TECHNICAL MANUAL

ProView™ Sequencing Software

Instructions for Use

7/20
TM625

ProView™ Sequencing Software

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

Promega

1. Description

1.A. ProView™ Sequencing Software

Proview™ Sequencing Software is a customizable Sanger sequencing viewer capable of displaying .ab1 files generated on many currently available capillary electrophoresis (CE) sequencers. This software can be used to view and edit sequence information, display electropherograms, generate reports, display embedded information and save edited files in multiple formats.

1.B. Recommended System Requirements

Table 1. Recommended System Requirements for Installation and Use of ProView™ Sequencing Software.

Item	Specification
CPU	2+ cores, 64-bit
Operating System	Windows® 10, 64-bit
Memory	2GB
Storage	20MB
Display Resolution	1920 × 1080

2. Download and Installation Instructions

2.A. Download the ProView™ Sequencing Software

The installer for the ProView™ Sequencing Software is available as a free download at:
www.promega.com/ProviewDownload/

2.B. Software Installation Instructions

1. To install ProView™ Sequencing Software, double-click the downloaded installer and follow the prompts to install the software on your personal computer (PC).

2. To install a desktop shortcut, select the box next to "Create a desktop shortcut" on the 'Setup' screen (Figure 1). Select **Next** to proceed.

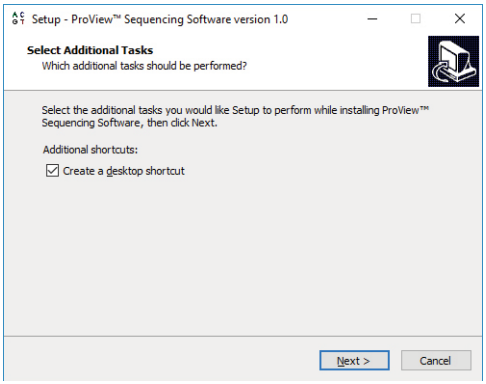


Figure 1. ProView™ Sequencing Software installation setup.

3. The software is now ready to be installed (Figure 2). Select **Install** to proceed or **Back** to change the installation options.

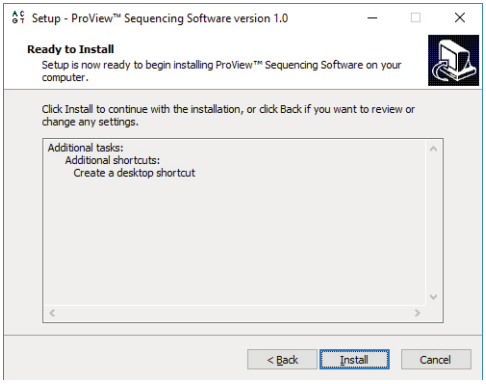


Figure 2. ProView™ Sequencing Software 'Ready to Install' screen.

2.B. Software Installation Instructions (continued)

4. Installation will proceed automatically (Figure 3). When installation is complete, the screen in Figure 4 will be displayed.

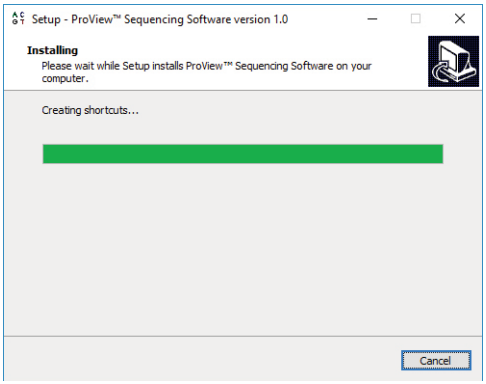


Figure 3. 'Installing' progress screen.

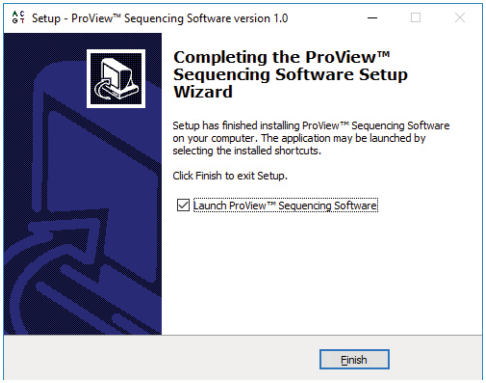


Figure 4. Installation complete.

5. To launch the software immediately, select the box next to "Launch ProView™ Sequencing Software". Otherwise, uncheck this box. Select **Finish** to complete the installation.

   Note: If you experience problems with the ProView™ Sequencing Software, try uninstalling and reinstalling the software. Alternatively, delete the 'ProView_settings.ini' file located in **C:\Users\<your_user_name>\AppData\Local\Promega\ProView** and restart the software.

