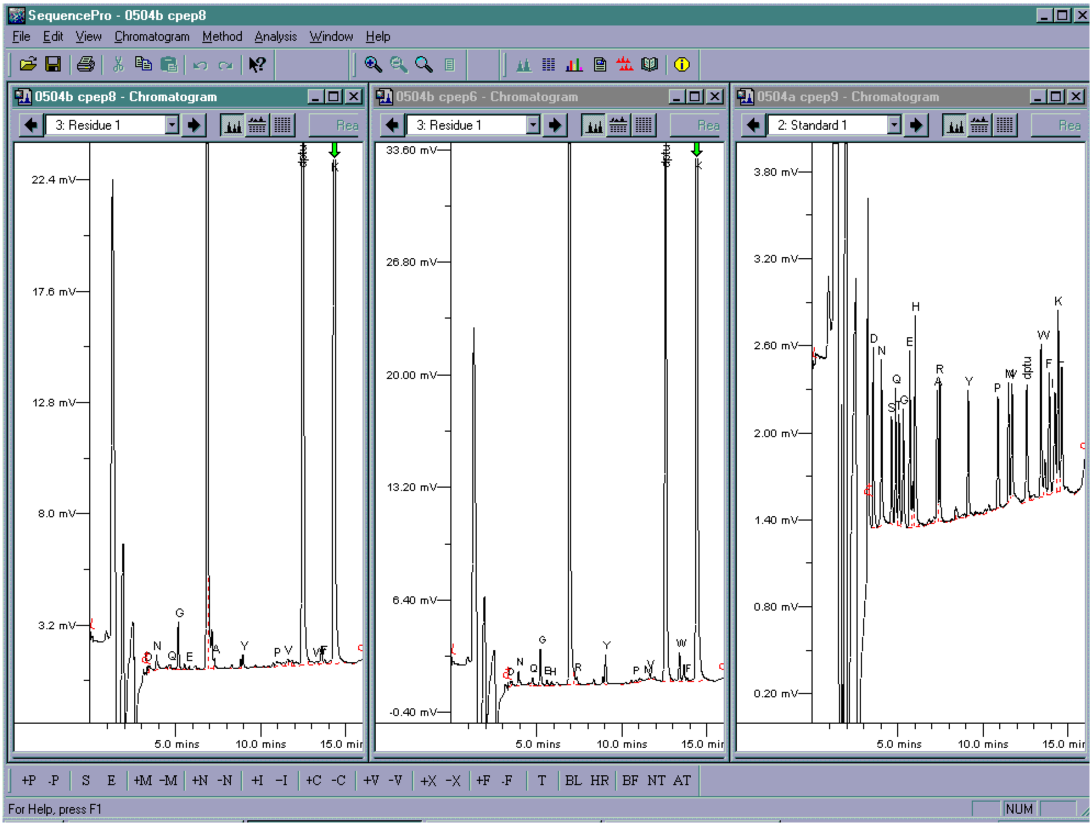


Tile Horizontal

This option displays open windows in a horizontal tile pattern, as follows:

Tile Vertical

This option tiles open windows vertically, as follows:



Using the Compare Command

Description

Use the Compare command from the View menu to:

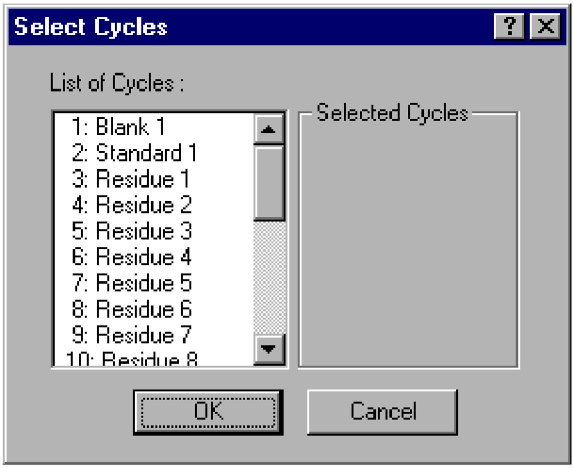
- Analyze up to six residue cycles
- Analyze one or more residue cycle(s) and the standard cycle

and determine the similarities and the differences between them.

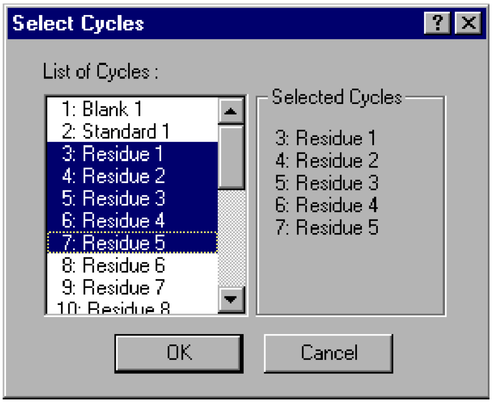
Procedure

To compare several cycles:

1. Select **Compare** from the View menu to open the Select Cycles dialog box.



2. Select the cycles you wish to compare. Residue cycles 1 through 5 have been selected below.

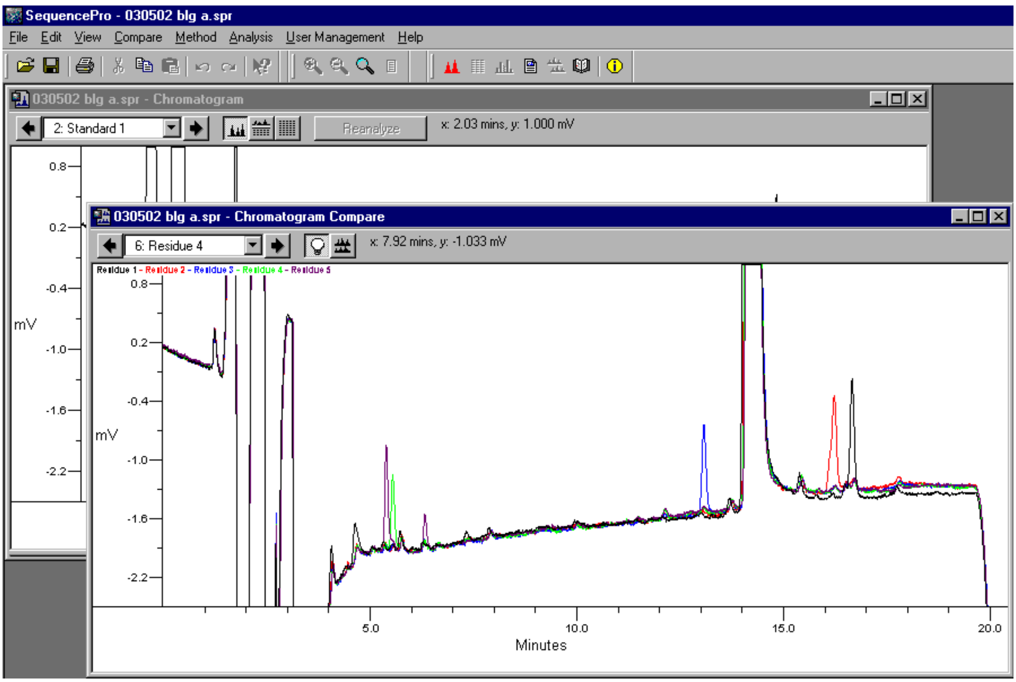


3. Click **OK** to open the Chromatogram Compare window.

Displaying Overlaid Cycles

To display overlaid cycles:

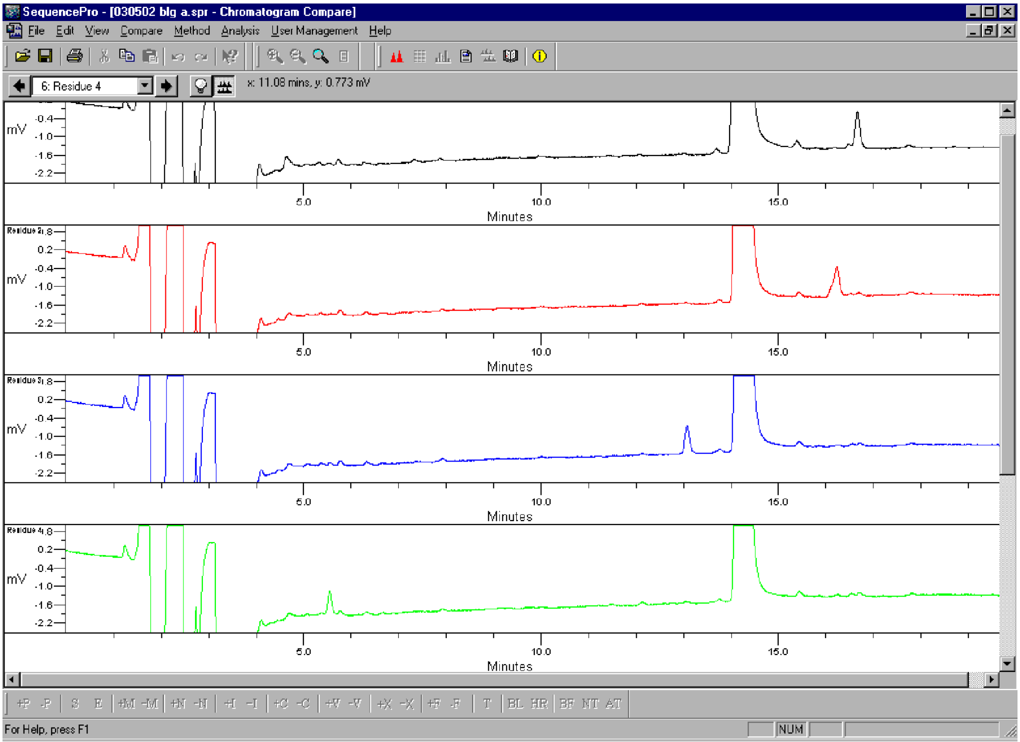
1. Click the **light bulb** button on the Chromatogram Compare toolbar to use the lightbox option. Selected cycle traces are overlaid in the chromatogram window.



2. Improve the alignment.
    a. Move the cursor over the chromatogram. The cross hairs will change to a hand.
    b. Click and hold the left mouse button to select the chromatogram and drag to the desired location.

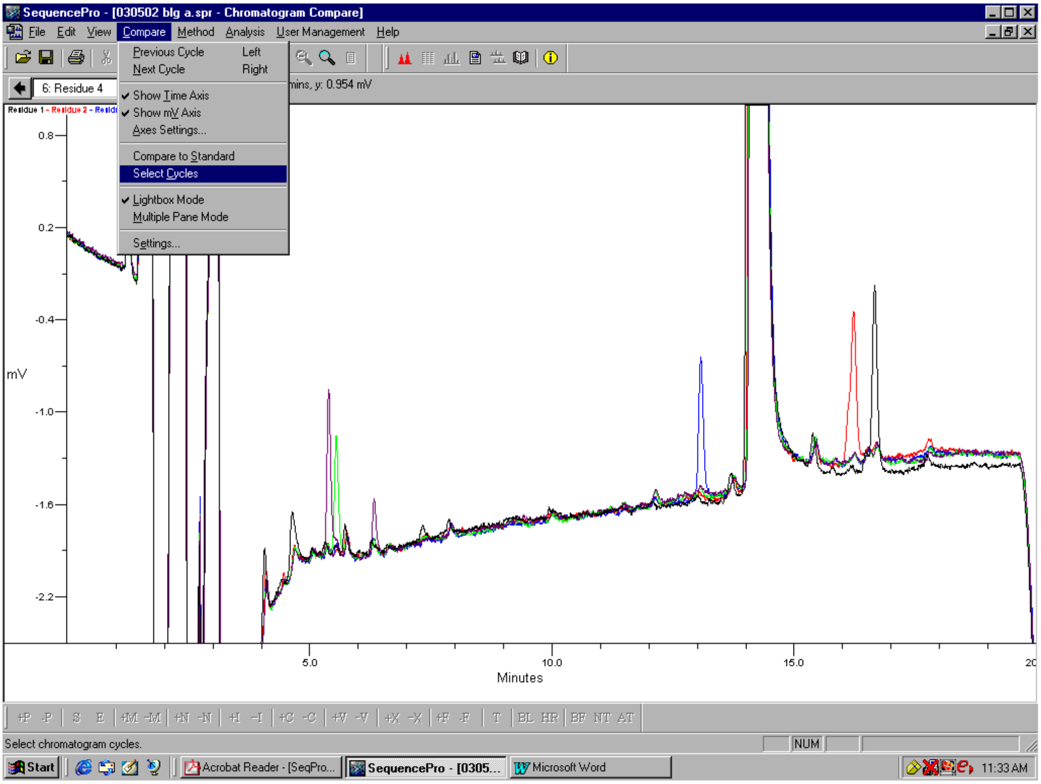
Displaying Multiple Panes

To display multiple panes click on the **chromatograms** button on the Chromatogram Compare toolbar.



Changing the Chromatogram Selection

When the Chromatogram Compare window opens, the Compare drop-down menu is available. The Compare drop-down menu takes the place of the Chromatogram drop-down menu in the Compare mode.



To change the chromatogram selection, select **Select Cycles**. You can also switch between lightbox and multiple pane display modes and activate the compare to standard mode using this menu.

Using the Axes Settings Options

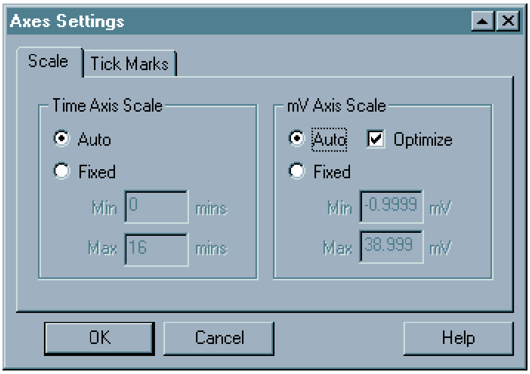
Description

The Axes Settings options allow you to set or edit the horizontal and vertical axes in the chromatogram window.

Setting Axes Properties

To set the axes properties:

1. From the Chromatogram menu, select **Axes Settings** to open the Axes Settings dialog box.



2. Enter the desired information under the Scale and Tick Marks tabs.

If...	Then...
you check both **Auto** and **Optimize** in the mV Axis Scale section	the chromatogram will scale so that the vertical axis shows the tallest amino acid peak in the cycle.

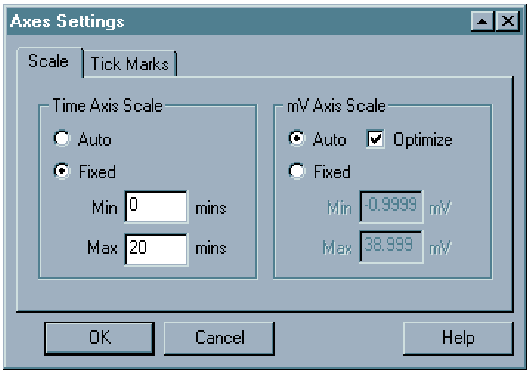
Note: You can view or edit these pages in any order.

Setting Scale Properties

The Scale page allows you to set the horizontal and vertical scales in the chromatogram window.

To set the scale properties of the chromatogram:

1. Open the Axes Settings dialog box to view the Scale page.



2. You can select the following from the Time Axis Scale (horizontal) section:

Select...	To set the...
Auto	horizontal axis to 30 sec before start of integration and 30 sec after integration stops, so that all amino acids are visible.
Fixed	minimum and maximum time settings in minutes.

3. You can select the following from the mV Axis Scale (vertical):

Select...	**To set the...**
Auto	maximum mV scale to match the height of the tallest peak in the chromatogram. Sets the minimum mV scale just below the lowest scale in the chromatogram.
Fixed	minimum and maximum settings in mV.

4. Click the **Tick Marks** tab or, if you are done, click **OK**.

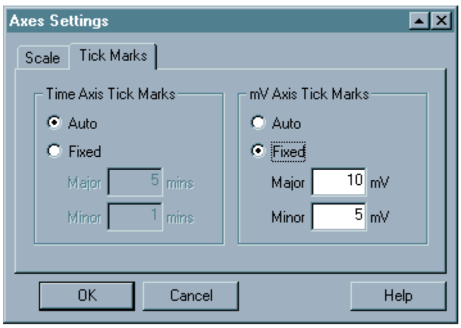
   For information on setting the tick mark properties, see “Setting Tick Mark Properties” on page 3-19.

Setting Tick Mark Properties

The Tick Marks page allows you to set the horizontal and vertical tick marks in the chromatogram window.

To set tick mark properties:

1. Click the **Tick Marks** tab in the Axes Settings dialog box to open the Tick Marks page.



2. Select the following from the Time Axis Tick Marks (horizontal) section.

Select...	**To...**
Auto	optimally set the major and minor tick marks. • Major tick marks have time labels. • Minor tick marks are small ticks.

Select...	To...
Fixed	set the major and minor tick mark settings.

3. You can select the following from the mV Axis Tick Marks (vertical) section:

Select...	To...
Auto	optimally set the major and minor tick marks. • Major tick marks have mV labels • Minor tick marks are small ticks.
Fixed	set the major and minor tick mark settings.

4. Click the **Scale** tab or, if you are done, click **OK**.

   For information on setting the scale properties, see the procedure on page 3-18.

Viewing Sample Information

Description

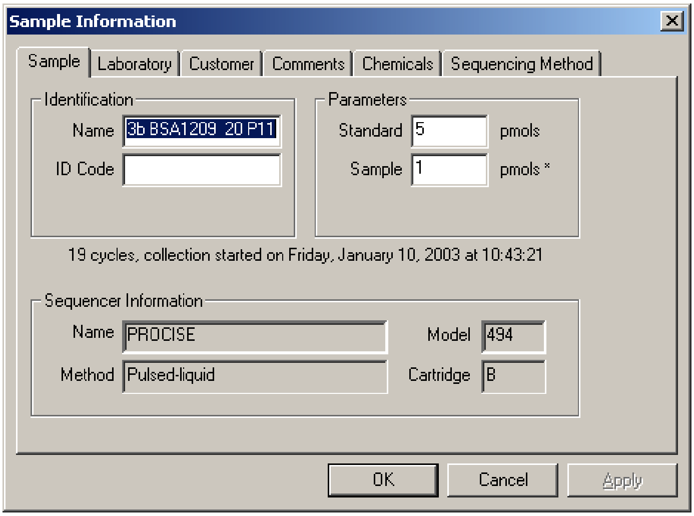
Use the Sample Information dialog box to view or modify information about a sample.

Procedure

To view sample information:

1. From the View menu, select **Sample Information**.

   The Sample Information dialog box appears.



2. Click each of the following tabs in the Sample Information dialog box and enter the required information on each page.

   Click a new tab after you complete each page.

   The following table lists where to find more information about each page.

For this topic...	See Page...
Using the Sample Page	3-23
Using the Laboratory Page	3-24
Using the Customer Page	3-25
Using the Comments Page	3-26
Using the Chemicals Page	3-27
Using the Sequencing Method Page	3-28

   Note: These pages may be viewed or edited in any order.

3. Click **OK** when you have completed viewing or editing information in all of the pages.

Using the Sample Page

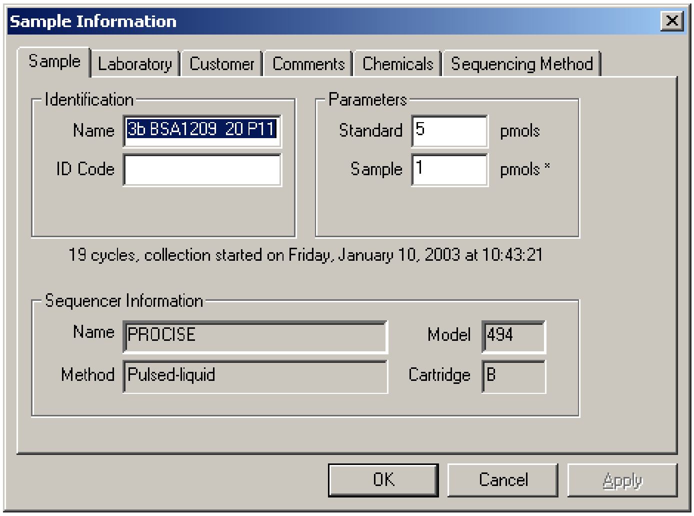
Description

Use the Sample page to view or edit information about the sample, as well as view information about the sequencer. The information entered in the Preferences dialog box is the default information.

Viewing Sample Information

To view sample information:

1. In the Sample Information dialog box, click the **Sample** tab to open the Sample page.



2. Enter, view, or edit the sample identification, parameters, or sequencer method information.

3. Click another tab or, if you are done, click **OK**.

Using the Laboratory Page

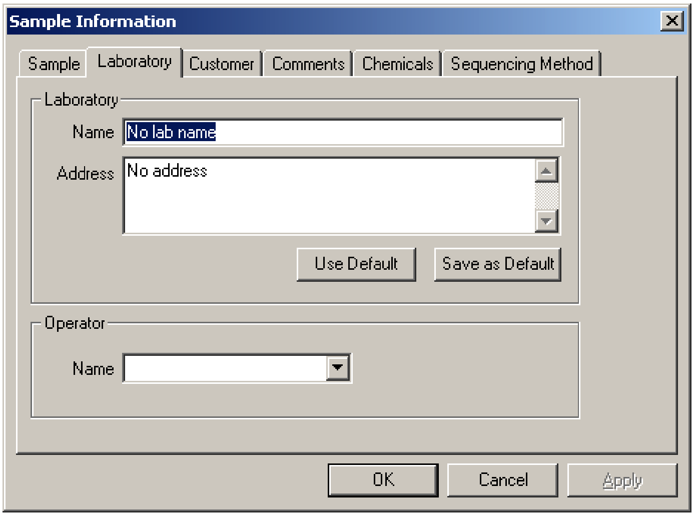
Description

Use the Laboratory page to view or enter information about your laboratory and the names of the operators. The information entered in the Preferences dialog box is the default information.

Viewing Laboratory Information

To view the laboratory information:

1. In the Sample Information dialog box, click the **Laboratory** tab to open the Laboratory page.



2. Enter, view, or edit laboratory and operator information.
3. Click the **Use Default** button to use the default name and address.
4. Click the **Save as Default** button to automatically update preferences, thus creating a new default identification.
5. Either:
   - Select the name of an operator from the Operator Name pull-down menu, or
   - Enter the operator name if it is not on the list

     Operator names are entered using the Preferences menu (see “Using the Laboratory Page” on page 2-4).
6. Click another tab or, if you are done, click **OK**.