3. Operating the ProView™ Sequencing Software

3.A. Software Navigation and Menus

Navigate the software using options in the Menu Bar and Navigation Pane (Figure 5).

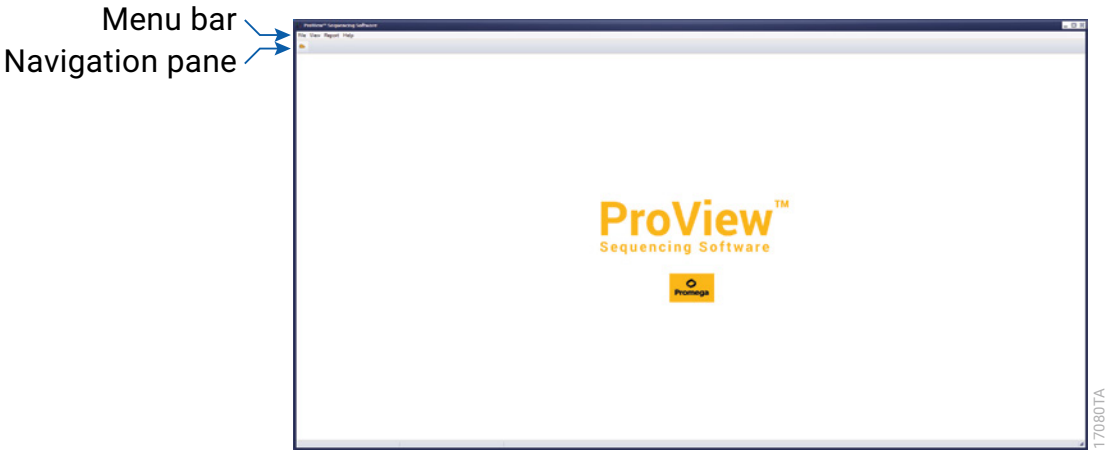


Figure 5. Main window showing Menu Bar and Navigation Pane.

3.B. Adding and Removing Files

1. To open files, select the folder icon in the Navigation Pane. Alternatively, select **Open** under File in the Menu Bar. The 'Load Files' window will appear (Figure 6).

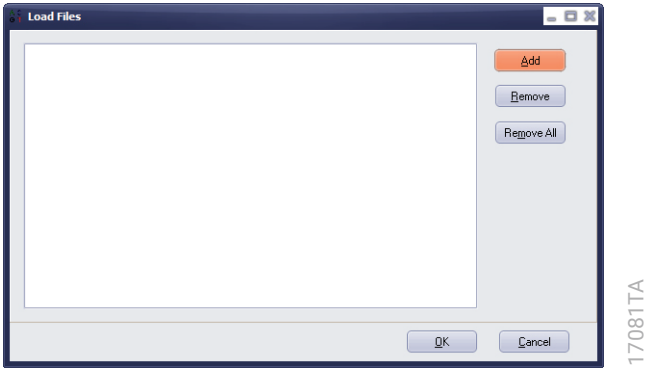


Figure 6. The 'Load Files' window.

3.B. Adding and Removing Files (continued)

2. Select **Add** and navigate to the files you want to open. Select the files and then **Open**. Alternatively, you can drag and drop files or folders from the Windows® Explorer into the 'Load Files' window (Figure 6). The ProView™ Sequencing Software will only load .ab1 files even if your folder contains other file types. Select **OK** to load the files.

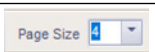
3. If there are files listed in the 'Load Files' window that you did not want to load, select one or more files and then **Remove** to delete them from the list. Alternatively, you can select **Remove All** to clear the file list in this window.

4. The files will appear in the Sample Table (Figure 7). Included in this table are the sample names as well as information about the samples. The columns in this table can be customized by selecting Settings under View (see Section 3.H). You can sort each column in alphabetical or numerical order by clicking on the column name. Click on the column name again to toggle between ascending and descending order.

3.C Viewing Electropherograms

1. When files are loaded, additional icons will appear on the Navigation Pane (Table 2).

Table 2. Navigation Pane Icons.

Icon	Name	Description
	Print Report	Opens the 'Print Report' window with options to print electropherograms (see Section 3.I).
	Open Trace Thumbnails	Opens the 'Trace Thumbnail' viewer to view thumbnails of each electropherogram (see Section 3.E).
	Display QV Mode	Determines how Quality Values (QV) are displayed when an electropherogram is shown. • Display Quality Values above each basecall as numbers • Display Quality Values above each basecall as bars • Hide Quality Values
	Mask Trimmed Data	Masks (grays out) data deemed to be of low quality, if this information is present in the file.
	Zoom In	Incrementally zooms in all currently displayed electropherograms.
	Zoom Out	Incrementally zooms out all currently displayed electropherograms.
	Page Down	Moves to the electropherogram for the next sample listed in the Sample Table. If the Page Size is set to display more than one electropherogram, Page Down moves to the next set of electropherograms.
	Page Up	Moves to the electropherogram for the previous sample listed in the Sample Table. If the Page Size is set to display more than one electropherogram, Page Up moves to the next set of electropherograms.
	Search	Searches the electropherograms for specific DNA sequences.
	Page Size	Selects how many electropherograms can be viewed at once (can be set to 1–4).

3.C Viewing Electropherograms (continued)

2. To open an electropherogram, double-click on the file from the Sample Table. The electropherogram will appear in the Viewer Pane at the bottom of the window (Figure 7). The size of the Viewer Pane can be adjusted by hovering the cursor over the bar separating the Sample Table from the Viewer Pane and holding the left mouse button while you move up or down.

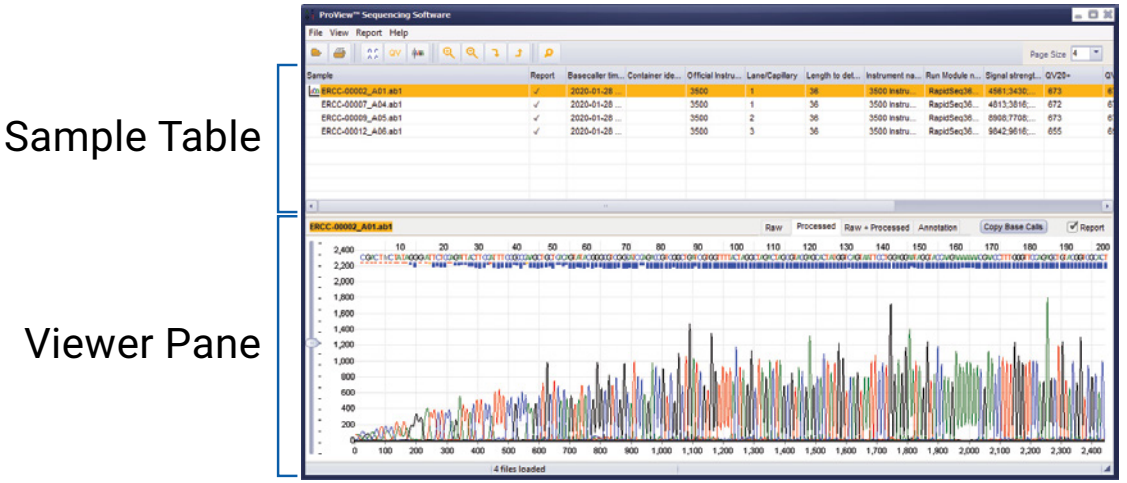


Figure 7. Sample Table and Viewer Pane.

3. Each electropherogram open in the Viewer Pane has a header that lists the file name. There are also four tabs to view your data. By default, ProView™ Sequencing Software displays the first 200 bases of your processed electropherogram. The tabs are listed in Table 3.

Table 3. Viewer Pane Tabs.

Tab	Description
Raw	Displays the raw data as it was collected on the instrument.
Processed	Displays the processed electropherogram with bases and quality values assigned to the peaks.
Raw + Processed	Divides the electropherogram window into two panels, with the raw data on the top and the processed data on the bottom.
Annotation	Displays information about the sequencing run that was embedded by the software during the run. The information that is displayed can be customized via Settings (see Section 3.H).

4. Select **Copy Base Calls** (Figure 8) to copy the entire sequence. Select the 'Report' checkbox to include electropherograms from the Quality Metric Report (see Section 3.I).