Using the Customer Page

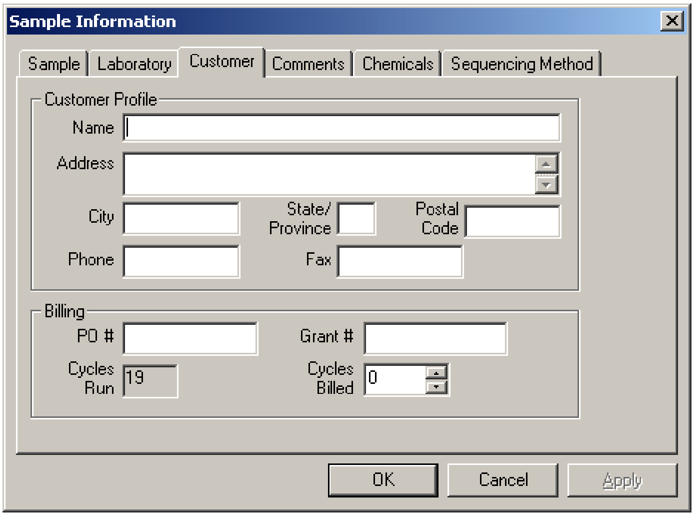
Description

Use the Customer page to enter customer profiles and billing information. The information entered in the Preferences dialog box is the default information.

Viewing the Customer Profiles

To view customer profiles and billing information:

1. In the Sample Information dialog box, click the **Customer** tab to open the Customer page.



2. Enter, view, or edit the customer profile and the billing information.
3. Click another tab or, if you are done, click **OK**.

Using the Comments Page

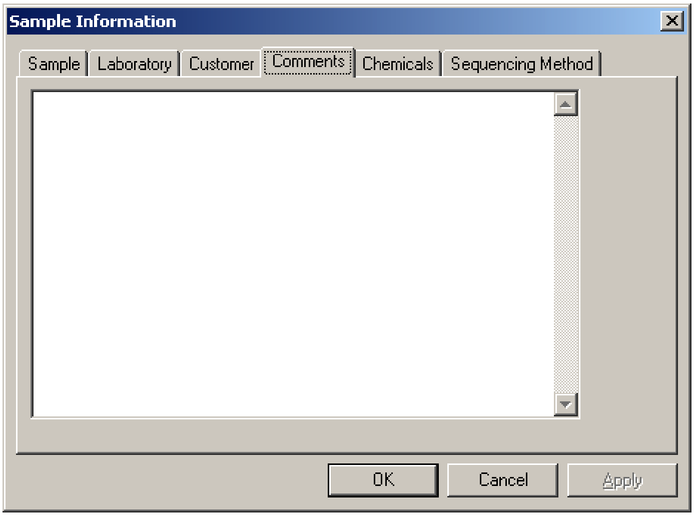
Description

Use the Comments page to view or write comments about your sample.

Writing Comments

To write comments:

1. In the Sample Information dialog box, click the **Comments** tab to open the Comments page.



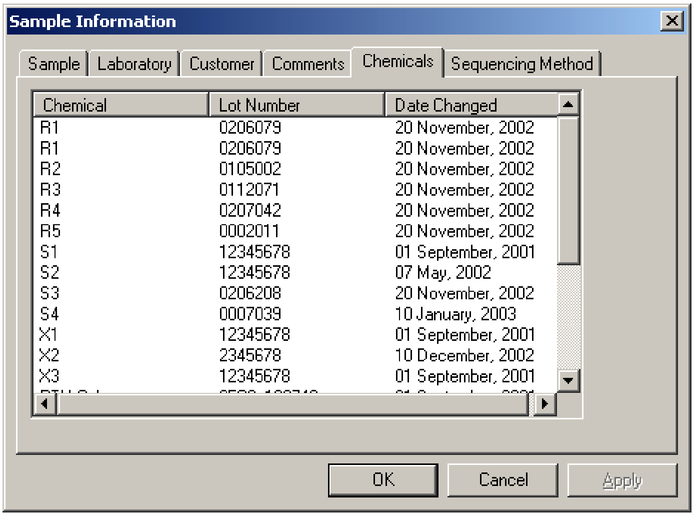
2. Enter your comments.
3. Click another tab or, if you are done, click **OK**.

Using the Chemicals Page

Description

Click the **Chemicals** tab to list all the information from the bottle change view of the protein sequencer pertaining to the currently opened SequencePro data file.

The list includes the name of the reagent, solvent or other consumable used in the analysis of the sample, the lot number, and the date of change.

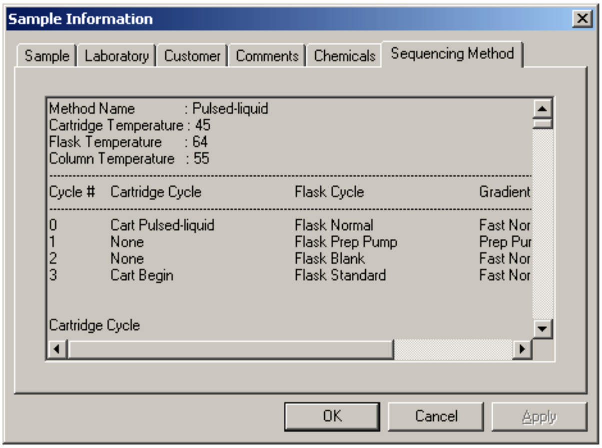


Using the Sequencing Method Page

Description

Click the **Sequencing Method** tab to list all the method information from the protein sequencer pertaining to the opened SequencePro software data file.

The list includes step by step listings of the method, cartridge and flask cycles and gradient.

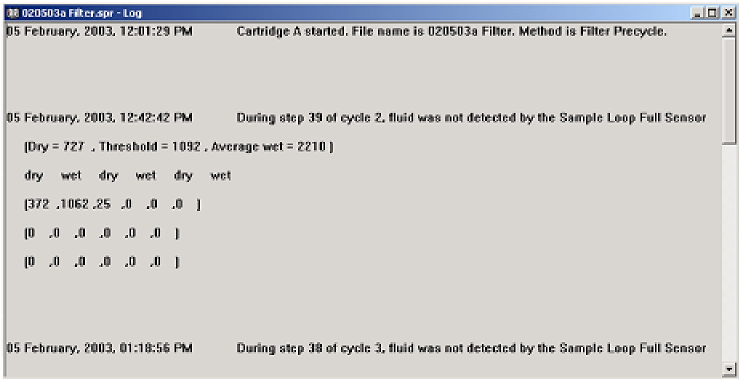


Viewing the Chromatogram Log

Description

The chromatogram log contains all the PROCISE software Event Log entries pertaining to the currently opened Sequence Pro software data file.

From the View menu, select **Chromatogram Log** to open the chromatogram Log window.



Analyzing Data Files

4

About This Chapter

Analysis of sequencing data acquired from protein sequencers includes calibration of standard peaks, integration of residual peaks, and sequence calling. Using the SequencePro® Data Analysis Application v2.1 software, you can view the data after each of these steps and, if necessary, correct any data analysis problems.

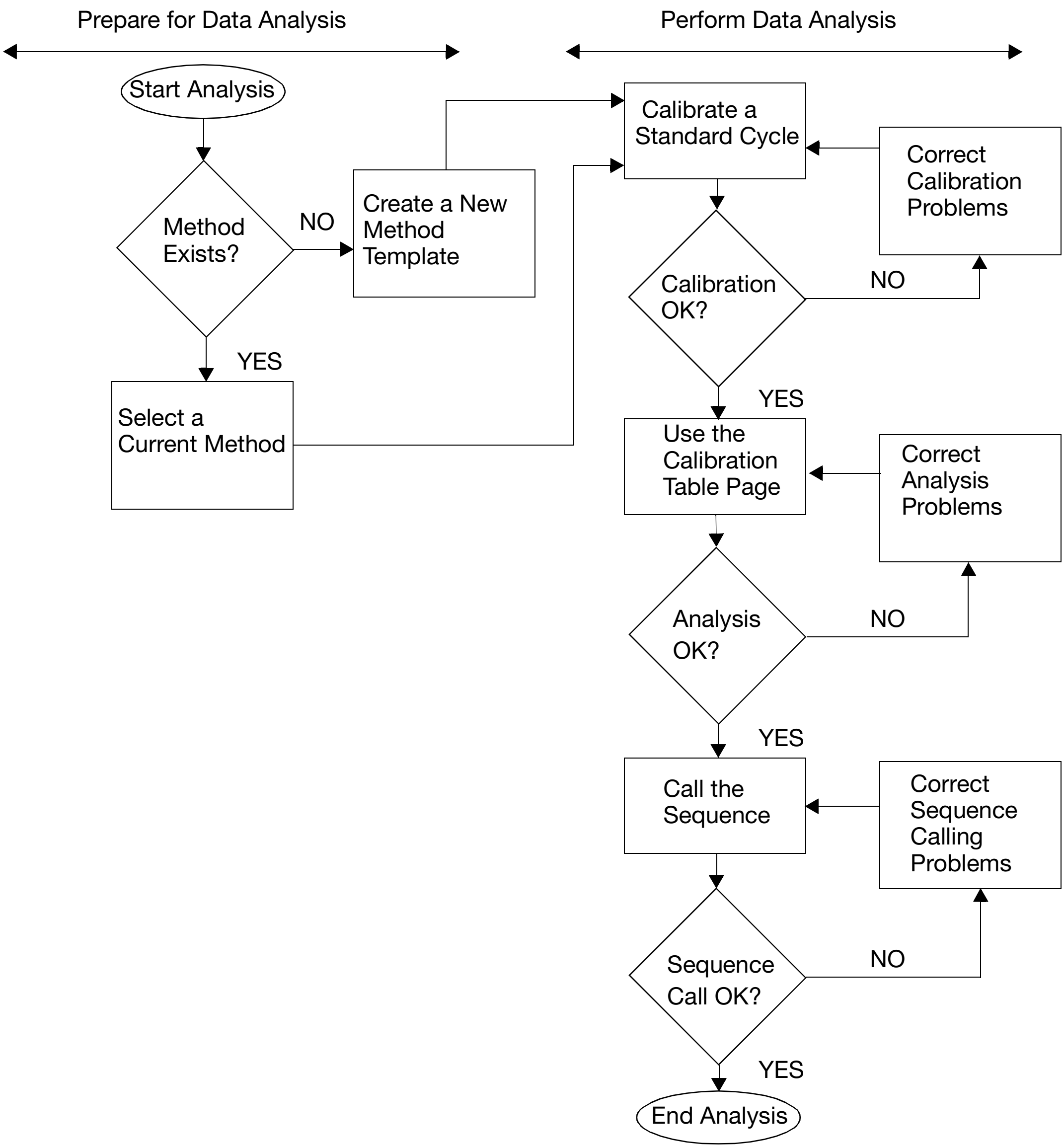
In This Chapter

This chapter contains the following topics:

Data Analysis Flow Diagram

Flow Diagram

This flow diagram illustrates the process of analyzing a data file.



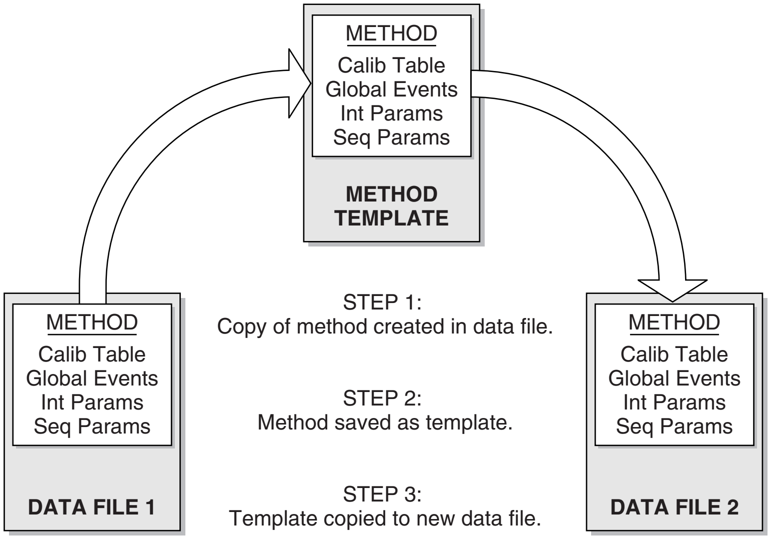
Using Methods

Definition

Methods are complete sets of data analysis parameters that can be shared by multiple SequencePro software data files.

Example of How Methods Are Used

The following figure illustrates how methods are used.



Where Methods Are Stored

Methods are stored by default in the Methods folder specified in the File Locations page of the Preferences dialog box. Method templates can also be stored in any folder on the disk or on the network.

What Methods Contain

The SequencePro software Methods contain the following information:

Methods contain...	For information, see...
Calibration table	"Using the Calibration Table Page" on page 4-15.
Global events	"Using the Global Events Page" on page 4-18.

Methods contain...	For information, see...
Integration parameters	"Using the Integration Page" on page 4-20.
Sequence calling options	"Using the Sequence Calling Page" on page 4-23.

The SequencePro software attaches the Methods you choose to your data files.

Creating, Selecting, and Editing Methods

The SequencePro software allows you to create, select, and edit Methods.

If you want to...	Then see...
Create a new Method template	"Creating a New Method Template" on page 4-5.
Select a previously created Method template and include it in the data file you are currently using	"Selecting a Current Method" on page 4-11.
Open and edit a previous created Method and include it in your current data file	"Editing the Current Method" on page 4-13.
Edit a Method template	"Using the Method Editor" on page 4-9.

Saving and Copying from and to a Method

Use the Method menu to:

- Save a current method as a template
- Copy a Calibration Table from a Method or Sample file (see "Copying a Calibration Table" on page 4-16)
- Copy standard peaks to a method to create a calibration table

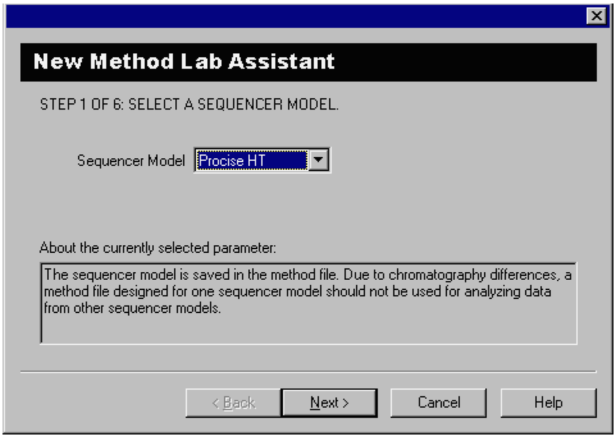
Creating a New Method Template

Creating a Method Template

To create a new Method template:

1. From the Method menu, select **Create Template**.

   The following dialog box appears.



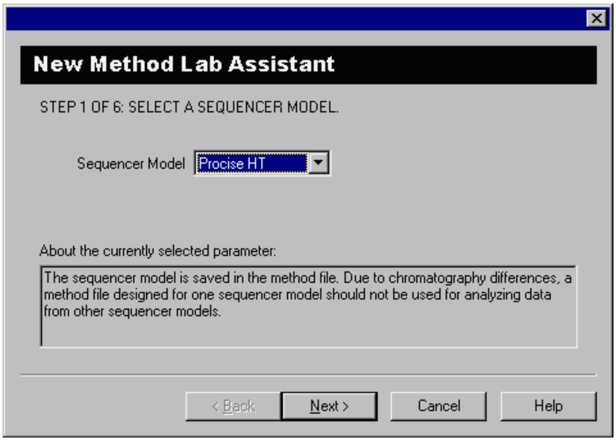
   The New Method Lab Assistant takes you through the following steps.

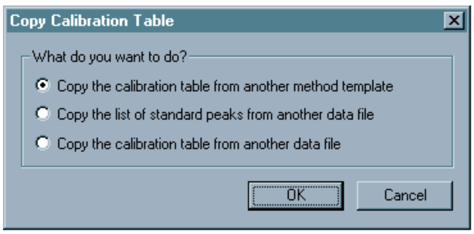
   During each step you can take any of the following actions:

Click	**Description**
Help	For more information.
Cancel	Discard the new method.
Back	Go to the previous New Method Lab Assistant step.
Next	Go to the Next Method Lab Assistant step.

2. From the Sequencer Model pull-down menu, select a sequencer model and click **Next**. The following dialog box appears.

   Note: If you do not want to import a calibration table at this time, click **Next** only. You can specify the calibration table later using the Method properties dialog.

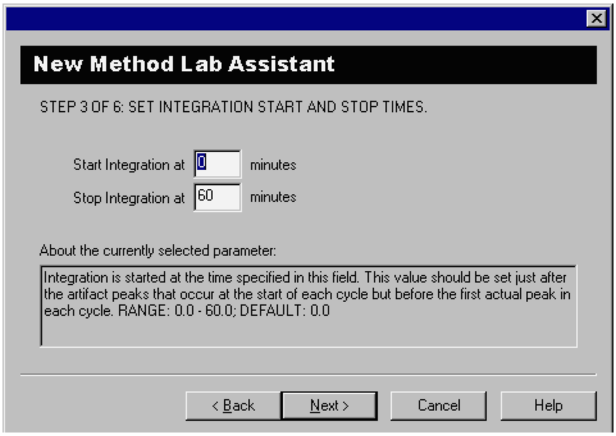


If you...	Then click...
want to import a calibration table	**Import Calibration Table** to import a calibration table from an existing method or data file. The following dialog box appears (see below).
	
do not want to import a calibration table	**Next**. You can specify the calibration table later using the Method properties dialog.

a. Click the button to select where you want to copy a calibration table from and click **OK**.

b. When the directory dialog box appears, navigate to the files from which you want to copy the calibration table.

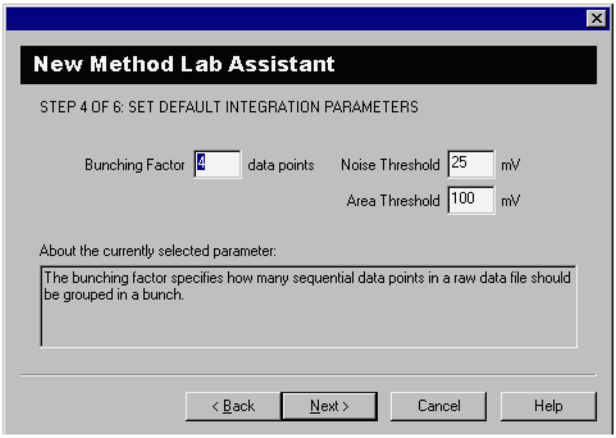
3. Set the Integration Start and Stop times.



Enter the times (in minutes) you want to start and stop integration in each cycle.

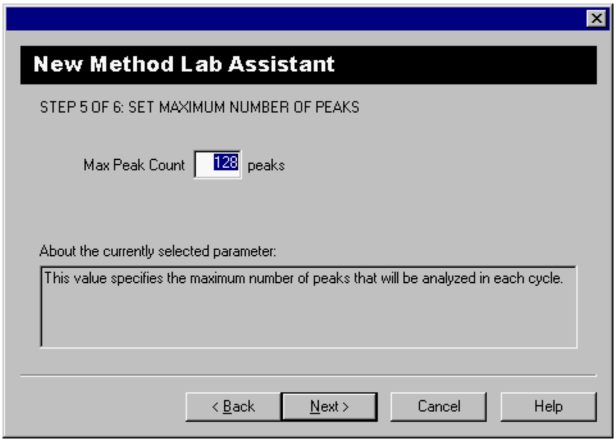
Note: You can modify these values later using the Method properties dialog.

4. Set the Default Integration Parameters.



Enter the default values for the bunching factor, noise threshold, and area threshold.

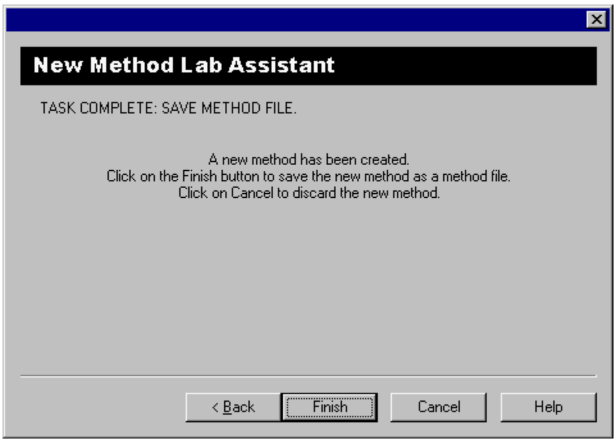
5. Set the Maximum Number of Peaks.



Enter a value for the maximum number of peaks you want to analyze.

Note: The maximum number of peaks allowed per cycle is 128.

The Task Complete: Save Method File dialog box opens. A new Method template has been created.



6. Click the **Finish** button to save the new method as a Method template in the Method folder.

   The new Method template is opened in the Method Editor window.

Using the Method Editor

Description

You can use the Method Editor to edit the current Method within a sample file or to edit an existing Method template.

The Method Editor allows you to edit parameters for the following:

You can edit these parameters...	For information, see...
Calibration table	"Using the Calibration Table Page" on page 4-15.
Global events	"Using the Global Events Page" on page 4-18.
Integration	"Using the Integration Page" on page 4-20.
Sequence calling	"Using the Sequence Calling Page" on page 4-23.

Opening the Method Editor

From the Method menu, select **Edit Current** or **Edit Template**.

Editing a Method Template

Description

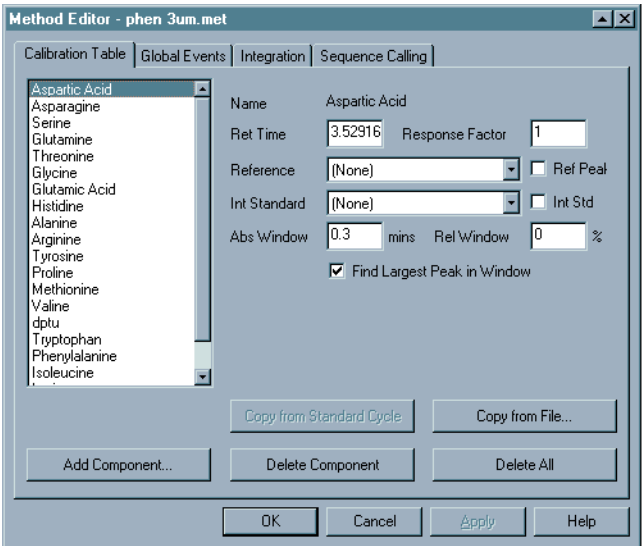
Use to open and edit a previously created Method template. The edited Method template will be used with all data files attached to it.

Procedure

To edit a previously created Method template:

1. From the Method menu, select **Edit Template**.

   The Method Editor dialog box appears.



2. In the Method Editor dialog box, click each of the following tabs, and type in your changes.

   The following table lists where to find more information about each tab:

For this topic...	See Page
Using the Calibration Table Page	4-15
Using the Global Events Page	4-18
Using the Integration Page	4-20
Using the Sequence Calling Page	4-23

   Click a new tab after you complete each page.

   Note: These pages may be completed in any order.

3. Click **OK** in the Method Editor dialog box when you have completed entering your changes.

Selecting a Current Method

Description

Allows you to copy the contents of a Method template into the current data file.

Procedure

To select a Method template to include in your current data file:

1. From the Method menu, select **Select Current**.

   The standard Open dialog box displays.

2. Locate and select a Method template.

   The Method is now attached to the data file.

Using Automatic Analysis

Description

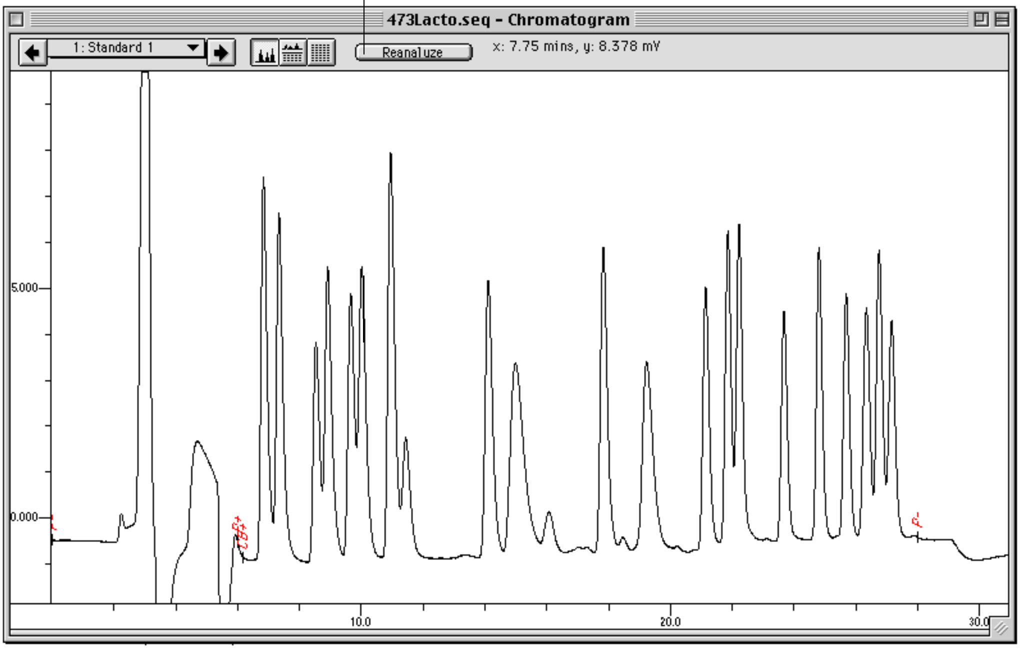
Automatic Analysis automatically keeps a data file analyzed whenever you:

- Add integration events
- Change analysis settings
- Modify peak names

Reanalyzing

If Automatic Analysis is off, click the **Reanalyze** button in the chromatogram window after making any changes (for example, adding an event). The updated chromatogram will be displayed.

Reanalyze button



Editing the Current Method

Description

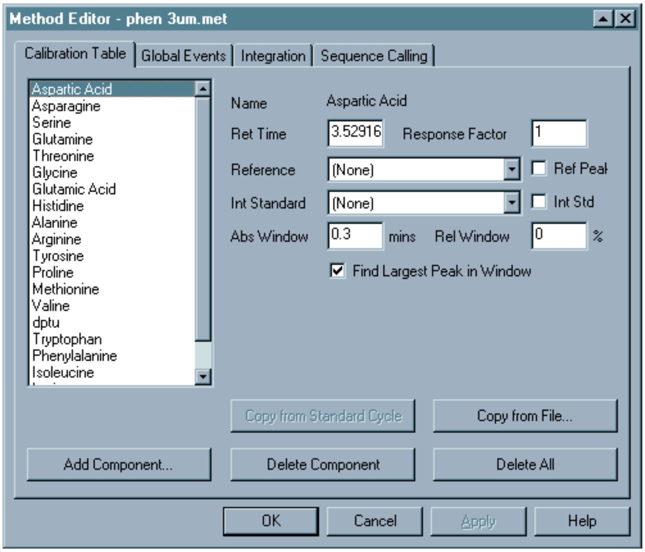
Allows you to select and edit the copy of the Method template included in the data file. The edited Method will be used only with the current data file.

Procedure

To edit the Method template included in your data file:

1. From the Edit menu, select **Edit Current**.

   The Method Editor dialog box opens.



2. Click each of the following tabs in the Method Editor dialog box and type in your changes on each page.

   The following table lists where to find more information about each tab:

For this topic...	See Page
Using the Calibration Table Page	4-15
Using the Global Events Page	4-18
Using the Integration Page	4-20
Using the Sequence Calling Page	4-23

   Click a new tab after you complete each page.

   Note: These pages may be completed in any order.

3. Click **OK** in the Method Editor dialog box when you have finished entering your changes.

Calibrating a Standard Cycle

Description

When the SequencePro software calibrates the standard cycles:

- All standard peaks are assigned the correct amino acid labels based on the contents of the Calibration table.
- All peaks are assigned the correct quantity (pmol) of standard for the height and area recorded.

Procedure

To calibrate the standard cycle, select Calibrate from the Analysis menu. If Automatic Analysis is on, the program automatically calibrates the standard cycle.

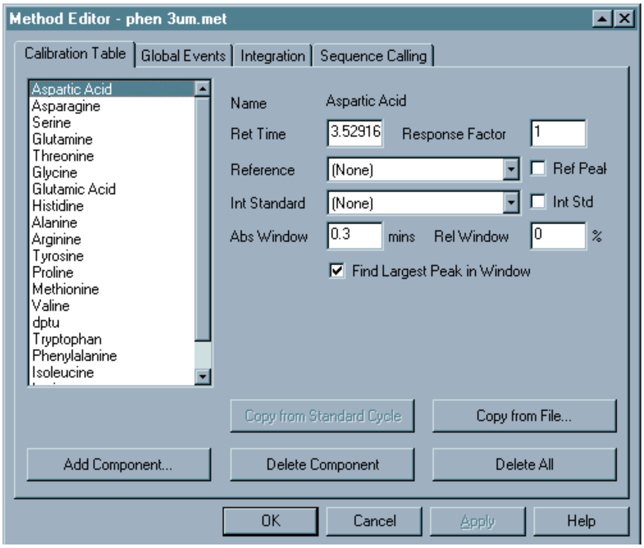
Using the Calibration Table Page

Description

Use the Calibration Table page to edit the calibration parameters.

Displaying the Calibration Table Page

To display the Calibration Table page, from the Method Editor dialog box, click the Calibration Table tab to open the Calibration Table page.

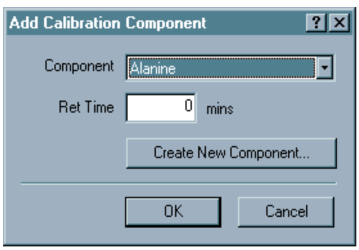


Adding a Component

To add a component:

1. From the Calibration Table page, click the **Add Component** button.

   The Add Calibration Component dialog box opens.



2. Select an amino acid from the Component pop-up menu.

3. Enter the retention time in the Ret Time text box, and click **OK**.
4. If the component you want to add is not listed in the pop-up menu:
   a. Click the **Create New Component** button. The Data Dictionary dialog box opens.
   b. Add the component to the Data Dictionary. See "Editing the Data Dictionary" on page 4-25.
   c. Return to step a to select the new component.
5. Click **OK**.
6. Click another tab or, if you are done, click **OK** in the Method Editor dialog box.

Copying from Standard

Use the Copy from Standard button to replace all the components in the list with the components from the Standard Cycle.

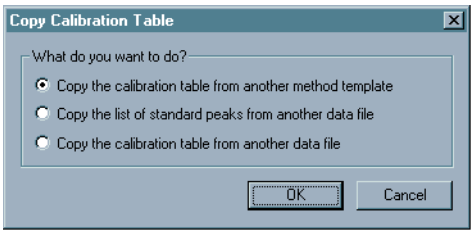
Copying a Calibration Table

Using the Method menu, you can also copy a Calibration Table from a Method or Sample file.

To copy a Calibration Table:

1. Open the Method Editor dialog box.
2. Click the **Copy from File** button in the Calibration Table page.

   The Copy Calibration Table dialog box opens.



3. Select the button next to the item that you want to do and click **OK**.

Deleting Components

To delete one component, select a component from the list and click the Delete Component button.

To delete all components, click **Delete All**.

Note: Click **Copy from Standard** to add the components from the Standard Calibration Table.

Integrating Residue Cycles

Description

When the SequencePro software integrates residue cycles, it compares the heights or areas of the residue peaks for each cycle to the heights or areas of the standard peaks. The amount of each amino acid in the residue cycle is then calculated.

Procedure

Select **Integrate** from the Analysis menu to have the SequencePro software integrate the residue cycles. If Automatic Analysis is on, residue cycles are automatically integrated.

Using the Global Events Page

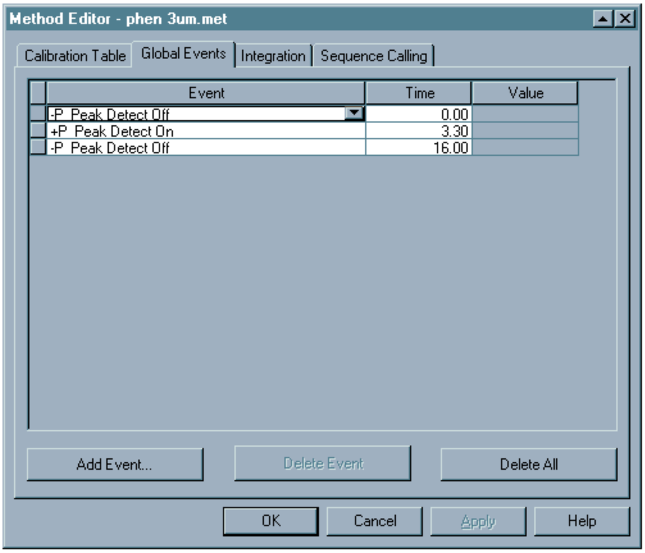
Description

Use the Global Events page to simultaneously edit events that apply to all cycles.

Editing Global Events

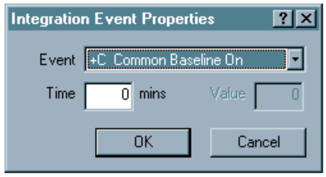
To edit global events:

1. From the Method Editor dialog box, click the **Global Events** tab to open the Global Events page.



2. You can take the following action:

If you want to...	Then...
delete an event	1. Select an event by clicking in the row header. 2. Click the **Delete Event** button.

If you want to...	Then...
add an event	1. Click the **Add Event** button. 2. The Integration Event Properties dialog box appears.  3. Select an event from the Event drop-down menu. 4. Enter the time and value of the event, then click **OK**. This event is now changed in all cycles.

3. Click another tab or, if you are done, click **OK** in the Method Editor dialog box.

Using the Integration Page

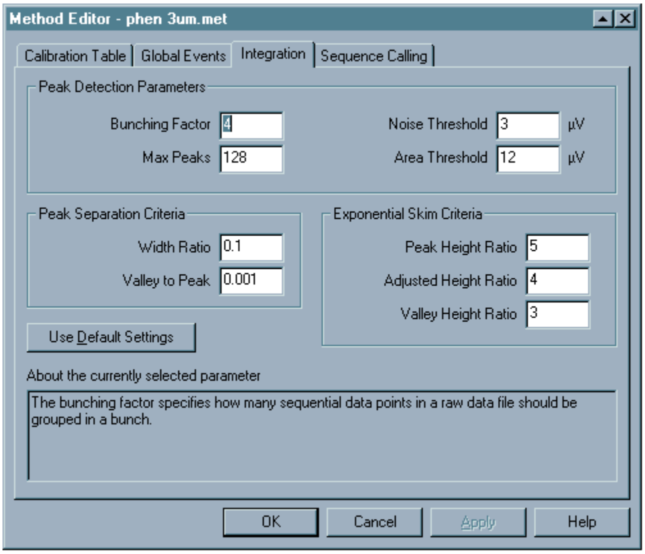
Description

Use the Integration page to edit parameters for peak detection, peak separation, and exponential skim.

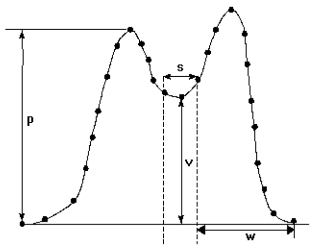
Editing Integration Parameters

To edit integration parameters:

1. From the Method Editor dialog box, click the **Integration** tab to open the Integration page.



2. In the Edit Peak Separation Criteria section, you can take the following actions (refer to “Understanding Peak Separation and Exponential Skim Parameters” on page A-13):

Field	**Description**
Width Ratio	The Width Ratio is the ratio of the distance between the end of the first peak and the start of the second peak to the width of the second peak at its base (w). See the figure below. If this ratio is greater than the set value, the peaks are considered to be separated. Otherwise, they are marked as overlapped. 
Valley to Peak	The Valley-to-Peak Ratio is the ratio of the height of the valley between peaks (v) to the height of the smaller peak (p). If this ratio is less than or equal to the set value, the peaks are considered to be separated. Otherwise they are marked as overlapped.

3. In the **Edit Exponential Skim Criteria** section, you can take the following action (refer to “Understanding Peak Separation and Exponential Skim Parameters” on page A-13):

Field	Description
Peak Height Ratio	The Peak Height Ratio is the ratio of the baseline-corrected height of the parent peak to the baseline corrected height of the child peak. This ratio must be greater than the set value for the child peak to be skimmed. To disable exponential skimming throughout a run, you can set this parameter to its maximum value (1.0e+06).
Adjusted Height Ratio	The Adjusted Height Ratio is the ratio of the height of the parent above its start point to the height of the child above the same point. This ratio must be greater than the set value for the child peak to be skimmed.
Valley Height Ratio	The Valley Height Ratio is the ratio of the baseline corrected height of the child peak (Hd) to the height of the valley between the parent and child peaks above the baseline (Hv). This ratio must be less than the set value for the child peak to be skimmed.

4. Click another tab or, if you are done, click **OK** in the Method Editor dialog box.

Using the Sequence Calling Page

Description

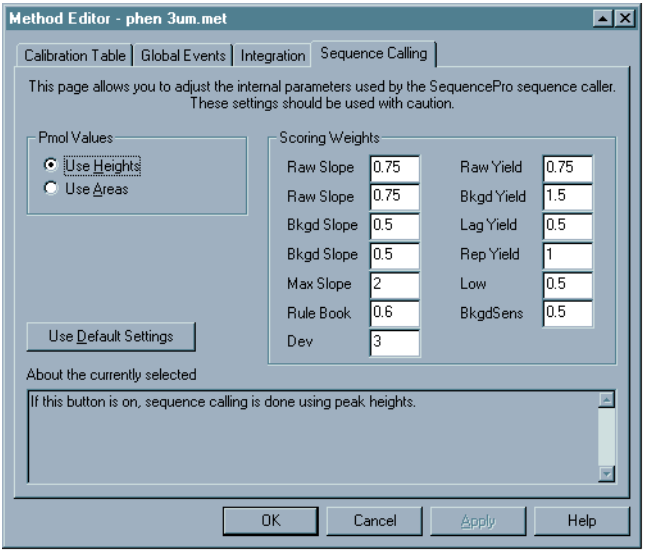
Use the Sequence Calling page to adjust the internal parameters used by the SequencePro software sequence caller.

Note: These settings should be used with caution.

Editing Sequence Calling Parameters

To edit sequence calling parameters:

1. From the Method Editor dialog box, click the **Sequence Calling** tab to open the Sequence Calling page.



You can take the following action:

If you want to use...	Then click...
the default settings	the **Use Default Settings** button.
peak height to determine the amount of each amino acid in the residue cycle	in the Pmol Values section, click the **Use Heights** button.
peak area to determine the amount of each amino acid in the residue cycle	in the Pmol Values section, click the **Use Areas** button.

2. In the **Scoring Weights** section, you can take the following action:

Value	Definition
Raw Slope 1	Score applied to the amino acid with the largest raw slope increase.
Raw Slope 2	Score applied to the amino acid with the second largest raw slope increase.
Bkgd Slope 1	Score applied to the amino acid with the largest background slope increase.
Bkgd Slope 2	Score applied to the amino acid with the second largest background slope increase.
Max Slope	Score applied to the amino acid with the maximum slope.
Rule Book	Score applied to the best match for the rule book criteria.
Dev	Deviation multiplier for calculating background and lag values.
Raw Yield	Score applied to the amino acid with the largest raw yield.
Bkgd Yield	Score applied to the amino acid with the largest background yield value.
Lag Yield	Score applied to the amino acid with the largest lag yield value.
Rep Yield	Score applied to the amino acid with the largest repetitive yield value.
Low	Score applied to the amino acid with the lowest repetitive yield value.
BkgdSens	Background sensitivity value used during sequence calling.

3. Click another tab or, if you are done, click **OK** in the Method Editor dialog box.

Editing the Data Dictionary

Contents

The Data Dictionary contains both component and sequence information. This section describes how to edit the Data Dictionary by adding or deleting components and sequences.

About the Components

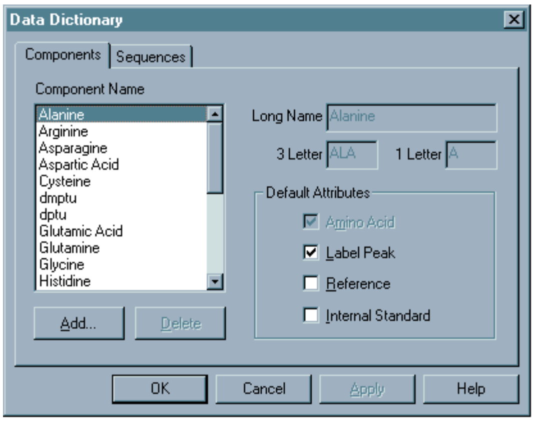
The Data Dictionary contains a list of components to be identified during the analysis process. You can add or delete components to the Data Dictionary or modify their parameters.

Adding Components to the Data Dictionary

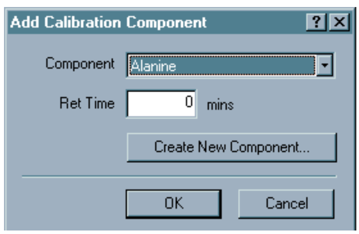
To add a component to the Data Dictionary:

1. From the View menu, select **Data Dictionary**.

   The Data Dictionary dialog box appears and the Components page is displayed.



2. Click the **Add** button to display the following dialog box.



3. Enter the long name for the component and click **OK**.

4. Enter the parameters of the new component in the appropriate fields:

New Component Parameter...	For example...
Long Name	Alanine
3-Letter	ALA
1-Letter	A

5. Click the appropriate check boxes in the **Default Attributes** section. By selecting these check boxes, these become the default settings for each component.

Select...	To...
Amino Acid	designate that peak as an amino acid.
Label Peak	make the label appear in the chromatogram window.
Reference	designate the peak as a reference peak.
Internal Standard	designate the peak as an internal standard.

6. Click **Add** to add the new component to the Data Dictionary.

   The component becomes the default in the method template and in the data file.

Deleting a Component from the Data Dictionary

To delete a component from the Data Dictionary:

1. From the View menu, select **Data Dictionary**.

   The Data Dictionary dialog box appears and the Components page is displayed.

2. Select the component you want to delete from the Component Name scroll box.

3. Click the **Delete** button.

   The component is deleted from the Data Dictionary.

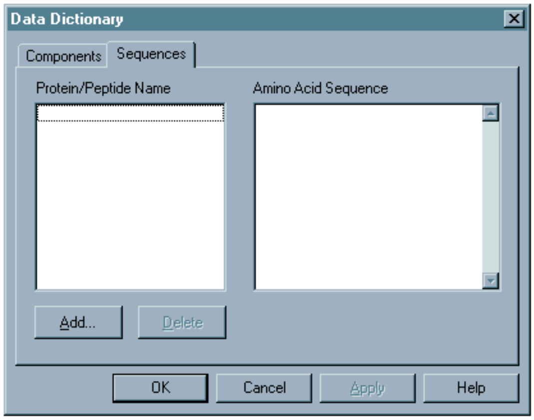
   Note: Standard Amino Acids cannot be deleted from the Data Dictionary, nor can the Amino Acid check box be changed.

Adding Sequences to the Data Dictionary

The Data Dictionary also contains sequence information that can be edited.

To add sequences to the Data Dictionary:

1. From the View menu, select **Data Dictionary** and click the **Sequences** tab to display the Sequences page.



2. Click the **Add** button to display the following dialog box.

3. Enter the protein/peptide name and click **OK**.

   The cursor moves to the Amino Acid Sequence window.

4. Enter the amino acid sequence.

5. Click **OK** to add the new sequence to the Data Dictionary.

Deleting a Sequence from the Data Dictionary

To delete a sequence from the Data Dictionary:

1. Select the sequence you want to delete from the Protein/Peptide Name scroll box.
2. Click the **Delete** button.

   The sequence is deleted from the Data Dictionary.

Calling the Sequence

Description

When the SequencePro software calls the sequence, it compares the relative heights or areas of residues in each cycle with the relative heights or areas of residues in the previous cycle. The residue with the largest change is called.

Procedure

To call the sequence, select Call Sequence from the Analysis menu. The SequencePro software automatically calls the protein sequence.

Correcting Calibration Problems

5

About This Chapter

This chapter describes how to correct analysis and calibration problems, specify reference peaks, edit and delete events, and modify integration parameters.

In This Chapter

This chapter contains the following topics:

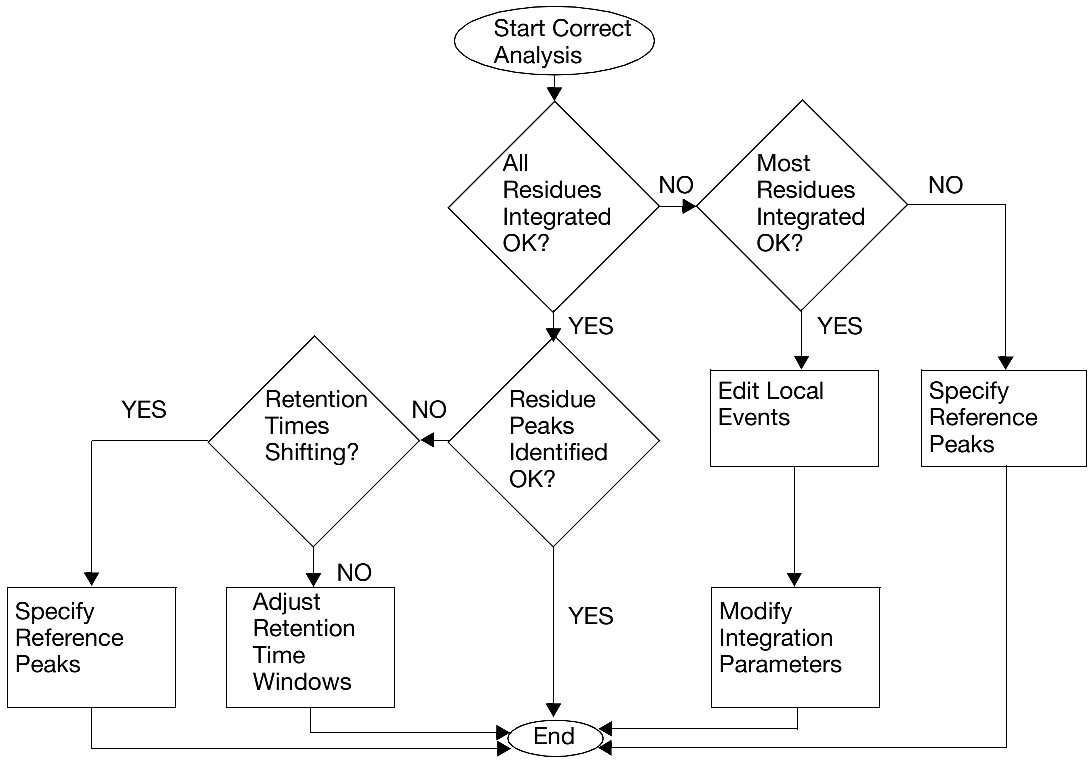
Correcting Analysis Problems

Correcting Process

If you find problems with the calibration of any of the standard cycles, you will need to make adjustments. This process involves visually inspecting the standard peaks, and then modifying the integration and retention times, as necessary.