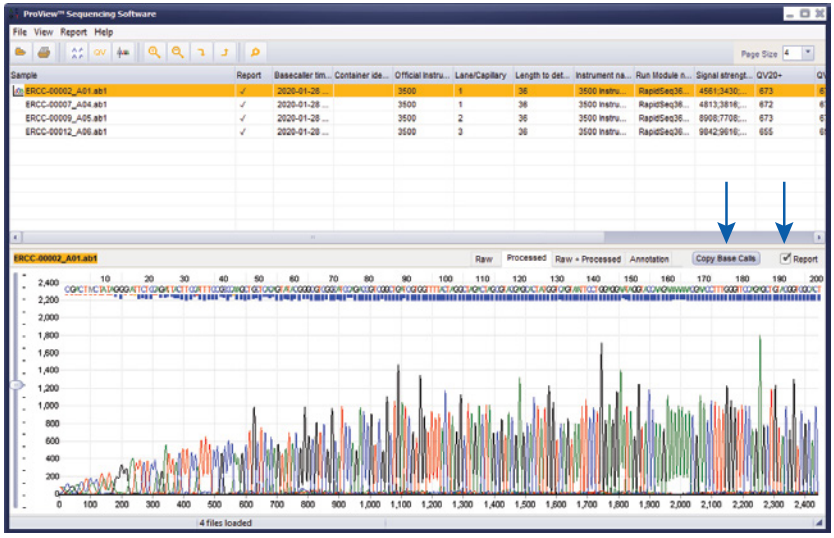


Figure 8. 'Copy Base Calls' button and 'Report' checkbox.

5. To scroll left and right through your electropherogram, right click on the electropherogram and drag the mouse left or right.

6. To zoom in on a section of the electropherogram, position the cursor at the left edge of the desired area, hold down the left mouse button and draw a box around the desired portion of the electropherogram. The image will zoom into the area selected. To zoom out to the full view, position the cursor anywhere within the electropherogram, hold down the left button and move the mouse to the left to draw a box. The entire electropherogram is displayed regardless of the size or position of the box.

   Note: The Y-axis scale is not affected by these boxes. Zooming only affects the X-axis scale using this method.

3.C Viewing Electropherograms (continued)

7. To adjust peak height (i.e., the Y-axis scale), adjust the slider on the left side of the electropherogram (Figure 9).

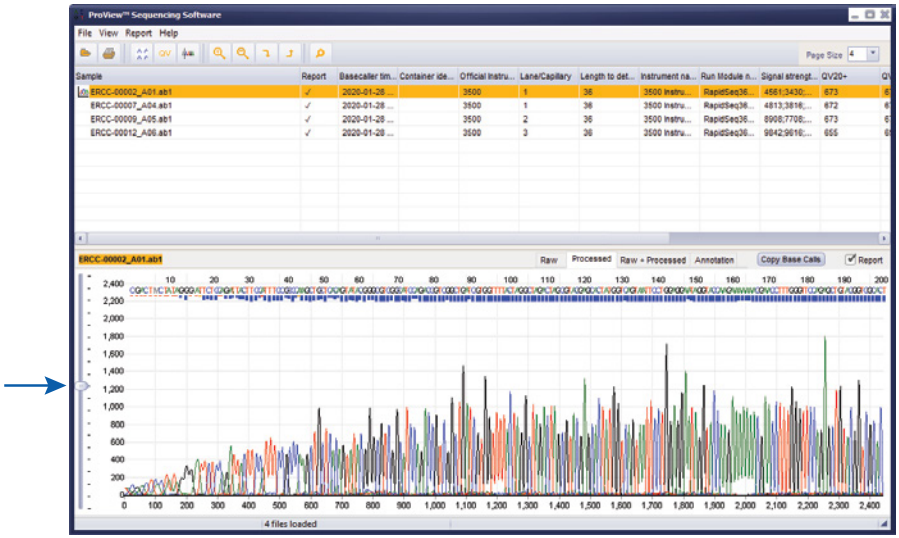


Figure 9. Y-axis-scale slider.

8. The scan and zoom functions described above are specific to the electropherogram. To zoom the X axis simultaneously on all displayed electropherograms, use the **Zoom In** and **Zoom Out** buttons in the Navigation Pane.

   Note: The **Zoom In** and **Zoom Out** buttons only affect electropherograms that are displayed.

3.D. Editing Sequence Information

1. To manually change a basecall, select the letter at the top of the electropherogram by double-clicking. This activates the edit function (Figure 10), where you can change the basecall to any IUPAC character (see Table 4).

Table 4. Valid IUPAC Bases.

Symbol	Bases Represented
A	A
C	C
G	G
T	T
W	A or T
S	C or G
M	A or C
K	G or T
R	A or G
Y	C or T
B	C, G or T
D	A, G or T
H	A, C or T
V	A, C or G
N	Any nucleotide

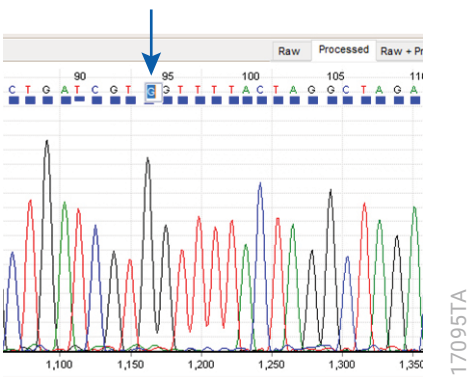


Figure 10. Base editing.