Flow Diagram

The following flow diagram illustrates the process of correcting calibration problems.



Adjusting Retention Time Windows

Description

Retention time windows define the time span in which a peak must occur in order to be identified as a given component. You can compensate for any shifting of the retention times of the peaks by widening the retention time window.

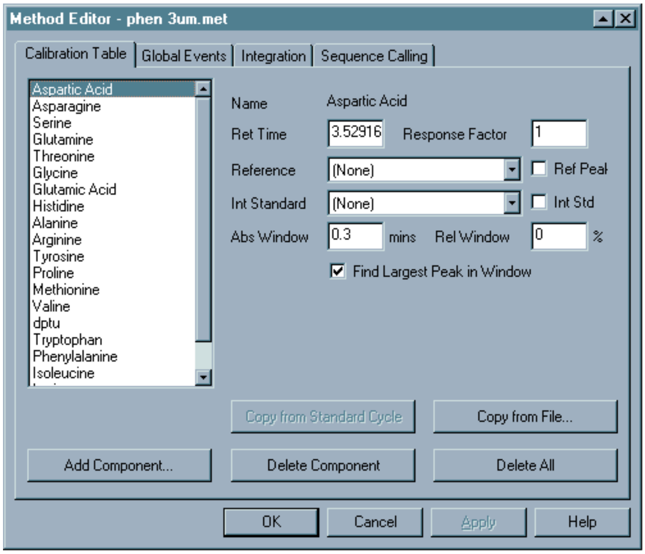
Procedure

Use the following procedure to open the Calibration Table tab and adjust the retention time window.

To adjust the retention time window:

1. From the Method menu, select **Edit Current**.

   The Method Editor dialog box appears and the Calibration Table tab is displayed.



2. Select the appropriate amino acid from the scroll box on the left.
3. If necessary, enter the new retention time in the Ret Time field, as shown in the example above.

4. Enter the new width of the retention time window:

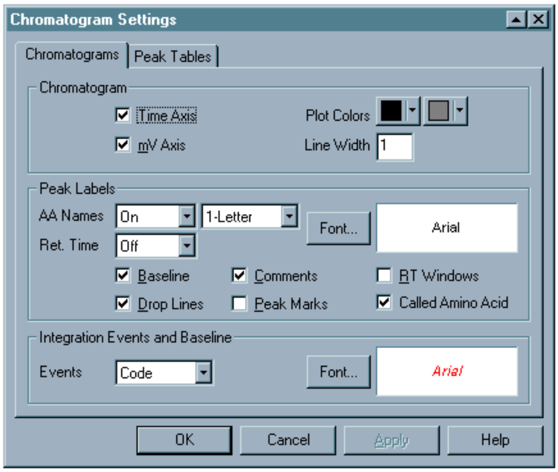
For a...	Enter...
constant window width	minutes in the **Abs Window** field.
variable window width	a percent of the retention time in the **Rel Window**.

5. Click **OK**.

Viewing Retention Times

To view the retention times on the chromatogram:

1. From the chromatogram window, select **Settings**.
2. Click the **Chromatograms** tab in the Chromatogram Settings dialog box that appears.



3. Select **Off** or **On** from the Ret. Time pop-up menu to specify if the retention time is to appear on the chromatogram.
4. Select the check box labeled **RT Windows** if you want the retention time window to appear on the chromatogram.
5. Click **OK**.

Specifying Reference Peaks

The Problem

Retention times for peaks can vary widely from run to run. Creating an excessively wide retention time window to find these peaks may cause windows to overlap. This complicates the peak identification process. and makes it more difficult to predict the peak identifications that the SequencePro® software will make.

What Is a Reference Peak?

A reference peak, like other single-peak components, has an expected retention time and a search window. However, it is usually readily identifiable because it is well separated from other peaks, or because it is the largest peak in that region of the chromatogram.

Why Use Reference Peaks?

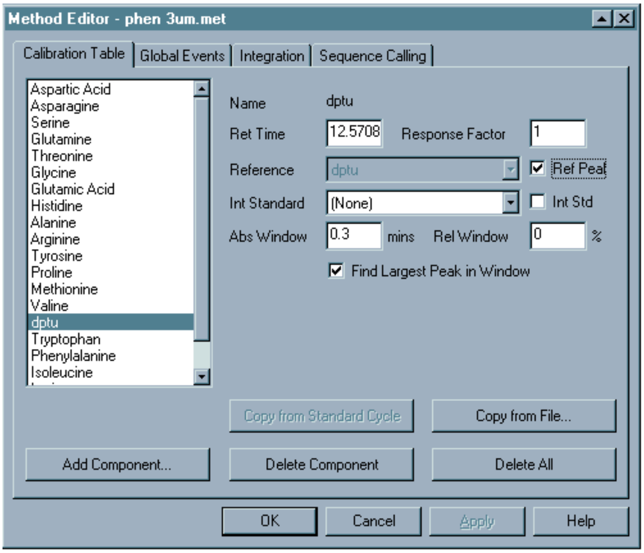
You can use reference peaks to compensate for shifts in peak retention times. Since all peaks shift by the same amount as the reference peak, you want to choose a reference peak that appears in every cycle, including the standard cycle and all residue cycles.

Selecting a Reference Peak

Note: When selecting a reference peak, always choose a component that is present in every cycle and shifts in the same general direction and time shift as the other peaks in the cycle.

To select a reference peak:

1. Click the **Calibration Table** tab in the Method Editor dialog box.
2. Click on the component you want to use as a reference peak, for example, dptu, in the scroll box on the left side (see the following figure).



3. Click the **Ref Peak** check box.

   An ‘X’ appears in the check box and the selected component is added to the list of reference peaks.

4. Click **OK**.

Assigning a Reference Peak

To assign the reference peak to an amino acid:

1. Click on an amino acid in the scroll box on the left side.
2. Select **dptu** (or another reference) from the Reference pop-up menu.
3. Repeat step 1 and step 2 to assign the reference peak to additional amino acids.
4. Click **OK**.

Editing and Deleting Global Events

Description

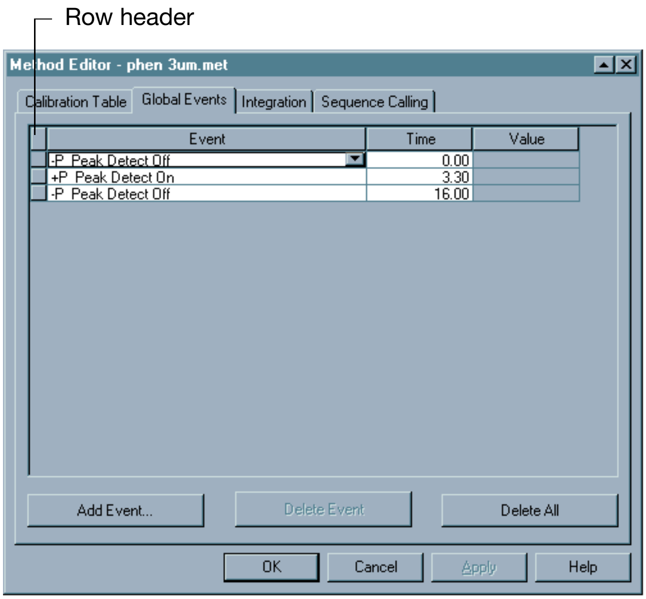
When you modify global events, you change the integration events for all cycles in the sample.

Editing Global Events

To edit global events:

1. Choose a method to edit:

   a. From the Method menu, select **Edit Current**. The Method Editor dialog box displays.

   b. Click the **Global Events** tab.

2. Add a global event:
   - Click the **Add Event** button, then select an event from the Event pop-up menu by clicking the row header, or
   - Shift-click and drag an event from the **Event** toolbar onto the chromatogram.

   The selected event appears in the Event list as shown above.

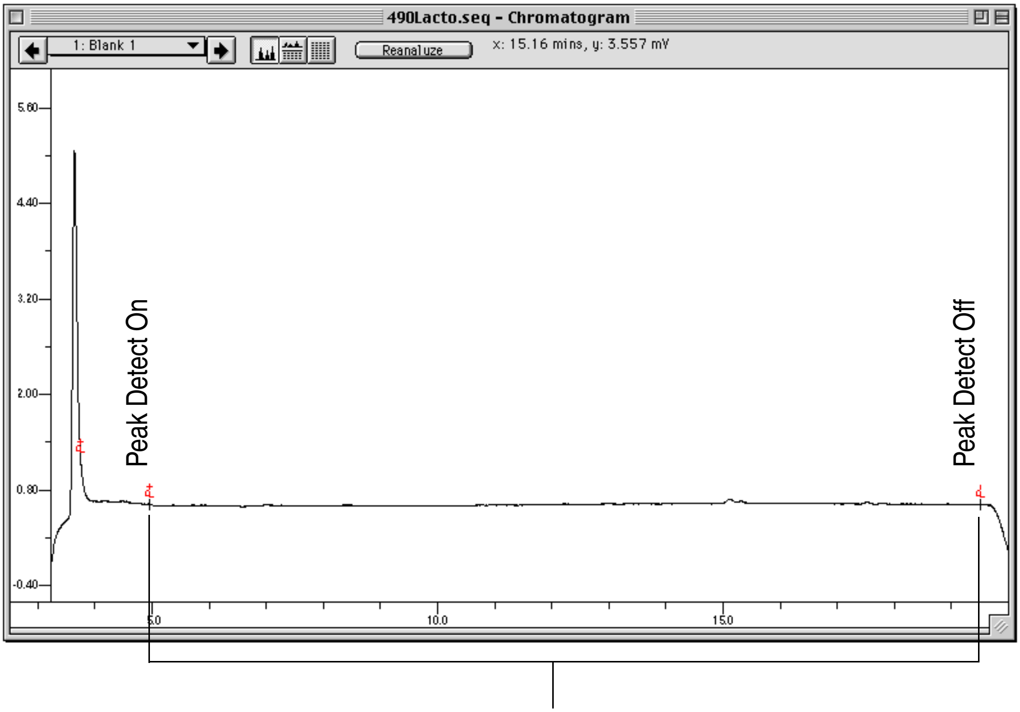
Deleting Global Events

To delete a global event:

If you want to delete...	Then...
a global event	1. Select an event from the **Event** list by clicking on its row header. 2. Click the **Delete Event** button.
all global events	Click the **Delete All** button.

Global Event Example

The following is an example of a global event.



These are global events. The short vertical line indicates where the event occurs.

Editing Local Events

Description

When you edit or add local events to a cycle, you change the integration results of only the active cycle. All other cycles remain unchanged.

Adding a Local Event

Note: For a detailed description of the Events toolbar button functions see Appendix A, “Peak Detection and Integration,” on page A-3.

To add or modify local events:

1. In the Events toolbar, click the appropriate icon.

   The cursor icon changes from an arrow to a one- or two-letter symbol with a short line.

2. Move the cursor to the appropriate place on the chromatogram, and then left-click to add the event.

   Changes to the chromatogram analysis parameters appear.

If automatic analysis is...	Then the...
enabled	sample is automatically reanalyzed.
not enabled	reanalyze button is activated, so you can choose when to perform analysis.

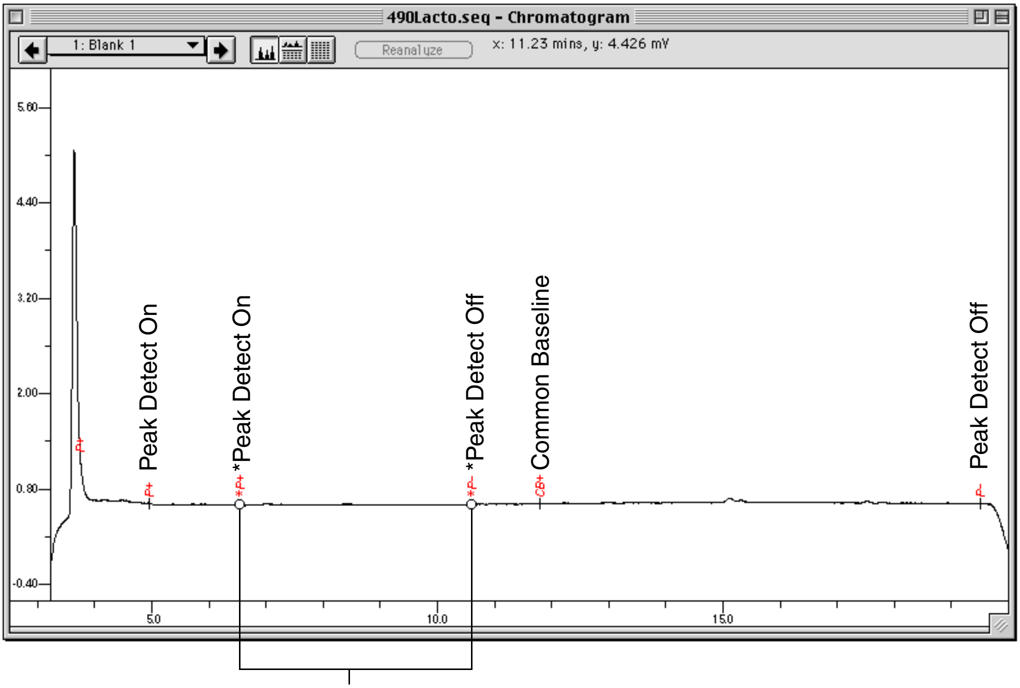
Note: Shift-click to add a global event to every cycle.

For a description of the:

- Buttons on the Events toolbar, see “Using the Events Toolbar” on page 2-21.
- Events toolbar, see Appendix A, “Peak Detection and Integration.”

Local Event Example

The following is an example of a local event.



These are local events. The circles indicate where the events occur.

Editing a Local Event

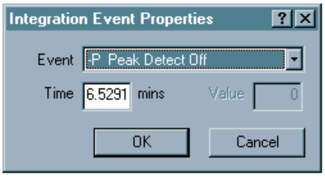
To edit a local event:

1. Click in the circle associated with the event.

   The circle turns black.

2. From the Analysis menu, select **Edit Event**.

   The Integration Event Properties dialog box opens.



3. From the Event pop-up menu, select a different event.
4. Change the time.
5. Click **OK**.

Deleting a Local Event

To delete a local event:

1. Click in the circle associated with the event.

   The circle turns black.

2. From the Analysis menu, select **Delete Event**.
3. Click **OK**.

Adding a Local Event to the Method

To add a local event to a method:

1. Click in the circle associated with the event.

   The circle turns black.

2. From the Analysis menu, select **Add Event**.

   The circle changes to a short line.

3. Click **OK**.

Modifying Integration Parameters

Description

When you modify integration parameters, you change the way all cycles in the sample are integrated.

Procedure

To modify integration parameters:

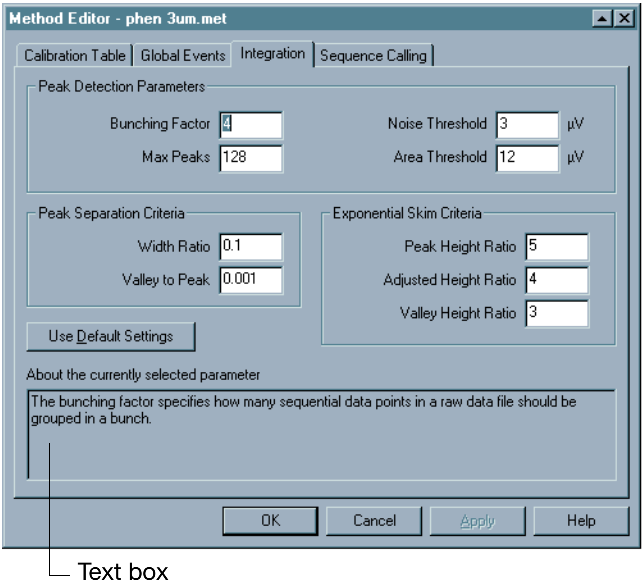
1. Choose a method to edit:

   a. From the Method menu, select **Edit Current**. The Method Editor dialog box appears.

   b. Click the **Integration** tab.

2. In the Peak Detection Parameters section, click on an integration parameter.

   A description of the parameter appears in the text box below (see also "Using the Integration Page" on page 4-20 for an overview of all the integration parameters).



3. Modify the parameter and then click **OK**.

   All cycles in the sample will be integrated with this modification.

Viewing Sequence Data

6

About This Chapter

This chapter describes the flow of the Sequence Calling process, and the tables that are used to display the results.

In This Chapter

This chapter contains the following topics:

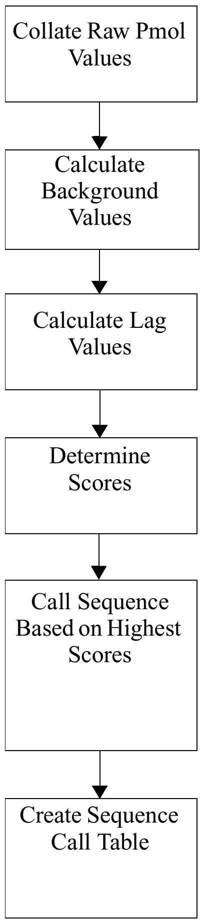
Sequence Calling Flow Diagram

Description

The SequencePro® Data Analysis Application v2.1 software identifies the called amino acid sequence by collating all of the raw pmol values for each residue. Values for background and lag are calculated for each amino acid and a scoring algorithm is used to determine the called amino acid in each residue cycle.

Flow Diagram

The following flow diagram illustrates the flow of data for sequence calling in the SequencePro software.



Viewing Tables in the Sequence Window

Tables You Can View

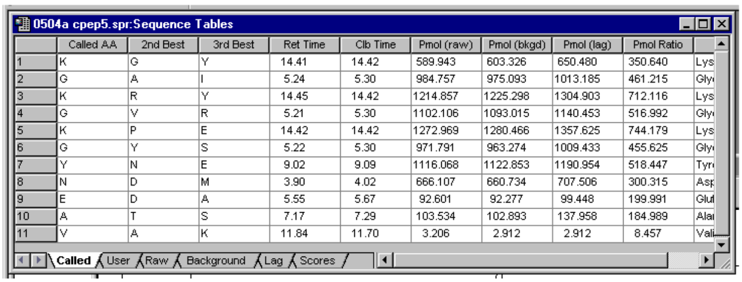
When the Sequence window is open, you can view the following sequence data tables using the Sequence Tables window:

For this table...	See page
Using the Sequence Call Table	6-4
Using the User Call Table	6-5
Using the Raw Picomole Table	6-6
Using the Background Corrected Table	6-7
Using the Lag Corrected Table	6-8
Using the Scores Table	6-9

Viewing the Sequence Tables Window

To view the Sequence Tables window:

1. While the Chromatogram window is open, from the View menu, select **Sequence Data**. The Sequence Tables window opens as shown below.



0504a cpep5.spr:Sequence Tables

	Called AA	2nd Best	3rd Best	Ret Time	Clb Time	Pmol (raw)	Pmol (bkgd)	Pmol (lag)	Pmol Ratio	
1	K	G	Y	14.41	14.42	589.943	603.326	650.480	350.640	Lys
2	G	A	I	5.24	5.30	984.757	975.093	1013.185	461.215	Gly
3	K	R	Y	14.45	14.42	1214.857	1225.298	1304.903	712.116	Lys
4	G	V	R	5.21	5.30	1102.106	1093.015	1140.453	516.992	Gly
5	K	P	E	14.42	14.42	1272.969	1280.466	1357.625	744.179	Lys
6	G	Y	S	5.22	5.30	971.791	963.274	1009.433	455.625	Gly
7	Y	N	E	9.02	9.09	1116.068	1122.853	1190.954	518.447	Tyr
8	N	D	M	3.90	4.02	666.107	660.734	707.506	300.315	Asp
9	E	D	A	5.55	5.67	92.601	92.277	99.448	199.991	Glu
10	A	T	S	7.17	7.29	103.534	102.893	137.958	184.989	Ala
11	V	A	K	11.84	11.70	3.206	2.912	2.912	8.457	Vali

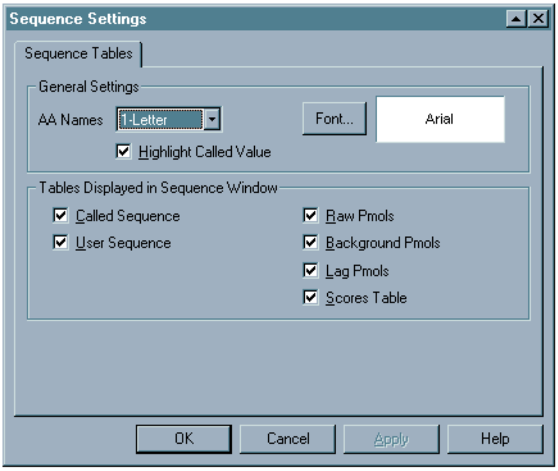
Called | User | Raw | Background | Lag | Scores

2. To view another table, click a tab in the lower left side of the window.

Changing Sequence Window Settings

To change the Sequence Window settings:

1. From the Sequence menu, select **Settings** to open the Sequence Settings dialog box.



2. Make the changes you want and then click **OK**.

Using the Sequence Call Table

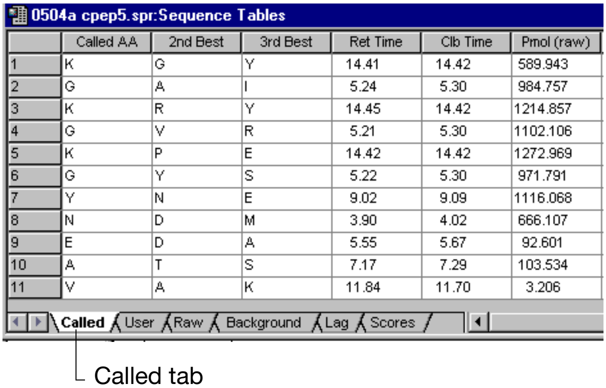
Description

The Sequence Call table contains the collated sequence call values for the called amino acid in each residue cycle.