3.D. Editing Sequence Information (continued)

2. Basecalls can also be manually inserted or deleted (Figure 10). To delete a basecall, select the letter by double-clicking and then delete it using the keyboard. To insert an extra base, select the area between two letters by double-clicking and enter any IUPAC value from Table 4.

   Note: An inserted base will be assigned a Quality Value of 1, but inserting bases will not change the QV20+, CRL or Trace Score. (See Table 11 for definitions of these terms.) Editing or deleting an existing base will not change the Quality Value assigned to this base.

3.E. Trace Thumbnail Viewer

The 'Trace Thumbnail Viewer' window displays the raw electropherograms of all samples (Figure 11). This offers quick visualization of many electropherograms at once and can be useful for troubleshooting or finding failed reactions within a large pool.

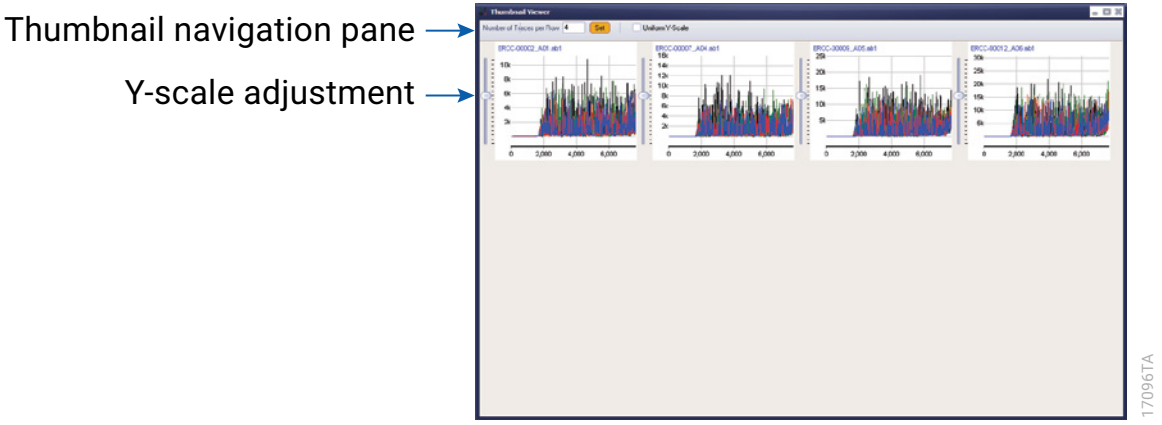


Figure 11. 'Trace Thumbnail Viewer' window.

The Thumbnail Navigation Pane at the top of the Trace Thumbnail Viewer can customize the view. The options provided are summarized in Table 5.

Table 5. 'Trace Thumbnail Viewer' Window Options.

Option	Description
Number of Traces per Row	Customizes the number of thumbnails that will fit in each row of the 'Trace Thumbnail Viewer' window. This may be set to any value 1–12.
Uniform Y-Scale	Normalizes the Y-axis scales in all thumbnails to the electropherogram with the highest signal. This function is used for finding reactions with low signal strength.

The Trace Thumbnail Viewer also includes a slider bar to adjust the Y-axis scale (Figure 11).

You can access this function through the Menu Bar or the Navigation Pane.

1. To open using the Menu Bar, select **View** and then **Open Trace Thumbnail Viewer**. To open through the Navigation Pane, select the **Open Trace Thumbnails** button (Table 2). The window shown in Figure 11 is opened.
2. Make the desired adjustments to the Navigation Pane and Y-axis scale.
3. To close the 'Trace Thumbnail Viewer' window, select the **X** on the top right of the window. This action will not close the ProView™ Sequencing Software.

3.F. Viewing Specific Dye Channels

ProView™ Sequencing Software allows you to hide or view individual dye channels in both raw and processed electropherograms. Hide or view individual dye channels as follows:

1. Select **View** in the Menu Bar. You can show and hide all dye channels at the same time or display individual dye channels (Figure 12).
2. Select the letter of the dye channel that you want hidden or displayed. A check next to the letter indicates that this dye channel will be displayed.

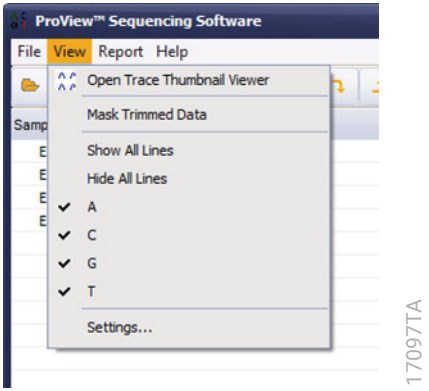


Figure 12. The 'View' menu.

3.G. Viewing Embedded Run Information

Sequencing platforms embed useful information in the .ab1 files generated on that instrument. For example, quality metrics, expiration dates and lot numbers of consumables, and information regarding the instrument protocols and data analysis can often be found in these files. There are two ways to view this data: Using the Sample Table and the 'Annotation' tab (Section 3.C). Definitions for these data fields can be found in the *ABIF File Format Specification and Sample File Schema* document published by Applied Biosystems. Each of the two methods for viewing embedded data can be independently configured to display only the desired information. The method for customization is described in Section 3.H.

3.H. Settings

To open the 'Settings' window, select **View** in the Menu Bar and then choose **Settings** (Figure 13).

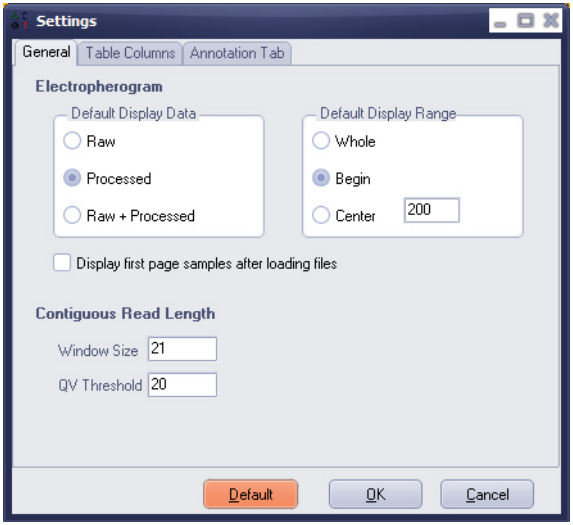


Figure 13. The 'Settings' window.

The 'Settings' window has three tabs, which are described in Table 6.

Table 6. 'Settings' Tabs.

Tab	Description
General	Contains general program settings regarding electropherogram display and the Contiguous Read Length (CRL) calculation.
Table Columns	Contains all possible fields that can be displayed within the Sample Table as defined by the ABIF file format (.ab1).
Annotation Tab	Contains all possible fields that can be displayed within the 'Annotation' tab of each electropherogram as defined by the ABIF file format (.ab1).

‘General’ Tab

The ‘General’ tab has two main areas: Electropherogram and Contiguous Read Length.

The ‘Electropherogram’ section includes multiple settings that can be adjusted. These are Default Display Data, Default Display Range and a checkbox for opening electropherograms automatically after loading files.

For Default Display Data, you can choose between the options in Table 7.

Table 7. Default Display Data.

Setting	Description
Raw	Displays the raw electropherogram without basecalls or mobility correction.
Processed (Default)	Displays the processed electropherogram after mobility correction with basecalls and quality values.
Raw + Processed	Displays both raw and processed electropherograms simultaneously by splitting the panel. The raw electropherogram is on top, and the processed electropherogram is on the bottom.

For Default Display Range, you can adjust the portion of the electropherogram that will be displayed when opened using the options in Table 8.

Table 8. Default Display Range.

Setting	Description
Whole	Displays the entire electropherogram.
Begin	Displays the beginning of the electropherogram, using the value in the ‘Window Size’ box.
Center	Displays the center of the electropherogram, using the value in the ‘Window Size’ box.
Window Size	Adjusts the size of the window for Begin and Center above, in bases.

If desired, the first samples in the Sample Table can be displayed automatically after loading. The number of electropherograms that will be displayed is controlled by the ‘Page Size’ value (Section 3.C). Select the ‘Display first page samples after loading files’ checkbox to display electropherograms automatically after loading files.

3.H. Settings (continued)

The Contiguous Read Length (CRL) section allows you to adjust the values for the CRL calculation (see Section 3.I).

Table 9. Contiguous Read Length Definitions.

Setting	Description
Contiguous Read Length (CRL)*	Longest stretch of contiguous bases in the sequence that has an average Quality Value (QV) score of greater than or equal to the QV Threshold over the set Window Size. **Note:** The Quality Value (QV) is a logarithmic measure of base calling error probability. For example, quality values of 10, 20 and 30 indicate an incorrect base call probability of 1 in 10, 1 in 100, and 1 in 1,000, respectively.
Window Size	The size of the sliding window, in bases, used for the CRL calculation.
QV Threshold	The threshold for the sliding window average Quality Value (QV) used for the CRL calculation.