Viewing the Sequence Call Table

To view the Sequence Call table:

1. In the Sequence Tables window, click the **Called** tab to open the Sequence Call table.


0504a cpep5.spr:Sequence Tables

	Called AA	2nd Best	3rd Best	Ret Time	Clb Time	Pmol (raw)
1	K	G	Y	14.41	14.42	589.943
2	G	A	I	5.24	5.30	984.757
3	K	R	Y	14.45	14.42	1214.857
4	G	V	R	5.21	5.30	1102.106
5	K	P	E	14.42	14.42	1272.969
6	G	Y	S	5.22	5.30	971.791
7	Y	N	E	9.02	9.09	1116.068
8	N	D	M	3.90	4.02	666.107
9	E	D	A	5.55	5.67	92.601
10	A	T	S	7.17	7.29	103.534
11	V	A	K	11.84	11.70	3.206

For information about changing the table settings, see "Changing Sequence Window Settings" on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Using the User Call Table

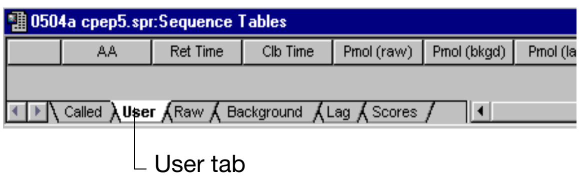
Description

The User Call table contains the collated sequence call values for the amino acids in each residue cycle called by the user.

Viewing the User Call Table

To view the User Call table:

1. In the Sequence Tables window, click the **User** tab to open the User call window.



For information about changing the table settings, see "Changing Sequence Window Settings" on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Using the Raw Picomole Table

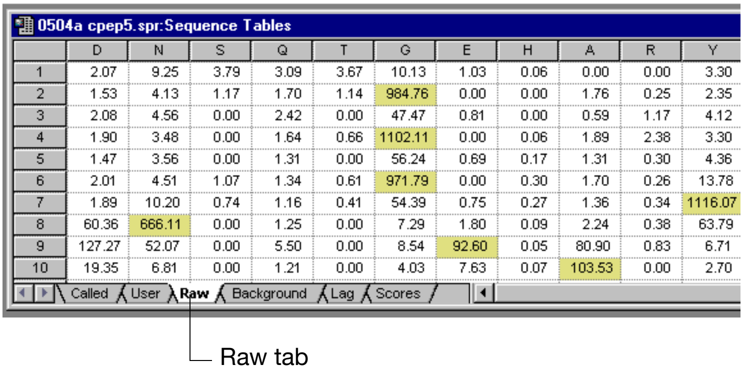
Description

The Raw Picomole table contains the collated raw picomole values for each amino acid identified in the residue cycles.

Viewing the Raw Picomole Table

To view the Raw Picomole table:

1. In the Sequence Tables window, click the **Raw** tab to open the Raw Picomole table.



	D	N	S	Q	T	G	E	H	A	R	Y
1	2.07	9.25	3.79	3.09	3.67	10.13	1.03	0.06	0.00	0.00	3.30
2	1.53	4.13	1.17	1.70	1.14	984.76	0.00	0.00	1.76	0.25	2.35
3	2.08	4.56	0.00	2.42	0.00	47.47	0.81	0.00	0.59	1.17	4.12
4	1.90	3.48	0.00	1.64	0.66	1102.11	0.00	0.06	1.89	2.38	3.30
5	1.47	3.56	0.00	1.31	0.00	56.24	0.69	0.17	1.31	0.30	4.36
6	2.01	4.51	1.07	1.34	0.61	971.79	0.00	0.30	1.70	0.26	13.78
7	1.89	10.20	0.74	1.16	0.41	54.39	0.75	0.27	1.36	0.34	1116.07
8	60.36	666.11	0.00	1.25	0.00	7.29	1.80	0.09	2.24	0.38	63.79
9	127.27	52.07	0.00	5.50	0.00	8.54	92.60	0.05	80.90	0.83	6.71
10	19.35	6.81	0.00	1.21	0.00	4.03	7.63	0.07	103.53	0.00	2.70

For information about changing the table settings, see “Changing Sequence Window Settings” on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Using the Background Corrected Table

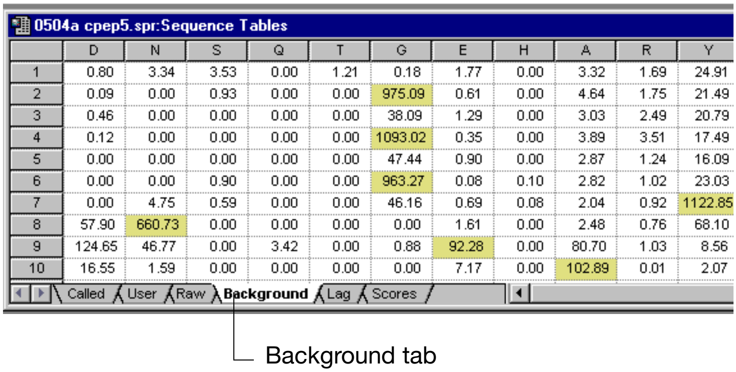
Description

The Background Corrected table contains the collated background corrected picomole values for each amino acid identified in the residue cycles.

Viewing the Background Corrected Table

To view the Background Corrected table:

1. In the Sequence Tables window, click the **Background** tab to open the Background Corrected table.



0504a cpep5.spr:Sequence Tables

	D	N	S	Q	T	G	E	H	A	R	Y
1	0.80	3.34	3.53	0.00	1.21	0.18	1.77	0.00	3.32	1.69	24.91
2	0.09	0.00	0.93	0.00	0.00	975.09	0.61	0.00	4.64	1.75	21.49
3	0.46	0.00	0.00	0.00	0.00	38.09	1.29	0.00	3.03	2.49	20.79
4	0.12	0.00	0.00	0.00	0.00	1093.02	0.35	0.00	3.89	3.51	17.49
5	0.00	0.00	0.00	0.00	0.00	47.44	0.90	0.00	2.87	1.24	16.09
6	0.00	0.00	0.90	0.00	0.00	963.27	0.08	0.10	2.82	1.02	23.03
7	0.00	4.75	0.59	0.00	0.00	46.16	0.69	0.08	2.04	0.92	1122.85
8	57.90	660.73	0.00	0.00	0.00	0.00	1.61	0.00	2.48	0.76	68.10
9	124.65	46.77	0.00	3.42	0.00	0.88	92.28	0.00	80.70	1.03	8.56
10	16.55	1.59	0.00	0.00	0.00	0.00	7.17	0.00	102.89	0.01	2.07

For information about changing the table settings, see “Changing Sequence Window Settings” on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Using the Lag Corrected Table

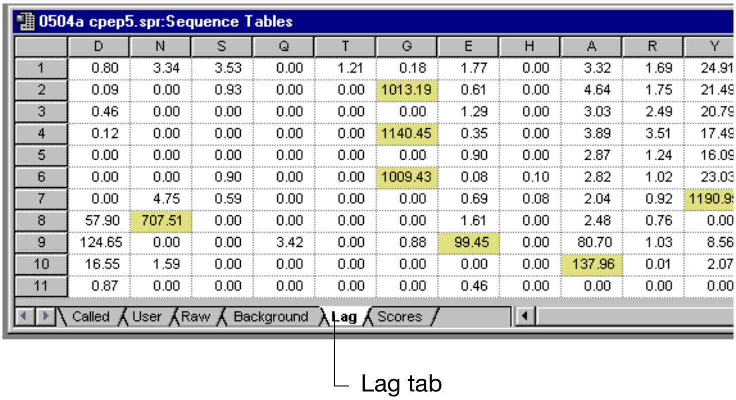
Description

The Lag Corrected table contains the collated lag corrected picomole values identified in the residue cycles.

Viewing the Lag Corrected Table

To view the Lag Corrected table:

1. In the Sequence Tables window, click the **Lag** tab to open the Lag Corrected table.



	D	N	S	Q	T	G	E	H	A	R	Y
1	0.80	3.34	3.53	0.00	1.21	0.18	1.77	0.00	3.32	1.69	24.91
2	0.09	0.00	0.93	0.00	0.00	1013.19	0.61	0.00	4.64	1.75	21.49
3	0.46	0.00	0.00	0.00	0.00	0.00	1.29	0.00	3.03	2.49	20.79
4	0.12	0.00	0.00	0.00	0.00	1140.45	0.35	0.00	3.89	3.51	17.49
5	0.00	0.00	0.00	0.00	0.00	0.00	0.90	0.00	2.87	1.24	16.09
6	0.00	0.00	0.90	0.00	0.00	1009.43	0.08	0.10	2.82	1.02	23.03
7	0.00	4.75	0.59	0.00	0.00	0.00	0.69	0.08	2.04	0.92	1190.9
8	57.90	707.51	0.00	0.00	0.00	0.00	1.61	0.00	2.48	0.76	0.00
9	124.65	0.00	0.00	3.42	0.00	0.88	99.45	0.00	80.70	1.03	8.56
10	16.55	1.59	0.00	0.00	0.00	0.00	0.00	0.00	137.96	0.01	2.07
11	0.87	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00

   For information about changing the table settings, see “Changing Sequence Window Settings” on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Using the Scores Table

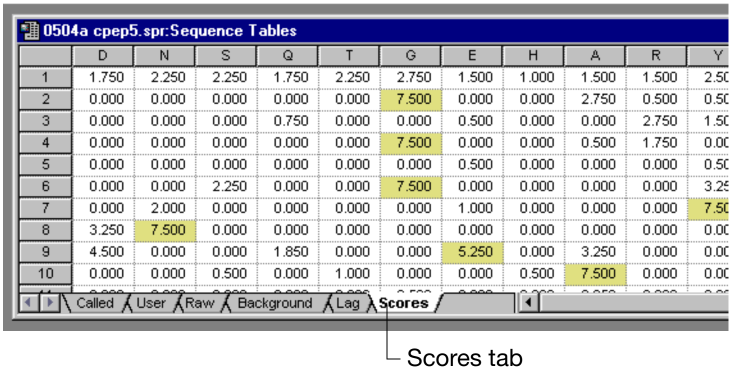
Description

The Scores table contains the total score values for each amino acid identified in the residue cycles.

Viewing the Scores Table

To view the Scores table:

1. In the Sequence Tables window, click the **Scores** tab to open the Scores table.



	D	N	S	Q	T	G	E	H	A	R	Y
1	1.750	2.250	2.250	1.750	2.250	2.750	1.500	1.000	1.500	1.500	2.50
2	0.000	0.000	0.000	0.000	0.000	7.500	0.000	0.000	2.750	0.500	0.50
3	0.000	0.000	0.000	0.750	0.000	0.000	0.500	0.000	0.000	2.750	1.50
4	0.000	0.000	0.000	0.000	0.000	7.500	0.000	0.000	0.500	1.750	0.00
5	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.50
6	0.000	0.000	2.250	0.000	0.000	7.500	0.000	0.000	0.000	0.000	3.2
7	0.000	2.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	7.50
8	3.250	7.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
9	4.500	0.000	0.000	1.850	0.000	0.000	5.250	0.000	3.250	0.000	0.00
10	0.000	0.000	0.500	0.000	1.000	0.000	0.000	0.500	7.500	0.000	0.00

For information about changing the table settings, see “Changing Sequence Window Settings” on page 6-4.

2. To view another table, click a tab in the lower left side of the window.

Viewing Graph Windows

What Graphs You Can View

When the Chromatogram window is open, you can view the following three graphs in the Graph window:

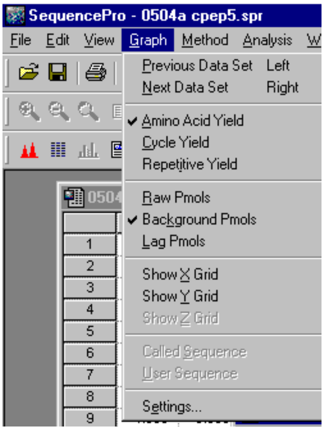
For This Graph	See Page...
Using the Amino Acid Yield Graph	6-15
Using the Cycle Yield Graph	6-16
Using the Repetitive Yield Graph	6-17

Viewing the Graph Window

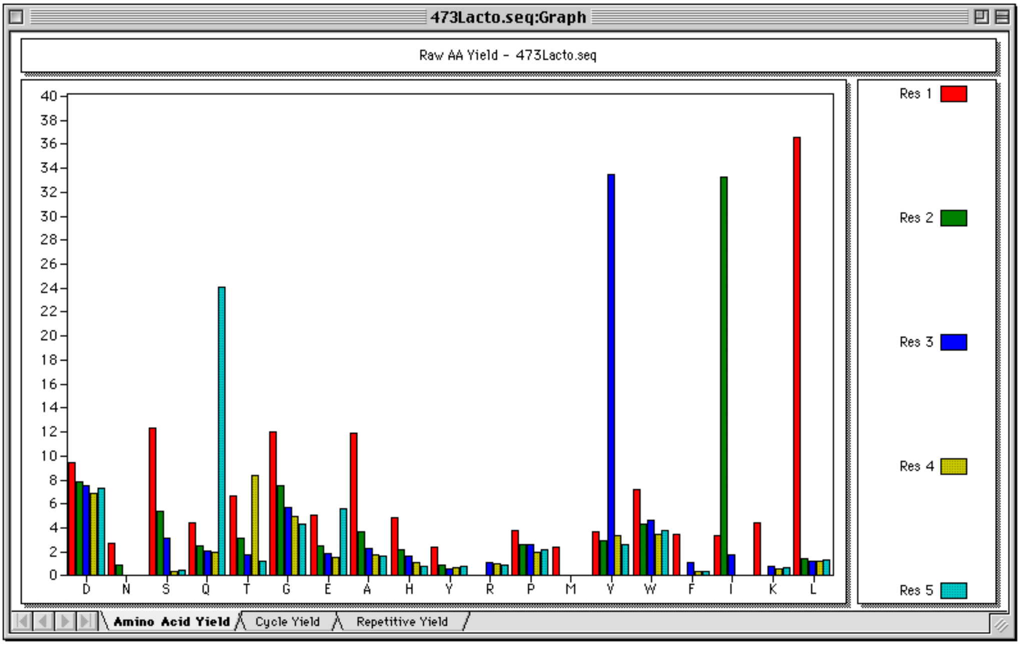
To view the Graph window:

1. From the View menu, select **Graphs** to open the Graph window.

   Note: While a graph is displayed, the Graph menu appears on the menu bar.



2. To view another graph, click a tab in the lower left side of the window.



Using the Graph Window

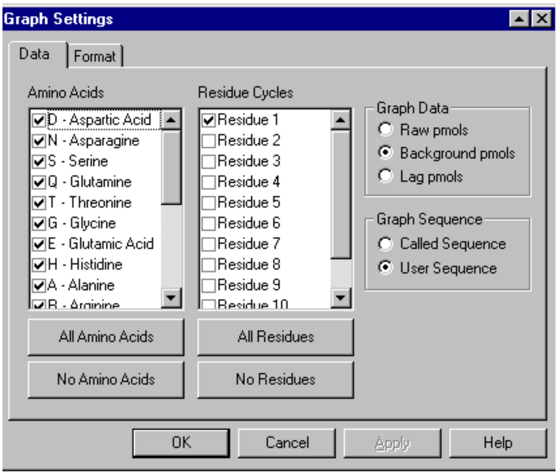
To use the Graph window:

To...	Then...
go from one cycle to the next	use the arrow keys on the key board.
view another graph	click a tab in the lower left side of the window. 

Changing Graph Windows Settings

To change graph window settings:

1. From the Graph menu, select **Settings**. The Graph Settings dialog box opens to the Data page.



Note: While a graph is displayed, the Graph menu appears on the menu bar.

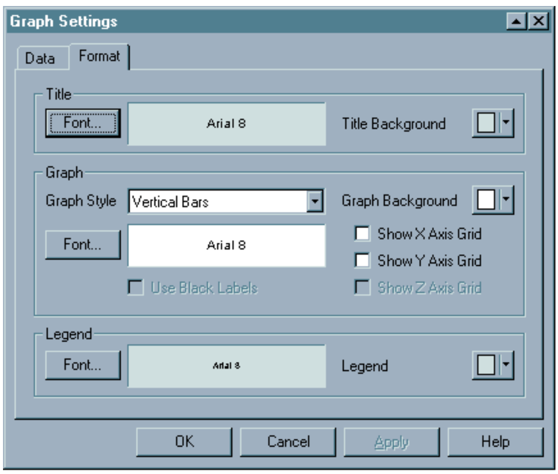
Note: You must check at least one check box for both Amino Acids and Residue Cycles.

2. From the Data page, you can take the following actions:

In this section…	You can click…
Amino Acids	either the: • Individual check boxes that you want to appear in the graph. • **All Amino Acids** button to select (check) all the options. • **No Amino Acids** button to deselect (uncheck) those options.

In this section...	**You can click...**
Residue Cycles	either the: • Individual check boxes that you want to appear in the graph. • **All Residues** button to select (check) all the options. • **No Residues** button to deselect (uncheck) those options.
Graph Data	the appropriate radio buttons for the graph data you want: • **Raw pmols** to display the raw picomole graph. • **Background pmols** to display the background picomole graph. • **Lag pmols** to display the Lag yield graph.
Graph Sequence	either the **Called Sequence** or **User Sequence** radio button, depending on the sequence you want to graph.

3. In the Graph Settings dialog box, click the **Format** tab to open the Format page.



4. From the Format page, you can take the following actions:

In this section...	**You can select the...**
Title	graph title font, the font style and color, and the title background color.
Graph	graph style, the font style and color, the graph background color. You can also click the check boxes to show the x- axis and/or the y-axis grid.
Static	legend font, the font style and color, and the legend background color.

5. Click the **Data** tab or click **OK**.

Using the Amino Acid Yield Graph

Description

The Amino Acid Yield graph displays the amount of each amino acid for a selected cycle in picomoles.

Viewing the Graph

To view the Amino Acid Yield graph:

1. In the Graph window, click the **Amino Acid Yield** tab to open the Amino Acid Yield graph (see "Graph Example" below).

   Note: The default graph displays all amino acids for a given residue.

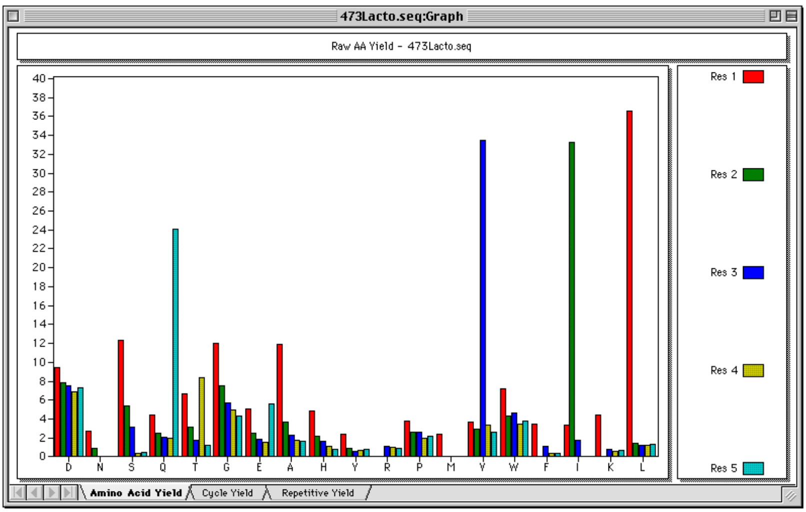
   For information about changing the graph settings, see "Changing Graph Windows Settings" on page 6-12.

2. You can take the following action:

To...	Then...
go from one cycle to the next	use the arrow keys on the key board.
view another graph	click a tab in the lower left side of the window.

Graph Example

The following is an example of an Amino Acid Yield graph.



Using the Cycle Yield Graph

Description

The Cycle Yield graph displays in picomoles the amount of an amino acid in each cycle.

Viewing the Graph

To view the Cycle Yield graph:

1. Click the **Cycle Yield** tab in the Graph window to open the Cycle Yield graph (see "Graph Example" below).

   Note: The default graph displays the amount (in picomoles) in every cycle for a given residue.

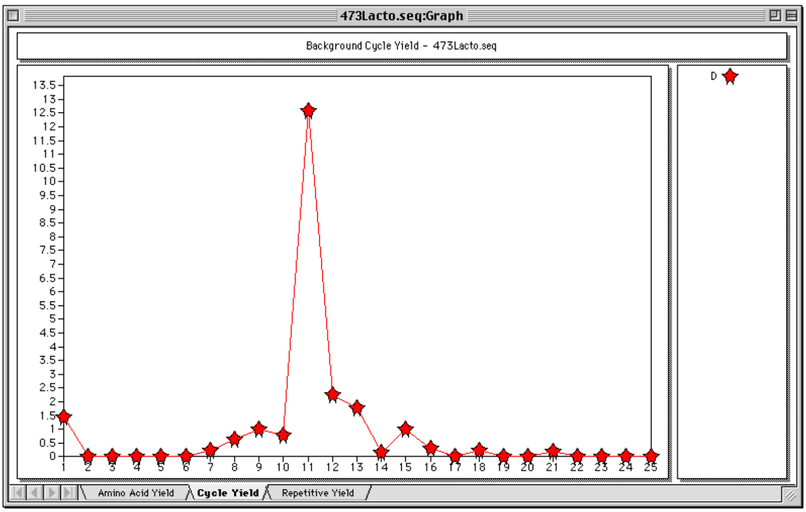
   For information about changing the graph window settings, see page 6-12.

2. You can take the following action:

To...	Then...
go from one cycle to the next	use the arrow keys on the key board.
view another graph	click a tab in the lower left side of the window.

Graph Example

The following is an example of a Cycle Yield graph.



Using the Repetitive Yield Graph

Description

The Repetitive Yield graph displays in picomoles the amount of the called amino acid in each cycle. The calculated average repetitive yield is plotted as a straight line.

Viewing the Graph

To view the Repetitive Yield graph:

1. Click the **Repetitive Yield** tab in the Graph window to open the Repetitive Yield graph (see "Graph Example" below).

   Note: The default graph displays the amount (in picomoles) in every cycle for a given residue.