By default, the Window Size is 21 and the QV Threshold is 20, the default values of the Spectrum Compact CE System. Other platforms may use different values for these calculations.

Note: A field with an * after the title is a value calculated within the ProView™ Sequencing Software rather than an embedded value. These may differ from the embedded values due to different default settings on different instruments.

'Table Columns' Tab

The 'Table Columns' tab contains all possible fields that can be displayed within the Sample Table. A check in the checkbox means that this field will be displayed in the Sample Table. There is also the option to **Select All** or **Deselect All**.

Note: A field with an * after the title is a value calculated within the ProView™ Sequencing Software rather than an embedded value (see Table 11). These may differ from the embedded values due to different default settings on different instruments.

'Annotation' Tab

The 'Annotation' tab contains all possible fields that can be displayed within the 'Annotation' tab of each electropherogram. There is also the option to **Select All** or **Deselect All**.

Note: A field with an * after the title is a value calculated within the ProView™ Sequencing Software rather than an embedded value. These may differ from the embedded values due to different default settings on different instruments.

3. After choosing your preferred settings, select **OK** to save them. Alternatively, select **Cancel** to close the settings dialog without any changes, or select **Default** to reset all settings to the default values.

3.I. Creating and Printing Reports

You can export or print reports from the ProView™ Sequencing Software by selecting one of the following two options from the Report menu in the Menu Bar.

Table 10. Report Options.

Report Option	Description
Export Quality Metric Report	The Quality Metric Report displays each sample that was selected to be included in the report. This is further described below.
Print Report	The Print Report option allows you to print electropherograms, with customization as to which files and how many bases are shown on one line. This is described further in Step 1 below.

The Quality Metric Report displays basic information and quality metrics about your electropherograms (Figure 14 and Table 11).

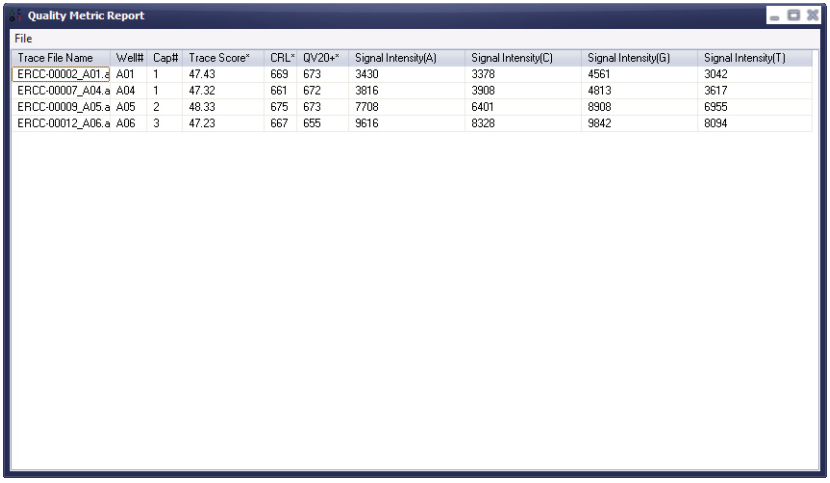


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Figure 14. 'Quality Metric Report' window.

3.I. Creating and Printing Reports (continued)

Table 11. Quality Metric Report.

Name	Description
Trace File Name	The file name of each sample.
Well#	The position of each sample in the plate or strip tube.
Cap#	The capillary number of the array that was used to inject this sample.
Trace Score*	The average Quality Value of all bases that were not trimmed (see Table 2), as calculated by ProView™ Sequencing Software.
CRL*	The contiguous read length of each sample as calculated by ProView™ Sequencing Software using the values set in the 'Settings' window (Figure 13).
QV20+*	The total number of bases in the electropherogram with a Quality Value ≥20 as calculated by ProView™ Sequencing Software. The Quality Value for each base is an estimate of basecaller accuracy.
Signal Intensity (A), Signal Intensity (C), Signal Intensity (G), Signal Intensity (T)	The average signal intensity for each dye channel.

Note: A field with an * after the title is a value calculated within the ProView™ Sequencing Software rather than an embedded value. These may differ from the embedded values due to different default settings on different instruments.

The Quality Metric Report can be exported as a Microsoft Excel®-formatted spreadsheet or printed via the 'Print' option.

1. To print your electropherogram(s), select **Print Report** under Report in the Menu Bar. The 'Print Settings' window will appear (Figure 15).

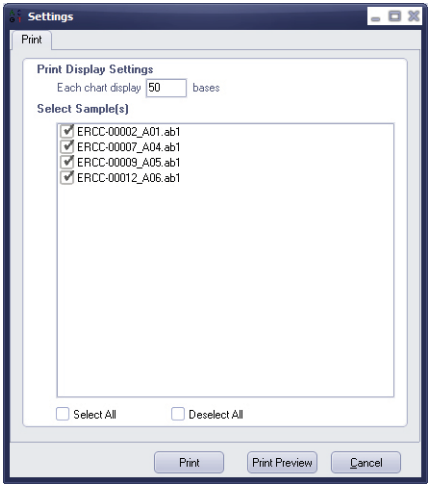

Figure 15. Print Settings.

2. To adjust how many bases are displayed on each line of the printout, adjust the 'Print Display Settings' box. To select or deselect files to be included in the printout, select or deselect the checkbox to the left of each file name. Choose **Print Preview** to see a preview of your printout (Figure 16). You can print from the 'Print Settings' window directly by selecting **Print**. You can also print from the 'Print Preview' window (Figure 16) using the **Print** icon at the top of the window.

3.I. Creating and Printing Reports (continued)

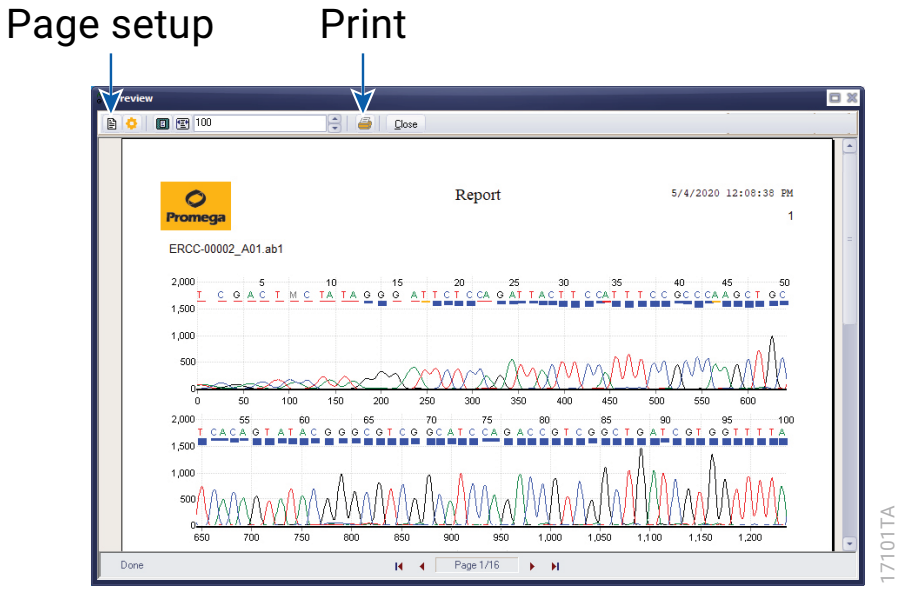

Figure 16. The 'Print Preview' window.

Access additional print settings by selecting **Page Setup** at the top of the 'Print Preview' window.

3.J. Saving Files

ProView™ Sequencing Software can save files in two formats: ab1 and fasta (Figure 17).

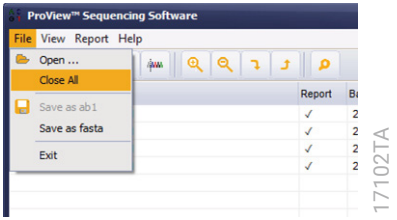

Figure 17. Saving the sequencing files.

1. If you edit the basecalls in an .ab1 file, you can save the edited file in the .ab1 format. This will save your edit and append the filename with a timestamp. Please note that the 'Save as ab1' option is greyed out unless an electropherogram is edited (see Section 3.D).
    a. To save an edited file as an ab1 file, select **File** and then choose **Save as ab1**. A window will open asking where to save the file. This will not overwrite the original file because the saved file will have a timestamp appended to the file name. Selecting **Save as ab1** will save an .ab1 file for each edited file but will not create new files for those electropherograms that were not changed.
2. Alternatively, if you only want to save the sequence information without the electropherogram, you can save files as FASTA files. To save as a FASTA file, select **File** and then choose **Save as fasta**. This will save the sequencing information in a FASTA-formatted file. This option saves all loaded files as FASTA files.

4. Help

The Help menu in the Menu Bar displays two options: Download Manual and About.

1. Select **Download Manual** to open your default browser to download the ProView™ Sequencing Software Technical Manual.
2. Choose **About** to display information on the currently installed version of ProView™ Sequencing Software (Figure 18).

Figure 18. 'About' window.