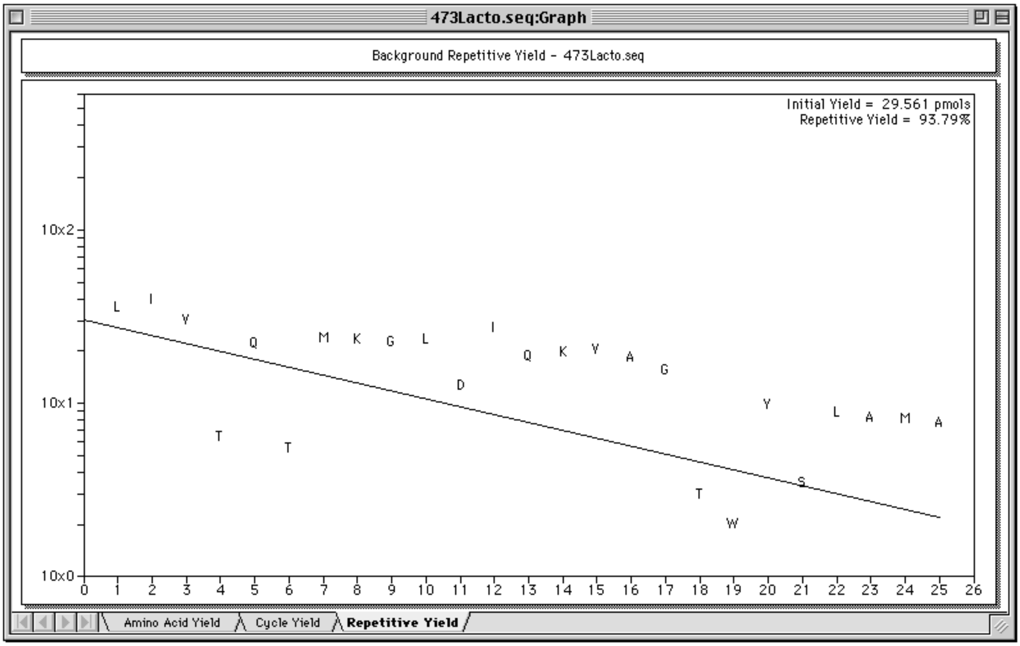
   For information about changing the graph settings, see page 6-12.

2. You can take the following action:

To...	Then...
go from one cycle to the next	use the arrow keys on the key board.
view another graph	click a tab in the lower left side of the window.

Graph Example

The following is an example of a Repetitive Yield graph.



Graphing User Sequence

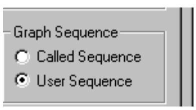
Procedure

To graph the user sequence:

1. From the Graph menu, select **Settings**.

   The Graph Settings dialog box opens to the Data page.

2. In the Graph Sequence section, click the **User Sequence** radio button and click **OK**.



Customizing Analysis Settings 7

About This Chapter

This chapter describes procedures used to customize analysis settings as you work with standard cycles. A flow diagram shows the proper sequence to follow to calibrate and edit the settings.

In This Chapter

This chapter contains the following topics:

Correcting Calibration Problems

Correcting Process

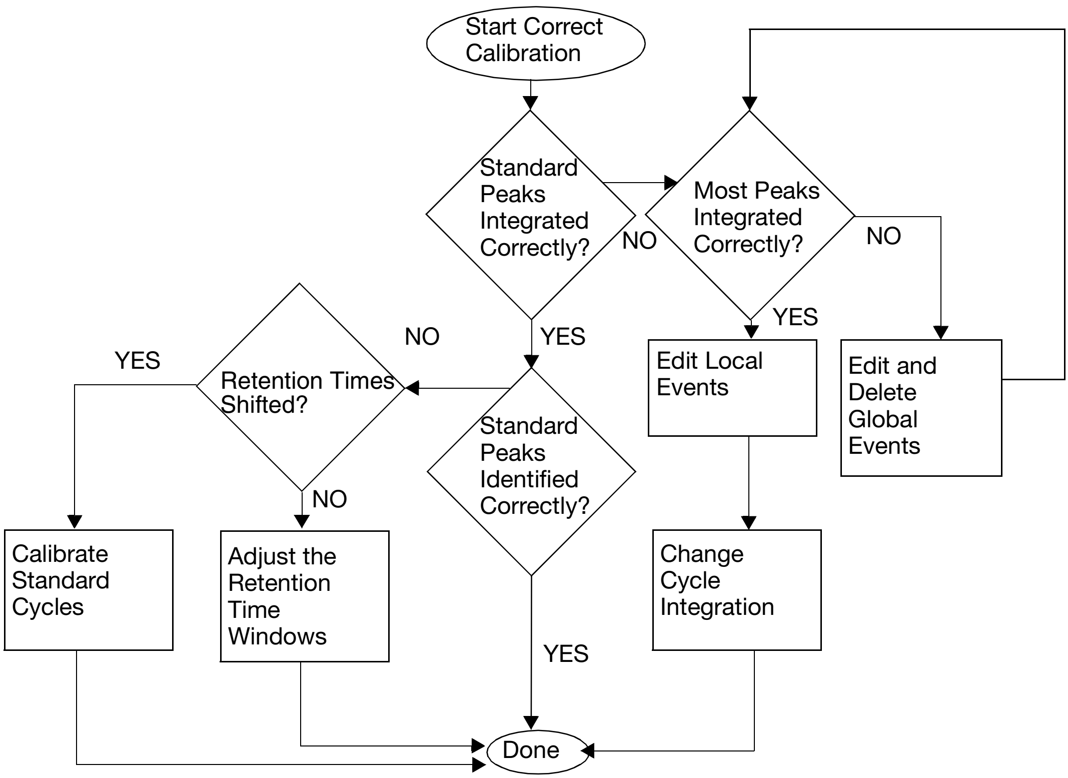
If you find problems with the calibration of the standard cycle, you will need to make corrections.

There are two ways to correct calibration problems:

- Visually inspecting the standard peaks, and then modifying the method to correct peak identification and integration of the standard cycle
- Correcting peak identification in the standard cycle and copying the list to standard peaks to the method

Flow Diagram

The following flow diagram illustrates the process of correcting calibration problems.



Adjusting the Retention Time Windows

Description

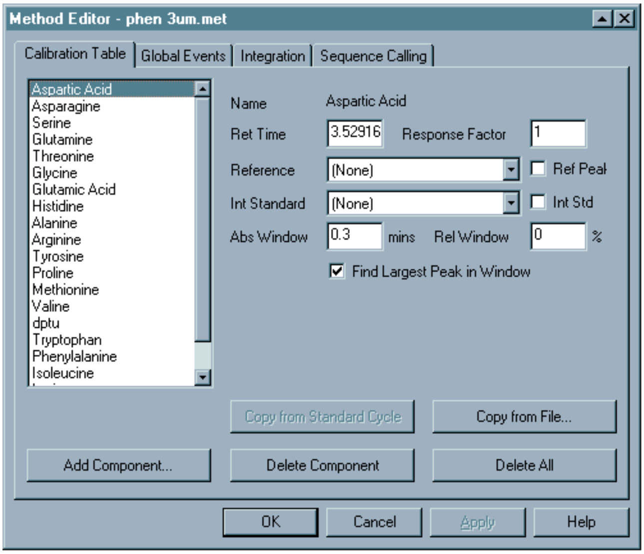
Retention time windows define the time span in which a peak must occur in order to be identified as a given component. You can compensate for any shifting of the retention times of the peaks by widening the retention time window.

Procedure

To adjust the retention time window:

1. From the Method menu, select **Edit Current**.

   The Method Editor dialog box opens and the Calibration Table tab is displayed.



2. Select the appropriate amino acid from the scroll box on the left.

3. If necessary, enter the new retention time in the Ret Time field as shown in step 1above.

4. Enter the new width of the retention time window:

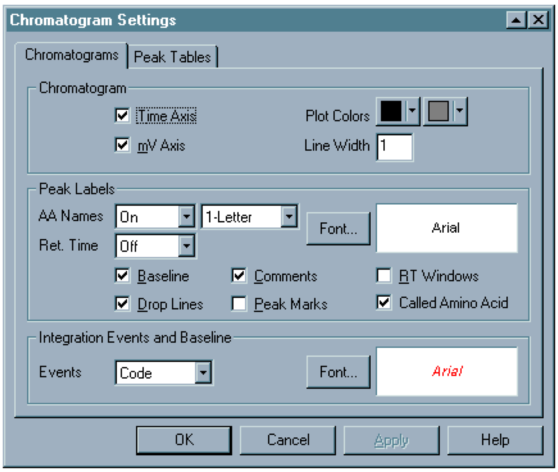
For a...	Enter...
constant window width	minutes in the Abs Window field.
variable window width	a percent of the retention time in the Rel Window.

5. Click **OK**.

Viewing Retention Times

To view the retention times on the chromatogram:

1. From the chromatogram window, select **Settings**.
   The Chromatogram Settings dialog box opens.



2. In the Chromatogram tab Ret. Time drop-down menu, select **Off** or **On** to specify if the retention time is to appear on the chromatogram.

3. Select the check box labeled **RT Windows** if you want the retention time window to appear on the chromatogram.

4. Click **OK**.

Specifying Reference Peaks

Overview

A reference peak, like other single-peak components, has an expected retention time and a search window. However, it is usually readily identifiable because it is well-separated from other peaks, or because it is the largest peak in that region of the chromatogram.

Note: Retention times for peaks can vary widely from run to run. Creating an excessively wide retention time window to find these peaks may cause windows to overlap. This overlap complicates the peak identification process and makes it more difficult to predict the peak identifications that the SequencePro® software will make.

Using Reference Peaks

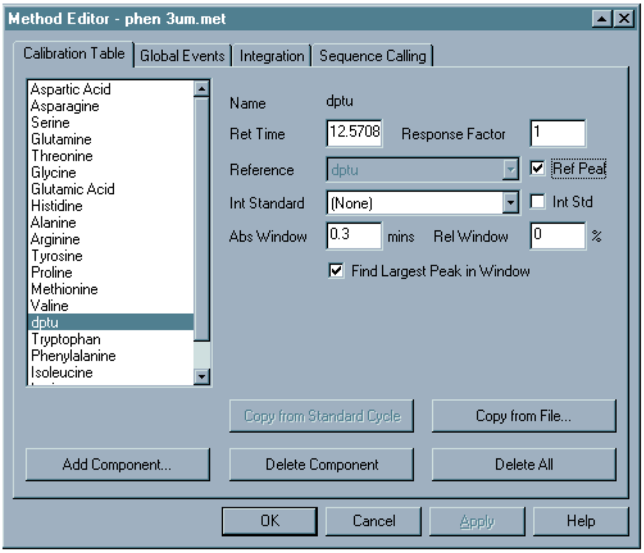
You can use reference peaks to compensate for shifts in peak retention times. Since all peaks shift by the same amount as the reference peak, you want to choose a reference peak that appears in every cycle, including the standard cycle and all residue cycles.

Selecting a Reference Peak

Note: When selecting a reference peak, always choose a component that is present in every cycle and shifts in the same general direction and time shift as the other peaks in the cycle.

To select a reference peak:

1. In the Method Editor dialog box, click the **Calibration** Table tab.
2. Click on the component you want to use as a reference peak, for example, dptu, in the scroll box on the left side.



3. Click the **Ref Peak** check box.

   A check mark appears in the check box and the selected component is added to the list of reference peaks.

4. Click **OK**.

Assigning a Reference Peak

To assign the reference peak to an amino acid:

1. Click on an amino acid in the scroll box on the left side.
2. Select **dptu** (or another reference) from the **Reference** pop-up menu.
3. Repeat step 1 and step 2 to assign the reference peak to additional amino acids.
4. Click **OK**.

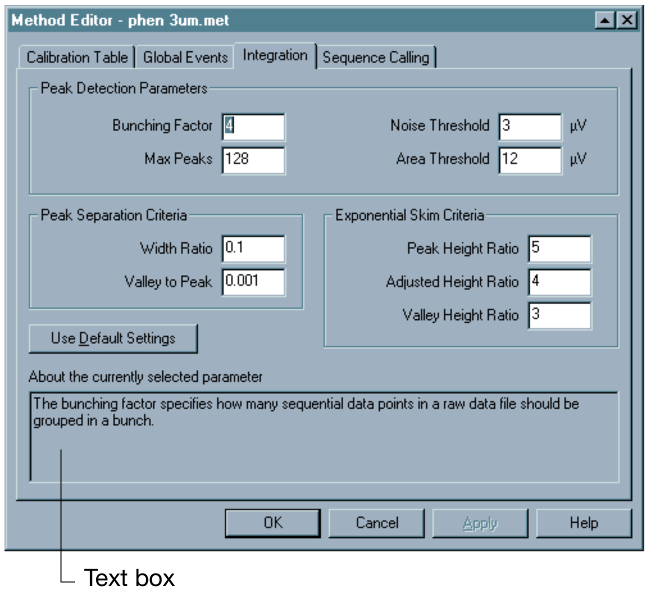
Changing Cycle Integration

When you modify integration parameters, you change the way all cycles in the sample are integrated.

Modifying Integration Parameters

To modify integration parameters:

1. Choose a method to edit:
   a. From the **Method menu**, select **Edit Current**. The Method Editor dialog box appears.



   b. Click the **Integration** tab.

2. In the Peak Detection Parameters section, click on an integration parameter.

   A description of the parameter appears in text box below (see also “Using the Integration Page” on page 4-20 for an overview of all the integration parameters).

3. Modify the parameter and then click **OK**.

   All cycles in the sample will be integrated with this modification.

Calibrating Standard Cycles

When the SequencePro software calibrates the standard cycles, it labels all the amino acid peaks and assigns the correct quantity of standard present for the recorded height and area.

Select **Calibrate** from the Analysis menu.

Creating a Report

8

About This Chapter

This chapter describes how to create, format, specify page layout, and save a report of the protein sequencing data.

In This Chapter

This chapter contains the following topics:

About Creating a Customized Report

Description

You can create a customized report of the protein sequencing data. A report consists of the following:

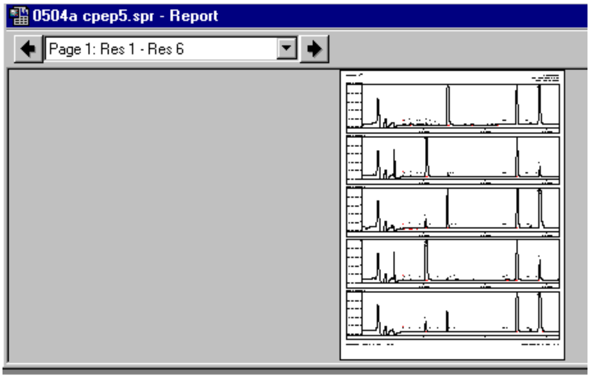
- Sample information
- Sequence call summary
- Chromatograms for selected cycles

Creating a Customized Report

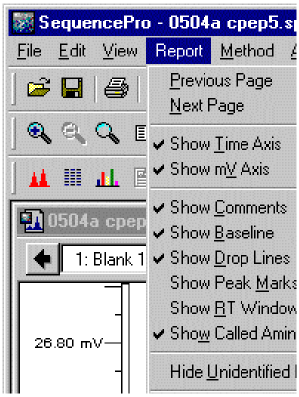
To create a customized report:

1. From the View menu, select **Report**.

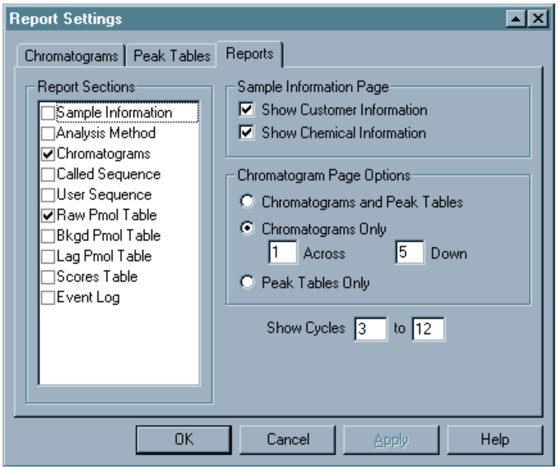
   The Report window opens.



2. When the Report window opens, the Report menu is available on the menu bar.



3. From the Report menu, select **Settings** to open the Report Settings dialog box.



4. In the Report Settings dialog box, click the boxes of the appropriate items.

   A checked box means that data will appear in the report.

5. Click **OK**.

6. From the File menu, select **Print**.

About Formatting Chromatograms in the Report

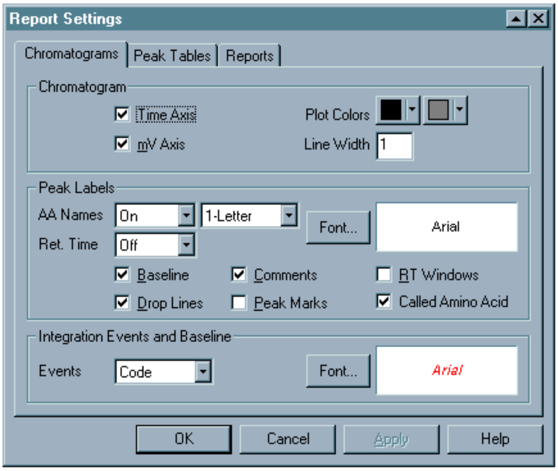
Description

Use the Chromatogram tab in the Report Settings dialog to format how the chromatogram appears in the report.

Formatting Chromatograms in the Report

To format chromatograms in the report:

1. While a report is displayed, from the Report menu, select **Settings** to open the Report Settings dialog box.
2. Click the **Chromatogram** tab.



3. In the Chromatogram section:

Use the...	To...
Time Axis and mV Axis check boxes	select the parameters that you want to appear.
Plot Colors button	compare chromatograms.
Line Width text box	enter the line width you want for the chromatogram.

4. In the Peak Labels section:

Use the...	To...
Name pull-down menu	select where you want the amino acid name to appear (On or Off).
Letter pull-down menu	select how you want the amino acid name to appear (1- Letter, 3-Letter, or Full Name).
Font button	select the font for peak labels.
Ret. Time pull-down menu	display the retention time above the label (On or Off).
Check boxes	to select the parameters you want to appear in the chromatogram: • Baseline • Drop Lines • Comments (indicated by yellow box above peak in chromatogram view, and by footnote in Report view) • Peak Marks • RT Windows • Called Amino Acid

5. In the Integration Events and Baseline section:

Use the...	To...
Events pull-down menu	select how you want to indicate the events information (Code, Full Text, or Off).
Font button	select a font, font style, and color, as in step 4.

6. To complete the procedure:

Click...	To...
Apply	apply the changes to the report. Then click **OK** to save the changes.
OK	apply the changes to the report and exit. The changes are saved.
Cancel	exit the dialog box.

About Formatting the Peak Table in the Report

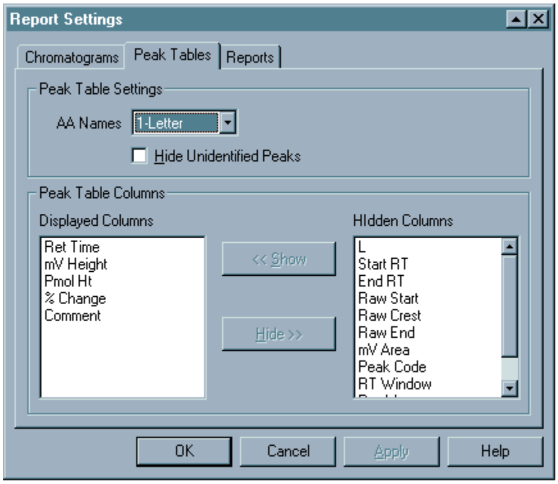
Description

Use the Peak Tables tab in the Report Settings dialog to format how the peak table appears in the report.

Formatting the Peak Table in the Report

To format the peak table in the report:

1. Once a report is displayed, select **Settings** from the Report menu to open the Report Settings dialog box.
2. Click the **Peak Tables** tab.



3. In the Peak Table Settings section, you can:

Use the...	To...
AA Names pull-down menu	select the Full Name, 1-Letter, or 3-Letter amino acid designation.
Hide Unidentified Peaks check box	not display the unidentified peaks in the peak table.

4. In the **Peak Table Columns Settings** section, you can take the following actions:

If you want to...	Then...
show a column	1. From the Hidden Columns list, select the column(s) you want to appear in the Peak Tables. 2. Click the **<<Show** button. The selected columns appear in the Displayed Columns list.
hide a column	1. Select a column(s) in the Displayed Columns list. 2. Click the **Hide>>** button. The selected columns are deleted from the Displayed Columns list.

5. To complete the procedure, you can:

Click...	To...
Apply	apply the changes to the report. Click **OK** to save the changes.
OK	apply the changes to the report and exit. The changes are saved.
Cancel	exit the dialog box.

About Specifying Page Layout

Specifying Page Layout

To specify the page layout:

1. With the Report window open, from the File menu, select **Print Setup**. The standard page setup dialog box opens.

   Note: The appearance of this page will vary with your operating system.

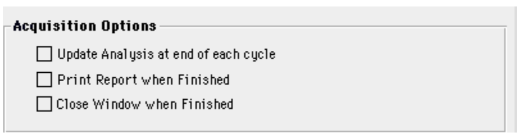
2. Select the options depending on what appears in the Print Setup dialog box.
3. Click **OK**.

About Specifying Automatic Printing

Specifying Automatic Printing

To set up the report to print automatically after a data analysis is complete, do the following:

1. From the View menu, select **Preferences**. The SequencePro Preferences dialog box appears.
2. Click the **Acquisition** tab.
3. Select the **Print Report when Finished** check box.



4. Click **OK**.

About Saving the Report

Saving the Report

Once the report is printed, select the Save command from the File menu to save it to a file you choose.

Peak Detection and Integration

A

About This Appendix

This appendix provides information about the stages in the peak detection and peak integration processes. Information is also provided about bunching factors, noise and area thresholds, and the twenty-four types of Events that the SequencePro® Data Analysis Application v2.1 software supports.

In This Appendix

This appendix contains the following topics:

Understanding Peak Detection and Integration

About Peak Detection

Peak detection is the process by which the SequencePro software examines the raw data points to determine where peaks exist. Peak detection consists of four stages:

Peak detection process	Stage	See Page
"Finding the Potential Peak Start"	Begin stage	A-4
"Confirming the Peak Start"	Confirm stage	A-4
"Finding the Peak Top"	Find Top stage	A-5
"Finding the Peak End"	Find End stage	A-7

About Integration

Integration is the process that accomplishes the following tasks:

- Sets the final positions of baselines for peaks
- Determines peak areas
- Adjusts peak areas, if necessary, by using exponential or tangential skims
- Calculates peak heights and retention times

The timed events you set, as well as the parameters in the Integration dialog box, affect peak detection and integration.

For more details, see "Affecting Integration with Timed Events" on page A-18.

Understanding the Stages in Peak Detection

In order to use timed events and peak detection and integration parameters correctly, you need to be familiar with the stages in the peak detection process. The following is an overview of each stage in peak detection.

Finding the Potential Peak Start

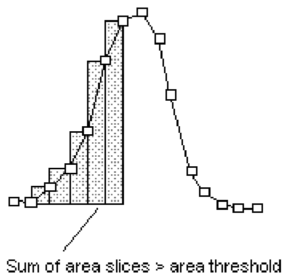
To find the potential start of a peak, the SequencePro software examines the difference in value between each bunched data point and the preceding one.

A potential peak start point has been found if the difference exceeds the current noise threshold value.

Confirming the Peak Start

How the SequencePro Software Confirms the Peak Start

After the SequencePro software finds a potential peak start, it begins to sum the differences between each bunched point and the last baseline point. Since each bunched point represents an area slice, the sum is the accumulated area for the potential peak, as shown in the following example.



Determining Whether the Peak Start Is Confirmed or Canceled

The peak start will be...	If...
confirmed	• It passes the area threshold test, or • The accumulated area exceeds the area threshold before a bunched point fails the noise threshold test.
canceled	the differences between bunched points must also continue to exceed the noise threshold.

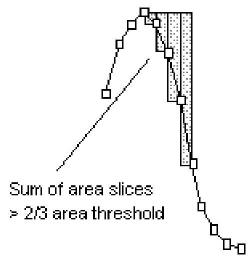
Finding the Peak Top

Note: The reported retention time for a peak is not simply the time of the point identified as the peak top. The reported retention time is determined from a quadratic fit based on the three highest data points.

How SequencePro Software Finds the Peak Top

The following table lists the process the SequencePro software uses to find the top of the peak.

To...	The software...	When...
find the top of the peak	first tries to identify a local maximum bunched point value.	a bunched point is lower than the previous one, the previous point is considered to be the potential peak top.

To...	The software...	When...
avoid finding a false peak due to noise	performs a confirmation test by summing the differences between the potential top and subsequent bunched points.	the sum exceeds two-thirds of the area threshold value the potential peak top is confirmed. 
		A higher bunched point is found before the area test is passed, a new potential top is identified and the area test restarted.

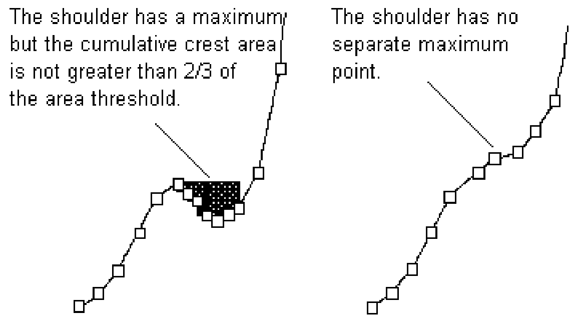
How the SequencePro Software Detects Shoulders

Because of this top-of-peak test, the choice of an area threshold value affects both peak confirmation and how shoulders are detected on the leading edge of a larger peak. Shoulders on the leading edge of a larger peak are not detected as peaks unless they have a discernible maximum point and a crest area that is greater than two-thirds of the area threshold.

Use the timed event S (split peak) to separate a shoulder that has no maximum point as a distinct peak.

Shoulder Not Detected Example

The shoulder is not detected as a peak in the figure below. In the first figure on the left, the Area Threshold value is too large. In the second figure, the shoulder has no maximum point.



Finding the Peak End

There are two indicators of a peak end:

- Two consecutive bunched point differences are less than the noise threshold, or
- The start of another peak is detected.

In either case, the lowest bunched point from the last five bunches is considered to be the actual end point of the peak.

Changing Peak Detection and Integration Parameters

Description

The Integration command in the Method Editor lets you edit the parameters that affect peak detection and integration.

About Peak Detection Parameters

Peak detection parameters are:

- Bunching factor
- Noise threshold
- Area threshold

About Peak Integration Parameters

Peak integration parameters, which are available when you expand the Integration dialog box, are:

- Width ratio
- Valley-to-peak ratio
- Peak height ratio
- Adjusted height ratio
- Valley height ratio

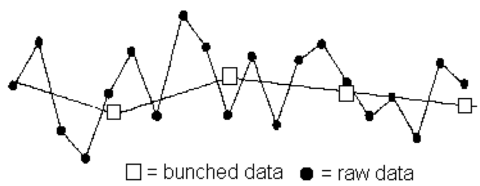
Establishing a Bunching Factor

Bunching Factor Description

The bunching factor specifies how many sequential data points in a raw data file should be grouped in a bunch. During peak detection, the SequencePro software counts bunches and averages the voltage values of the points in each bunch. The resulting averages are assigned to bunched points.

Bunching Factor Example

The following figure shows how bunching smooths out raw data, which helps prevent the SequencePro software from identifying baseline noise as peaks.



Using the Bunching Factor

Bunching Factor and Over-Sampling

A bunching factor also compensates for over-sampling, that is, collecting more points than are necessary in a peak. Ideally, peaks should have about 20 points from start to end. This provides the best balance between acceptable processing time and correct peak detection and integration.

About the Sampling Rate

The following table lists information about the sampling rate.

Sampling Rate Guidelines

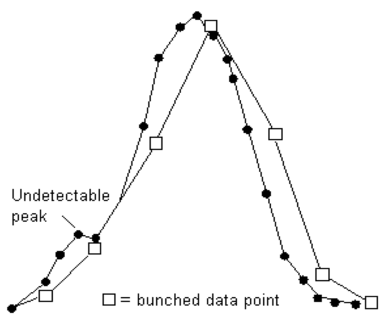
If...	Then...
the sampling rate remains constant throughout a run	you may not be able to avoid over-sampling because peak widths may vary, perhaps broadening later in the run. The broader a peak, the more points it is likely to have; thus, it may be over sampled.

Sampling Rate Guidelines *(continued)*

If...	Then...
both narrow and broad peaks occur in the same run	increase the bunching factor one or more times by using a timed event. You specify the event (increasing the bunching factor) and the time in the run when the event will take place.
you set the bunching factor too high	this can lead to small, unresolved peaks being smoothed out completed, so they are undetected. The goal in setting a bunching factor by timed event is to maintain the number of points across the peaks reasonably close to the 20-point optimum.

Lack of Bunching Example

Too few bunched data points can smooth small peaks, making them invisible to peak detection.



The SequencePro software uses bunched data points to identify the potential start, top and end of a peak. After finding the potential peak values, it reverts back to the raw data points to pinpoint the actual peak start, top, and end. Therefore, there is no loss of resolution in calculating baseline positioning or peak integration.

Setting the Noise Threshold

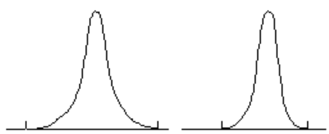
About Noise Threshold

The noise threshold (NT) parameter enables the SequencePro software to discriminate between baseline noise and peaks. If the difference between two consecutive bunched data points is greater than the noise threshold, the software recognizes the potential start of a peak.

The lower the noise threshold, the more sensitive peak detection will be. Conversely, raising the noise threshold decreases sensitivity. If the threshold is too high, however, the SequencePro software will not be able to detect some peaks.

Setting Noise Threshold and the Effect on Peak Detection

The following table lists examples of setting noise threshold and how it affects peak detection.

If the noise threshold is...	Then...
low	the earlier the peak start is detected, but the later the peak end. The data values at the end taper off to a point that is below the noise threshold.
high	peaks start and end more abruptly. A higher noise threshold requires a more abrupt rise between data values before a peak start can be detected. By the same token, the peak end is found sooner as differences between consecutive data values quickly reach the threshold. The following figure show how increasing the noise threshold affects peaks detection.  Lower noise threshold Higher noise threshold

Setting the Area Threshold

About Area Threshold

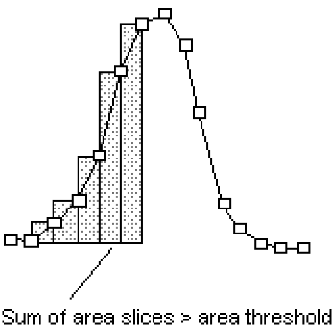
Area threshold (AT) is used to discriminate between noise spikes and peaks. Expressed in microvolts, this parameter is used after the noise threshold to confirm the potential start of peaks that pass the noise threshold test.

After passing the noise threshold test once, pairs of bunched data points must continue to exceed the noise threshold, and the cumulative sum of the bunched area slices on the leading edge must eventually exceed the area threshold.

Testing Area Threshold

The following figure illustrates a peak that passes the area threshold test. The sum of the bunched area slices on the leading edge exceeds the area threshold, so the peak is detected.

A peak is confirmed when it passes the area threshold test.



Using Area Threshold

Higher area threshold values make it harder to confirm a peak, and the SequencePro software may not detect smaller peaks at all, especially those that appear as shoulders on the leading or trailing edges of larger peaks.

A good policy when starting out is to use a low noise threshold and a high area threshold. These thresholds maintain a high degree of sensitivity in detecting peak starting and ending points, but still screens out noise spikes. Refer to Setting Noise and Area Thresholds in the “Editing Methods and Results Graphically” help file.

In addition to confirming the start of a peak, the area threshold also helps determine the top of a peak.

Understanding Peak Separation and Exponential Skim Parameters

About Peak Separation and Exponential Skim Parameters

The values for Peak Separation and Exponential Skim Criteria options have been optimized for normal protein sequencing data. Although the Integration page of the Method Editor dialog box displays additional parameters, they seldom need to be modified.

IMPORTANT! Peak Separation and Exponential Skim Criteria options should only be modified with caution. If you change these parameters without fully understanding the potential consequences, you may obtain invalid results.

Determining Peak Separation Criteria

Two parameters in the Peak Separation Criteria group box determine whether two adjacent peaks will be considered as overlapped or separated:

- Width Ratio
- Valley-to-Peak Ratio

This determination affects how the baseline is drawn beneath the peaks. A set of overlapped peaks is called a cluster and, by default, will share a common baseline. Peaks that are separated will each have an individual segment of the baseline.

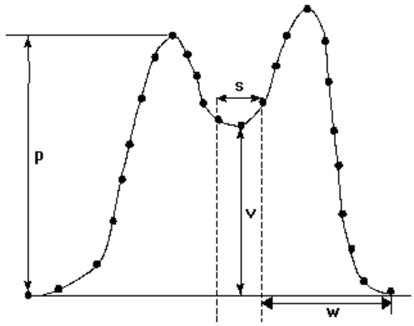
About Width Ratio

The Width Ratio is the ratio of the distance between the end of the first peak and the start of the second peak to the width of the second peak at its base (w). See the figure on the following page. If this ratio is greater than the set value, the peaks are considered to be separated. Otherwise, they are marked as overlapped.

About Valley-to-Peak Ratio

The Valley-to-Peak Ratio is the ratio of the height of the valley between peaks (v) to the height of the smaller peak (p).

If this ratio is less than or equal to the set value, the peaks are considered to be separated. Otherwise they are marked as overlapped.



Using Exponential Skim Criteria

The parameters in the Exponential Skim Criteria group box determine whether an exponential skim line will be used to calculate the area of a child peak eluting on the trailing edge of a parent peak.

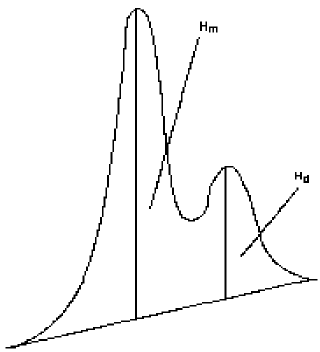
The following table lists when the SequencePro software will not use these parameters:

If an...	Then this...
+X timed event is in effect	forces an exponential skim under all circumstances.
-X timed event is in effect	prevents an exponential skim.

About Peak Height Ratio

The Peak Height Ratio is the ratio of the baseline-corrected height of the parent peak to the baseline corrected height of the child peak. This ratio must be greater than the set value for the child peak to be skimmed. To disable exponential skimming throughout a run, you can set this parameter to its maximum value (1.0e+06).

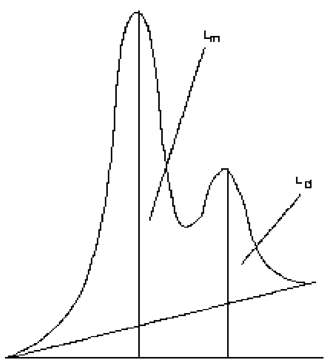
The child peak will not be skimmed if H_m divided by H_d is less than the set value for the Peak Height Ratio.



About Adjusted Height Ratio

The Adjusted Height Ratio is the ratio of the height of the parent above its start point to the height of the child above the same point. This ratio must be greater than the set value for the child peak to be skimmed.

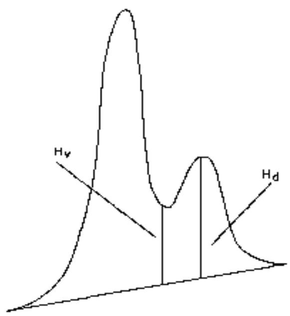
The child peak will not be skimmed if L_m divided by L_d is less than the set value for the Adjusted Height Ratio.



About Valley Height Ratio

The Valley Height Ratio is the ratio of the baseline corrected height of the child peak (H_d) to the height of the valley between the parent and child peaks above the baseline (H_v). This ratio must be less than the set value for the child peak to be skimmed.

The child peak will not be skimmed if H_d divided by H_v is greater than the set value for the Valley Height Ratio.



Using Events

About Baseline Timed Events

Baseline timed events alter the way the SequencePro software detects and integrates peaks during the run. Therefore, you can use them to help maintain consistent results when peak characteristics and baseline noise characteristics change.

About Local and Global Events

The SequencePro software supports two types of timed events.

Timed Event	Description
Local Event	Added directly to a chromatogram in the chromatogram window and apply only to the cycle in which they appear. Use the Events toolbar to add a Local Event.
Global Event	Contained in the method template and applied to every cycle in a sequencing run. Use the Method Editor to add a Global Event.

Other SequencePro Events

There are 24 events available in the SequencePro software. For events that appear with both a plus and a minus sign, selecting the positive event enables it; selecting the negative event disables it.

Note: Only the first three events (bunching factor, noise threshold, and area threshold) have values associated with them.

The following table provides a brief description of each timed event. They are described in more detail on pages A-19 to A-48. If you are not familiar with how to use timed events to optimize peak detection and integration, refer to this topic before starting the procedures.

Timed Events

Event	Description	Value
BF	Set bunch factor to value	1 to 99
NT	Set noise threshold to value	1 to 999999
AT	Set area threshold to value	5 to 10^{15}
+P	Enable peak detection	–

Timed Events *(continued)*

Event	Description	Value
–P	Disable peak detection	–
+N	Enable negative peak detection	–
–N	Disable negative peak detection	–
+I	Inhibit end of peak detection	–
–I	Allow end of peak detection	–
+CB	Turn on non-forced common baseline	–
–CB	Turn off non-forced common baseline	–
E	Force the end of current peak	–
S	Force start of new peak	–
BL	Force baseline to current peak	–
+HF	Force horizontal toward baseline	–
–HF	Stop horizontal toward baseline	–
HR	Force horizontal backward baseline	–
+M	Start manual integration window	–
–M	End manual integration window	–
+V	Turn on valley-to-valley baselines	–
–V	Turn off valley-to-valley baselines	–
+X	Force an exponential skim	–
–X	Prevent an exponential skim	–
T	Force a tangential skim	–

Affecting Integration with Timed Events

The following pages summarize how each timed event, or pair of timed events, affects the way the SequencePro software detects and integrates peaks.

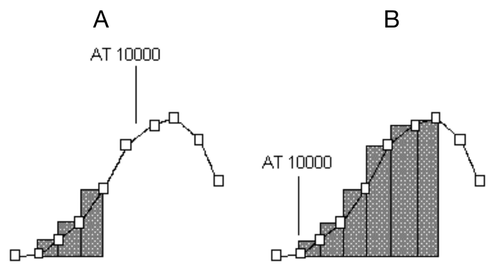
Using Bunching Factor, Noise Threshold, and Area Threshold Events

Resetting Peak Detection Values

Using the bunching factor (BF), noise threshold (NT), and area threshold (AT) timed events allows you to reset the peak detection values. These events take effect at the time they are scheduled: they do not work retroactively.

The following table describes the figure below.

Figure...	Shows...
"A"	the current area threshold is low, which enables the SequencePro software to confirm the peak before the AT event occurs. The new area threshold value will not affect the confirmation of the peak. The AT event is scheduled too late to affect confirmation of the current peak.
"B"	the AT event is scheduled during peak confirmation, so the SequencePro software uses the new, larger area threshold to determine where the peak actually starts. In this figure, the new area threshold takes effect.



Using BF, NT, or AT

For best results, schedule the BF, NT, or AT timed events on the baseline away from peaks.

Example

If you schedule these events at times too close to a transition from one peak detection stage to another, detection may differ significantly from chromatogram to chromatogram because of small shifts in retention time, or random noise.

Using BF

The BF event helps compensate for changes in peak width over the course of a run.

Example

In packed-column, isothermal gas chromatography, peaks tend to broaden as the run progresses. In these cases, scheduling this event at least once can help maintain the ideal 20 bunched points per peak. The value set for the bunching factors is the number of raw data points averaged together to produce a single bunched point. This reduces the effective sampling rate for peak detection.

Using NT

The NT event helps compensate for changes in baseline noise. You can increase the noise threshold to decrease peak detection sensitivity, or decrease the noise threshold to increase sensitivity.

Example

Suppose the beginning of a chromatogram is noisy because of fast-eluting contaminants in the solvent, and this noise diminishes later in the run.

Optimizing Peak Detection

Set the default noise threshold high enough to screen out the initial noise, and schedule the NT event to decrease the noise threshold at the time when the noise diminishes.

Using AT

The AT event also affects peak detection sensitivity. If you are scheduling an NT event to change the noise threshold, you should probably schedule an AT event at the same time to change the area threshold.

Example

If you are lowering the noise threshold to gain sensitivity, you want to lower the area threshold as well. Otherwise, the SequencePro software may not confirm the smaller potential peaks that the lower noise threshold allows it to detect.

Note: Applied Biosystems recommends that you set the area threshold to five times the value of the noise threshold.

Disabling or Enabling Peak Detection (+P/–P)

About Peak Detection

The pair of timed events +P and –P turn peak detection on and off, respectively, allowing you to enable and disable peak detection during a run. For example, if valve switching causes noise spikes in the middle of a chromatogram, you can schedule a –P event before the affected region and a +P event after it to avoid the detection of false peaks.

Enabling Peak Detection

The following table lists what happens if peak detection (+P) is enabled in the SequencePro software:

If the SequencePro software is...	Then...
at the point where it is searching for a peak ending point (*i.e.*, after the peak crest) when the –P event occurs	when the –P event occurs, the peak will end at exactly that point, and all peak detection will stop.
at any other point in peak detection	the process will stop and the current peak will not be detected.

Using +P and –P

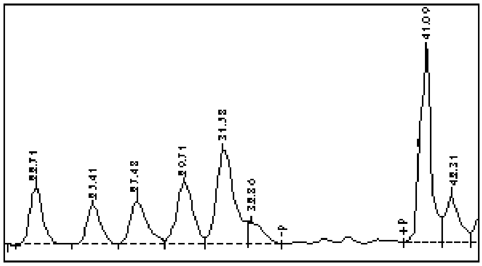
The following table lists the effects of the +P and –P event.

This event...	Has no effect...
+P	unless peak detection has been disabled by a –P event.
–P	if peak detection is currently disabled.

+P and -P Examples

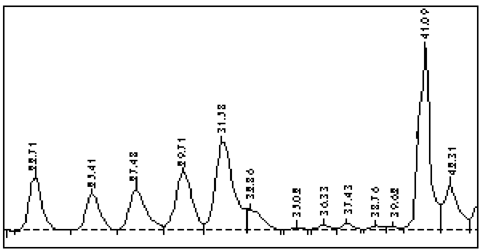
Example Showing Peaks Without –P and +P Events

Without –P and +P events, the SequencePro software detects insignificant peaks (from 35.02 to 39.62 minutes).



Example Showing With –P and +P Events

With –P and +P events, peak detection is disabled in this region.



Enabling or Disabling Negative Peak Detection (+N/–N)

About Negative Peak Detection

The pair of timed events +N and –N turn negative peak detection on and off, respectively. They allow you to enable and disable the detection of negative peaks during a run. By default, the SequencePro software detects only positive peaks, so you need to add a +N event in order to detect negative peaks. The program will continue to detect positive peaks after a +N event takes effect.

The SequencePro software detects a potential negative peak if:

- A bunched data point value decreases from the previous bunched point value by more than the noise threshold, and
- If both points lie below the "theoretical" baseline used to separate positive and negative peaks. It also applies the area threshold to negative peaks.

Using +N and –N

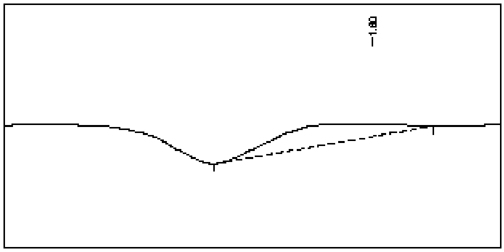
The –N event takes effect immediately if peak detection is in the Begin or Confirm stages. Otherwise, it is delayed until the end of the current peak is found.

This event...	Has no effect...
+N	if you schedule a +N event to occur when negative peak detection is already enabled.
-N	when negative peak detection is enabled.

+N and –N Examples

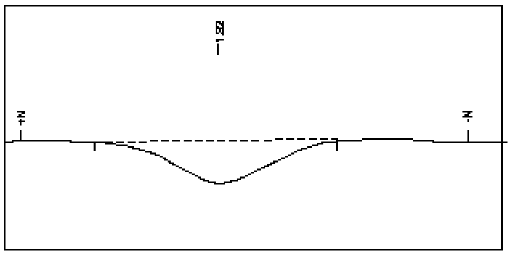
Example Showing Peaks Without +N and –N Events

Without the +N and –N events, the negative peak is not detected, and a false positive peak is found.



Example Showing Peaks with +N and –N Events

With the +N and –N events, the negative peak is detected, and then negative peak detection is disabled.



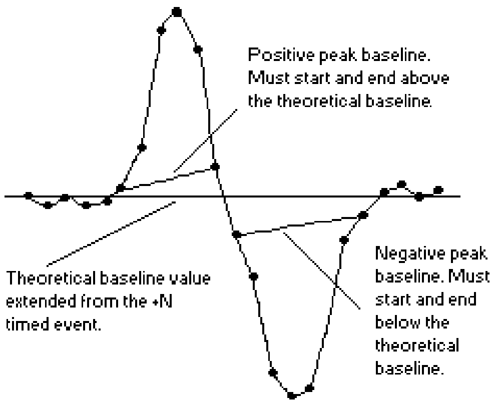
Detecting a Negative Peak Following a Positive Peak

To detect a negative peak that follows a positive peak, the SequencePro software must determine the baseline point at which the positive peak ends and the negative peak begins. The same is true for detecting a positive peak that follows a negative peak.

This theoretical baseline is set at the voltage level of the bunched point within whose data range the +N event occurs. The theoretical baseline is not likely to coincide with actual raw data point values when it intersects peaks on the chromatogram, so the SequencePro software will use the closest actual data. Positive peaks will be forced to start and end on raw data points above this baseline, and negative peaks will be forced to start and end on raw data points below this baseline. As a result, the areas between the theoretical baseline and peak baselines will be excluded from the peak areas.

Finding the Dividing Line

The following figure shows how the SequencePro software finds the dividing line between a positive and negative peak.



Inhibiting or Allowing End-of-Peak Detection (+I/–I)

About End-of-Peak Detection

The pair of timed events +I and –I inhibit and allow, respectively, the stage in peak detection when the peak end is detected. When +I is in effect, the SequencePro software does not attempt to determine the ending point of a peak. The –I event re-establishes peak detection for ending points.

Using +I and –I Events

Use +I and –I events when isomers of a compound elute as shoulders on the trailing side of the main peak, and if you want all isomers to be treated as part of the main peak. In this case, you can schedule the +I event to prevent the SequencePro software from finding the end of the main peak, and schedule the –I event to allow peak end detection again after the last isomer.

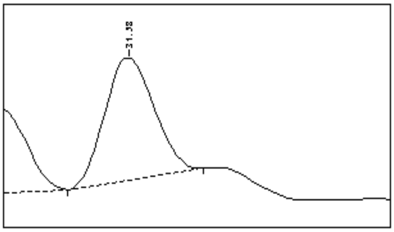
+I and –I Examples

The +I event forces the SequencePro software to remain in the Find End stage. The result is that it will neither find a peak end nor detect the start of the next peak until the event is switched off. The entire chromatogram, from the time of a +I event to the time of a –I event will be perceived as part of the same peak.

The +I event takes effect immediately, but has no impact on processing until peak detection naturally enters the Find End stage. The –I event takes effect immediately, and allows the SequencePro software to find the first peak end it encounters, based on the current noise threshold.

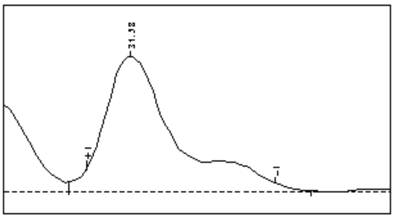
Example Showing Peaks without the +I and –I Events

Without the +I and –I events, the peak at 31.58 minutes ends naturally, but the shoulder on its trailing edge goes undetected.



Example Showing Peaks with the +I and –I Events

With the +I and –I events, the shoulder is included in the peak.



Forcing or Discontinuing Common Baseline (+CB/–CB)

About Common Baseline Events

The +CB event causes the software to assign an overlap flag of 1 to all peaks that occur while the event is in effect.

Using Common Baseline Events

The following table lists the how the SequencePro software treats peaks during integration if a +CB event is enabled.

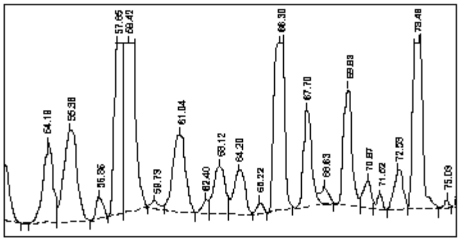
<table>
<tr><th>Stage</th><th>Description</th></tr>
<tr><td>1.</td><td>The SequencePro software treats all peaks as though they are clustered, even if they do not meet the criteria for being overlapped.</td></tr>
<tr><td>2.</td><td>The software then draws a common baseline for this series of peaks, as shown in the following example.
<table>
<tr><th>Event</th><th>Description</th></tr>
<tr><td>+CB</td><td>Takes effect at the time it is scheduled and applies to the current peak.
The peak on which the event is located, if any, as well as to subsequent peaks.</td></tr>
<tr><td>–CB</td><td>Discontinues the +CB event.
The SequencePro software continues to evaluate each peak against the overlap criteria and assigns overlap flags of 0 or 1, as warranted.
This event takes effect immediately and applies to the current peak and subsequent peaks.</td></tr>
</table></td></tr>
</table>

+CB and –CB Examples

Example Showing Peaks without the +CB Event

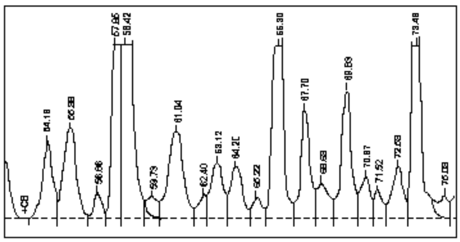
Without the +CB event, each peak has a separate baseline.

Note: Even when the +CB event is in effect, the SequencePro software will check the artificial cluster for valleys that penetrate the forced baseline. If such valleys are present, it redefines the baseline, where necessary, to eliminate penetration.



Example Showing Peaks with the –CB Event

With the +CB event (shown at the far left, below), all peaks share a common baseline.



Forcing or Discontinuing Valley-to-Valley Baselines (+V/–V)

About Valley-to-Valley Baselines

The +V event assigns an overlap flag of 0 to all peaks that occur while the event is in effect.

Using Valley-to-Valley Baselines

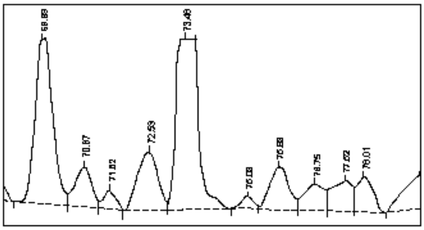
The following table lists how the SequencePro software treats peaks during integration if a +V event is enabled.

<table>
<tr><th>Stage</th><th>Description</th></tr>
<tr><td>1.</td><td>The SequencePro software treats all peaks as though they are resolved, even if they meet the criteria for being overlapped.</td></tr>
<tr><td>2.</td><td>The software draws an individual baseline for each peak, extending from valley to valley.
No peaks are clustered.
<table>
<tr><th>Event</th><th>Description</th></tr>
<tr><td>+V</td><td>Takes effect at the time it is scheduled and applies to the current peak, as well to subsequent peaks.</td></tr>
<tr><td>–V</td><td>Discontinues the +V event.
The SequencePro software resumes evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1, as warranted.
This event takes effect immediately and applies to the current peak and subsequent peaks.</td></tr>
</table>
</td></tr>
</table>

+V and –V Examples

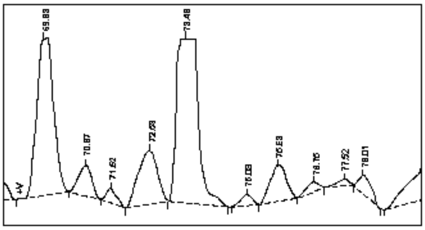
Example Showing Peaks without the +V Event

Without the +V event, some peaks are clustered, having a common baseline.



Example Showing Peaks with the +V Event

With the +V event, each peak has its own individual baseline, even if it is overlapped.



Forcing Baseline to Point (BL)

About the Baseline to Point Event

The BL event forces the baseline to the start of the current peak. The current peak is the one on which the event occurs, or the one that follows the event, if it occurs between peaks.

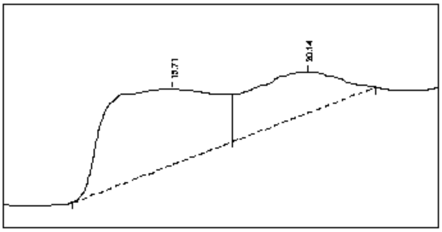
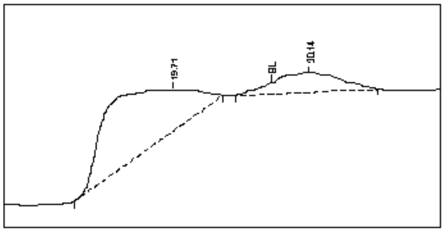
Using Baseline to Point Events

The following table lists how the SequencePro software treats peaks if a BL event is enabled.

<table>
<tr><th>Stage</th><th>Description</th></tr>
<tr><td>1.</td><td>The BL event causes the SequencePro software to assign an overlap flag of 0 to the peak preceding the current peak.
Result: The preceding peak is treated as though it is resolved from the current peak.</td></tr>
<tr><td>2.</td><td>The SequencePro software terminates the peak cluster at the end of the preceding peak by ending the cluster baseline there.
Because the baseline is forced into this position, the baseline for the current peak is also forced to start at or near the peak start.
<table>
<tr><th>If this event occurs when peak detection is in the...</th><th>Then the baseline is forced to start at the...</th></tr>
<tr><td>Begin or Confirm stages</td><td>bunched point representing the data bunch at the event’s time.</td></tr>
<tr><td>Find Top or Find End stages</td><td>starting point of the current peak.</td></tr>
</table>
</td></tr>
</table>

BL Event Examples

The following table lists examples using the BL event.

The figure below...	Shows...
	no BL event has been scheduled. A common baseline has been drawn for the two peaks because they meet the overlap criteria.
	the effect of the BL event when it occurs during the **Find Top** or **Find End** stages. The preceding peak is assigned a 0 overlap flag and thus has a valley-to-valley baseline. As a result, the baseline of the current peak is forced to begin at the peak starting point. The table below lists what happens if the BL event is enabled.

If the BL event is...	Then...
not enabled	the two peaks are clustered and integrated by using drop lines.
enabled	a baseline is forced to begin at the peak start.

Repositioning Theoretical Baselines

You can use the BL event to reposition the theoretical baseline used for negative peak detection when a negative peak and a positive peak are contiguous.

The following table lists where the theoretical baseline is moved in relation to where the BL event is placed:

If the BL event is...	Then the theoretical baseline is...
placed within the negative or positive peak	moved to the level of the peak start.
outside either peak	set at the level of the point within whose range the event is scheduled.

Forcing or Discontinuing Horizontal Baseline Forward (+HF/–HF)

About the Horizontal Baseline Forward Event

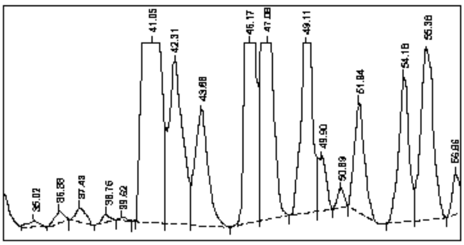
The +HF event projects a horizontal baseline from the time of the event to the end of the chromatogram, or until the event is turned off by a –HF event.

During integration, the SequencePro software does not adjust the baseline to avoid penetration by valleys (as shown by the valley between 43 and 46 minutes in the following example). Thus the baseline established by the +HF event always remains horizontal.

+HF and –HF Examples

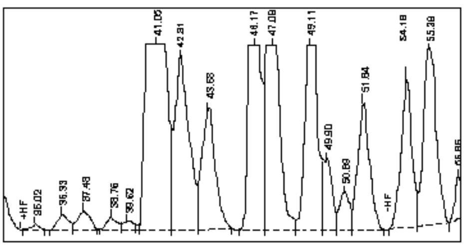
Example Showing Peaks without the +HF Event

Without the +HF event, each peak has a separate baseline.



Example Showing Peaks with the +HF Event

With the +HF event, all peaks share a common baseline that does not deviate from the horizontal. The –HF event restores normal baseline treatment.



Using the +HF Event

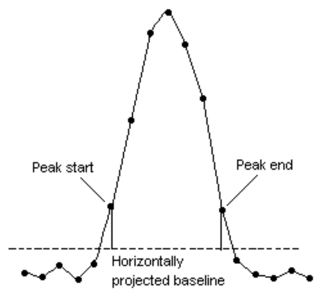
The following table lists the process of how the +HF event works:

Stage	Description
1.	The +HF event takes effect at the time the event is scheduled. The horizontal baseline is projected from the level of the bunched point within whose data range the event occurs.
2.	In sections of the chromatogram where the +HF event is in effect, peaks cannot have starting or ending points below the baseline.
3.	If the baseline intersects a peak, it is not likely to intersect exactly at a raw data point, so the peak starting and ending points will usually lie some distance above the projected baseline Result: The area between the projected baseline and peak starting and ending points will be excluded from the peak area, as shown in the example.

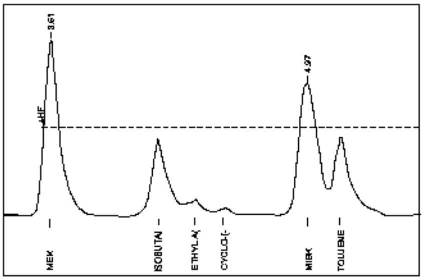
+HF Examples

The following table lists examples using the +HF event.

+HF Event Examples

The figure below...	Shows...
	the peak starting and ending points of the +HF event must lie above the horizontally projected baseline.

+HF Event Examples *(continued)*

The figure below...	**Shows...**
	that you must be careful where you place the +HF event. If the projected baseline is set too high, as in this example, large areas of peaks will be submerged below the baseline and, therefore, will not be integrated. Some peaks may be submerged entirely and go undetected because the SequencePro software removes the peak data from the result file. **Note:** To avoid this problem, do not schedule a +HF event on a peak: place it on the baseline just before the start of a peak. The following table describes using the +HF event.

The following table provides information on how the +HF event works.

If...	**Then...**
a +HF event is scheduled at a point that is too high on the chromatogram	peaks may not be integrated.
a –HF event is scheduled	the +HF event is discontinued. The SequencePro software continues evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1 as warranted.
the SequencePro software is searching for a peak ending point when either event is encountered	the current peak will end at the event. Otherwise, the current peak (if any) will be discarded.

Scheduling +HF and –HF Pairs

You may schedule any number of +HF/–HF pairs in a chromatogram, but they must not be overlapped.

If...	Then...
two +HF events are scheduled in a row, without an intervening -HF event	the second event will be ignored. The lack of an intervening –HF event might even prevent the SequencePro software from drawing a baseline.

Forcing a Horizontal Baseline Backward (HR)

About the Horizontal Baseline Backward Event

The HR timed event projects a baseline backward from the time of the event. The baseline is set at the level of the bunched point within whose data range the HR event occurs. The SequencePro software does not adjust the baseline to prevent penetration by valleys; thus the baseline remains horizontal.

Note: To achieve the best result, you should not schedule the HR event on a peak: place it on the baseline between peaks or after any peak. This helps avoid baselines that are too high or too low.

Using Horizontal Baseline Backward

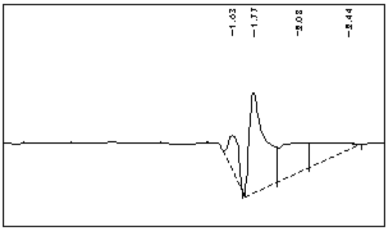
The following table lists the how the SequencePro software treats peaks if a HR event is enabled:

Stage	Description
1.	Where the HR event is in effect, peaks cannot have starting or ending points below the baseline.
2.	If the baseline intersects a peak, the intersection is not likely to occur exactly at a raw data point. Therefore, the peak starting and ending points will usually lie some distance above the projected baseline. As a result, the area between the projected baseline and the peak starting and ending points will be excluded from the peak area. This result is due to the algorithm in the SequencePro software that requires peak boundaries to occur at raw data points and not between points.

HR Event Examples

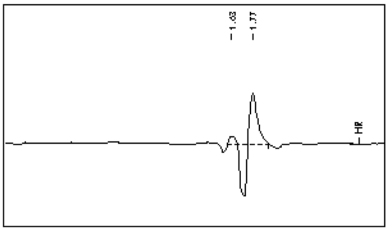
Example Showing Peaks without the HR Event

Without the HR event, peaks may have separate baselines.



Example Showing Peaks with the HR Event

With the HR event, a common baseline projects backward to the start of the chromatogram.



Using the End Peak Here (E) Event

About the End Peak Here Event

The E event establishes a peak end at a selected point. You can use this event in conjunction with the +I and –I events to ensure that a peak ends at the desired point.

Scheduling the End Peak Here Event

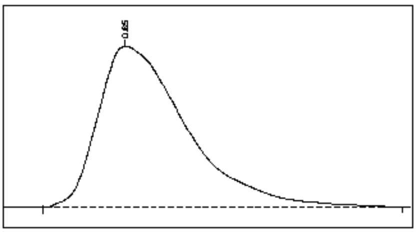
The following table lists scheduling an E event:

If the SequencePro software is...	Then the...
in the Begin or Confirm stages at the time the E event is scheduled	event will have no effect.
not in the Begin or Confirm stages at the time the E event is scheduled	last point of the current bunch becomes the ending point. After this event, the SequencePro software re-enters the Begin stage.

E Event Examples

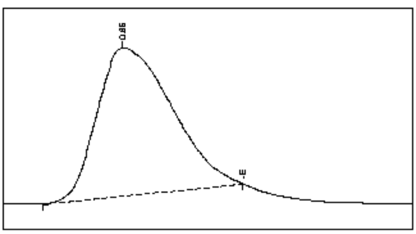
Example Showing a Peak without the E Event

Without the E event, the peak end is found automatically.



Example Showing a Peak with the Event

With the E event, the peak end is set arbitrarily.



Using the Split/Start Peak (S)

About the Split/Start Peak Event

The S event starts a new peak at the time when the event occurs, regardless of whether a peak would normally be detected and confirmed at this position. This event is typically used to force shoulders to be split from the parent peak, as shown in the following example.

Scheduling Split/Start Peak Events

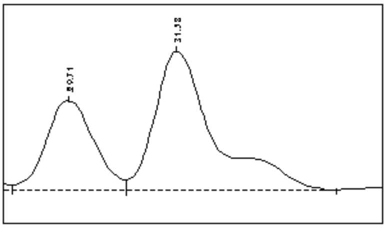
The following table describes scheduling an S event:

If the SequencePro software is...	Then...
in the Begin or Confirm stages at the time the S event occurs	the Last raw data point of the current bunch (located at the time the event is scheduled) becomes the peak starting point. Also, the SequencePro software enters into the Find Top stage.
at the Find Top stage when the S event is scheduled	it forces the present peak to end on the last point of the current bunch, and a new peak is forced to begin.

Split/Start Peak Event Examples

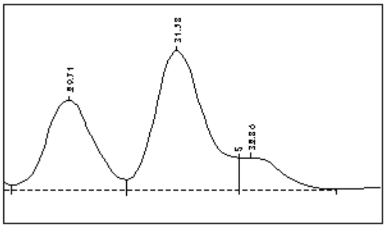
Example Showing Peaks without the S Event

Without the S event, the shoulder on the peak at 31.58 minutes is not detected as a separate peak.



Example Showing Peaks with the S Event

With the S event, the shoulder is detected and split off.



Using Start Manual Integration/End Manual Integration (+M/–M)

About Manual Integration Events

The timed events +M and –M allow manual integration of peaks. Manual integration of peaks may be required if you want to start a peak's baseline at a particular point.

Using the Manual Integration Events

The following table lists where to place the +M/–M events:

Place the...	**The SequencePro software will...**
+M event where you want a peak's baseline to start	draw the baseline between the points on which the events have been placed. If they are not placed exactly on two points, the SequencePro software will connect the points within whose area the events are positioned. Each point represents an area slice extending backward from the point to the preceding point.
–M event at the end of the baseline	

Using Force Exponential Skim (+X) Events

About Force Exponential Skim Events

To ensure that the SequencePro software creates an exponential skim, schedule the +X event anywhere within the parent peak on which you want the skim to start.

Knowing When +X Will Have No Effect

The following table lists when the +X event will have no effect or when it will not work:

The +X event will...	If...
have no effect	unless you place it between the starting and ending points of a peak.
not work	placed on the baseline outside a peak.

Using the +X Event

The following table lists how to use the +X event:

If you...	Then...
schedule more than one +X event on the same peak	only one event will be recognized.
place +X events on two adjacent peaks in the same cluster	the SequencePro software will ignore the first one because it cannot decipher multiple skims when they converge.
place +X events on nonadjacent peaks in the same cluster	the SequencePro software will draw exponential skims. However, what is drawn from the first peak will not extend past the start of the next peak with a +X event.

Using Prevent Exponential Skim (–X) Events

About Prevent Exponential Skim Events

Scheduling a –X event anywhere on a peak ensures that the SequencePro software will not create an exponential skim that originates on that peak. This event does not prevent the software from drawing a skim beneath the peak, if the skim originated on a preceding peak.

Knowing When –X Will Have No Effect

Note: If you schedule more than one –X event on the same peak, only one event will be recognized.

The following table lists when the –X event will have no effect or when it will not work:

The –X event will...	If...
have no effect	not placed between a peak's starting and ending points.
not work	placed on the baseline outside a peak.

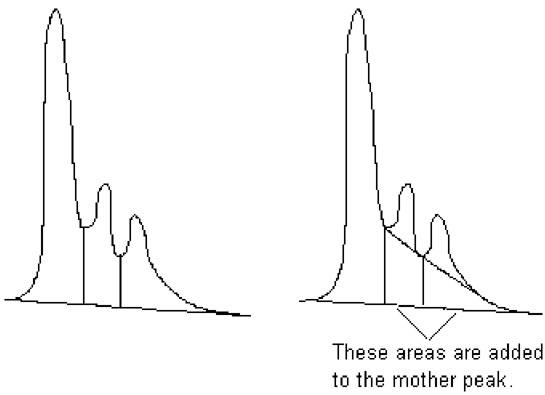
Tangential Skim (T)

About Tangential Skim Events

The following table describes how the SequencePro software creates a tangential skim:

Stage	Description
1.	The SequencePro software draws a straight baseline from the starting point to the ending point of each child peak of a designated parent peak, as shown in the following example. Such baselines are drawn for each child peak that occurs before the end of the cluster.
2.	The software checks to see that the new baselines are not penetrated by the child peaks.
3.	New areas for the child peaks are calculated, and the difference between the old and new areas (the area between the tangent baselines and the cluster baseline) is added to the parent peak.

The example below shows peaks before and after a tangential skim.



Adjusting Peak Areas

To adjust peak areas by using a tangential skim, use a T timed event. Unlike an exponential skim, the SequencePro software never creates a tangential skim automatically.

Knowing When T Will Have No Effect

The following table lists when the T event will have no effect or when it will not work:

The T event will...	If...
have no effect	not placed between a peak's starting and ending points.
not work	placed on the baseline outside a peak.

Scheduling More Than One T Event

The following table describes scheduling T events:

If you...	Then the...
schedule more than one T event on the same peak	effect will be the same as having one T event.
place T events on two adjacent peaks in the same cluster **Note:** Avoid scheduling T events on more than one peak in a cluster.	the first one will be ignored.

Software Warranty Information B

This appendix contains the following:

Computer Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, its SequencePro® Data Analysis Application v2.1 software will perform substantially in accordance with the functions and features described in its accompanying documentation when properly installed on the instrument system for which it is designated, and that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the software product will be free of defects in materials and workmanship under normal use. If buyer believes that it has discovered a failure of the software to satisfy the foregoing warranty, and if buyer notifies Applied Biosystems of such failure in writing during the ninety (90) day warranty period, and if Applied Biosystems is able to reliably reproduce such failure, then Applied Biosystems, at its sole option, will either (i) provide any software corrections or "bug-fixes" of the identified failure, if and when they become commercially available, to buyer free of charge, or (ii) notify buyer that Applied Biosystems will accept a return of the software from the buyer and, upon such return and removal of the software from buyer's systems, terminate the license to use the software and refund the buyer's purchase price for the software. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media. Applied Biosystems does not warrant that the software will meet buyer's requirements or conform exactly to its documentation, or that operation of the software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for software installed by Applied Biosystems personnel. For all software installed by the buyer or anyone other than Applied Biosystems, the applicable warranty period begins the date the software is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation outside of the environmental or use specifications, or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

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